The Ecology and Conservation of the Eurasian Red Squirrel (*Sciurus vulgaris*) in an Urban Environment

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Copyright Statement

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Abstract

Urban growth and intensification are rapidly increasing on a global scale, altering the available habitat for wildlife and driving many species to decline. Urban areas were historically disregarded as suitable wildlife habitat; however, they can be biodiverse if managed appropriately as many species have the behavioural flexibility to adapt to these environments. This research investigates the ecology of an urban-adaptable species, the Eurasian red squirrel (*Sciurus vulgaris*), using Formby, Merseyside, as a case study site. Chapter One provides background information and a systematic literature review regarding the biology, causes of decline, and urban ecology of the red squirrel. Chapter Two examines the red squirrel population in Formby (population demographics, distribution, and abundance), using data collected during live-capture trapping and long-term datasets from the Lancashire Wildlife Trust. Chapter Three assesses the resources and risks for the red squirrels in the study site (supplemental feeding, habitat quality, and mortality threats, as identified in Chapter One), using data collected from a public survey, seed crop abundance surveys, and post-mortem examinations. Chapter Four analyses the impact of these resources and risks on the home ranges of the red squirrels, using data collected through radio-tracking and spatial analyses using a geographic information system. Overall, the findings indicated a high-density population, particularly in the adjacent peri-urban woodlands, which has suffered from two squirrelpox virus outbreaks in 2007/08 and during this study in 2018/19. The peri-urban woodland squirrels were found to have small home ranges, suggesting they did not have to move far to access resources within the high-quality woodland habitat, whereas the urban squirrels had larger home ranges that suggest they had to travel further to exploit the scattered urban greenspaces. Therefore, the population may be at risk if urban intensification leads to the loss of the remaining greenspaces, as well as risks from future disease outbreaks. Finally, in Chapter Five, conservation management strategies are recommended to benefit the red squirrels in the study site and other strongholds, as well as urban wildlife more broadly.

Thesis Outline

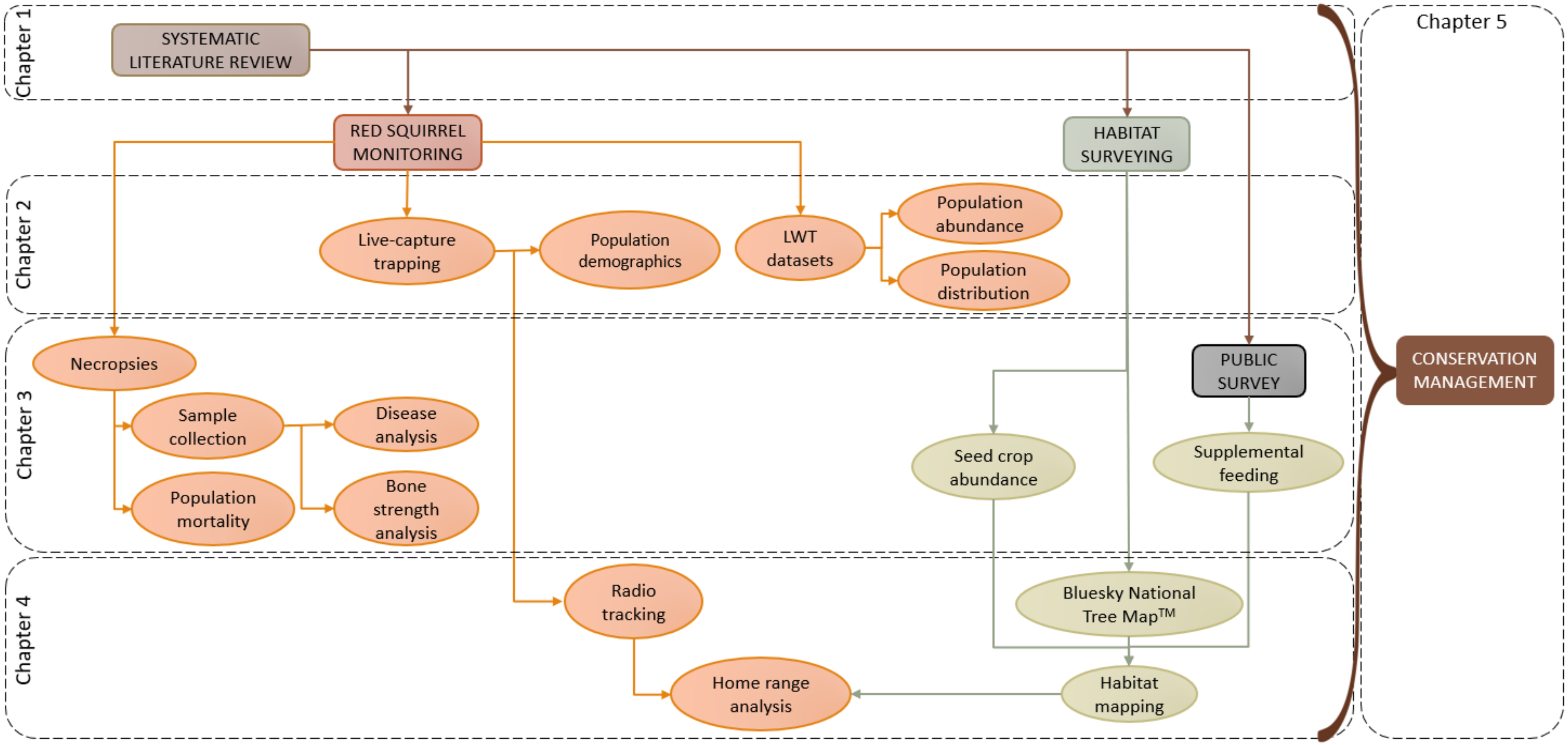


Figure i. Thesis outline providing an overview of what will be covered in each chapter, how the chapters are interlinked, and how these will inform the discussion of conservation management in Chapter Five.

Contributions to Data Collection

CHAPTER TWO

Population distribution data were collected from public sightings reported to the Lancashire Wildlife Trust. Distance transect data, used to calculate population abundance, were collected by Lancashire Wildlife Trust staff and volunteers as part of their bi-annual monitoring surveys. Both datasets were provided by Rachel Cripps from the Lancashire Wildlife Trust.

CHAPTER THREE

Seed crop abundance data were predominantly collected by masters' students (Jamie Smith in 2017, Ruth Pengelly in 2018, and Curtis Wright in 2019). Some of the necropsies were conducted by undergraduate and masters' students (Craig Dickson, Naomi Frost, Joseph Knightley, Charlotte Moore, and Charlotte Wheeler). Testing of the samples for squirrelpox virus and adenovirus were conducted by Dr Dave Everest (Animal & Plant Health Agency) and the sample for leprosy by Dr Anna Schilling (Royal (Dick) School of Veterinary Studies, University of Edinburgh). Bone strength data were collected with assistance from Dr Alex Kemp.

CHAPTER FOUR

Additional radio-tracking data was collected from mid-August until mid-November 2019 by Lancashire Wildlife Trust volunteers.

Contents

List of Figures

Powell 2000). The x-axis is the cumulative percentage of the number of fixes (the total number of fixes per individual is noted in brackets on each graph) and the y-axis is the home range area (ha). Using individual 5B53 as an example, the orange dashed line represents if all areas of the home range were randomly used, while the solid blue line shows the point of maximum difference between this and the utilisation distribution curve. Below this inflection point represents the most intensively used area (i.e. the core area, shown by the dotted green lines), which indicates the approximate percentage of fixes required to represent the core area and the approximate size of the core area (ha). Note that A9C5 was later excluded from the spatial analyses as the KDE_{LSCV} model failed to converge.

Figure 4.9. 100% MCP home ranges (grey polygons) and 70% core areas (coloured polygons, with different colours representing different individuals) in the urban area in 2019. Canopy cover, locations of supplemental feeders, and roads are also mapped (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Aerial Digimap Service (2019)).

Figure 4.10. Comparison of 100% home range sizes between (A) male and female red squirrels in the woodlands and (B) males inhabiting the urban area and woodlands.

Figure 4.11. The effect of the proportion of canopy cover on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

Figure 4.12. The effect of mean tree height (m) on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

Figure 4.13. No effect of the distance to the nearest road (m) on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

Figure 4.14. No effect of the distance to the nearest supplemental feeder (m) on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

List of Tables

List of Appendices

Appendix I: Fingland K, Ward SJ, Bates AJ, Bremner-Harrison S (2022) A systematic review into the suitability of urban refugia for the Eurasian red squirrel *Sciurus vulgaris*. *Mammal Review* 52: 26–38.

Appendix II: *Mammal Review* Supporting Information Appendix S1: Key findings from the final dataset of published articles (n = 25), determined from the Abstracts and Discussions, and conclusions drawn regarding whether or not urban habitats are feasible refugia for red squirrels.

Appendix III: Research Licences and Permissions.

Appendix IV: Fieldwork Protocols.

Appendix V: Supplemental Feeding Survey Forms.

Appendix VI: Mead D, Fingland K, Cripps R, Migeuz RP, Smith M, Corton C, Oliver K, Skelton J, Betteridge E, Dolucan J, *et al.* (2020) The genome sequence of the Eurasian red squirrel, *Sciurus vulgaris* Linnaeus 1758. *Wellcome Open Research* 5: 18.

Appendix VII: Mead D, Fingland K, Cripps R, Migeuz RP, Smith M, Corton C, Oliver K, Skelton J, Betteridge E, Dolucan J, *et al.* (2020) The genome sequence of the eastern grey squirrel, *Sciurus carolinensis* Gmelin, 1788. *Wellcome Open Research* 5: 27.

Appendix VIII: Power Analysis for Home Range Analysis (Chapter Four).

Abbreviations

AD	Alert distance
AIC	Akaike information criterion
APHA	Animal and Plant Health Agency
AWERB	Animal Welfare and Ethical Review Body
FDR	False discovery rate
FED	Fatal exudative dermatitis
FID	Flight initiation distance
GIS	Geographic information system
GLM	Generalised linear model
IUCN	International Union for Conservation of Nature
KDE	Kernel density estimation
LSCV	Least-squares cost-validation
LWT	Lancashire Wildlife Trust
MCP	Minimum convex polygon
NNR	National Nature Reserve
NTU	Nottingham Trent University
PCR	Polymerase chain reaction
PIT	Passive integrated transponder
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
SAC	Special Area of Conservation
SD	Standard deviation
SE	Standard error
SQPV	Squirrelpox virus
SSSI	Site of Special Scientific Interest
TEM	Transmission electron microscopy
VED	Vertical escape distance

1.0. Chapter One: Introduction

A growing body of evidence suggests that a sixth ‘mass extinction’ event is currently underway as a result of anthropogenic activities, which threatens global ecosystem services and therefore human health and well-being (Ceballos et al. 2015). The dominant direct driver of global biodiversity loss has been identified as land/sea use change, including the intensification of management for food production and the expansion of urban areas and associated infrastructure, both of which are linked to a growing population and increasing consumption (IPBES 2019, Jaureguiberry et al. 2022). Furthermore, the United Kingdom (UK) is one of the world's most nature-depleted countries (Burns et al. 2023). Therefore, it is imperative to urgently implement mitigation strategies to address these drivers of biodiversity loss.

Part A of this chapter provides background information regarding the Eurasian red squirrel (*Sciurus vulgaris* Linnaeus 1758; hereafter referred to as the red squirrel) in the UK, including their biology and causes of decline, to support the discussions in subsequent chapters. Part B, which was published in *Mammal Review* (Appendix I; Fingland et al. 2022), reviews the current published research and synthesises key topics regarding the urban ecology of red squirrels, in order to evaluate whether urban areas can be a suitable habitat for the species. Part C details the overall aims and objectives of this research project.

Part A: Overview of Red Squirrels in the United Kingdom

1.1. BACKGROUND INFORMATION

1.1.1. The Eurasian Red Squirrel

The red squirrel is the UK’s only indigenous squirrel species, with a wide geographic range that extends from Ireland and Portugal (Mathias & Gurnell 1998, Teangana et al. 2000) east to Japan (Noda et al. 2016). They are arguably one of the most charismatic and iconic species in the UK, with a high cultural value to the public (Gurnell & Pepper 1991).

The red squirrel was historically widespread across the UK, although their numbers and distribution declined following the introduction of the Eastern grey squirrel (*Sciurus carolinensis* Gmelin 1788; hereafter referred to as the grey squirrel) from North America in the late 19th century and extensive deforestation (Bosch & Lurz 2012), which will be discussed in more detail in section 1.1.2.1 (see p. 6). Subsequently, red squirrels have been classified as Endangered on the first official Red List for British Mammals (Mathews et al. 2018). Without long-term conservation management, it has been estimated that red squirrels could be extirpated from mainland Britain by 2030–2040 (Natural England 2010). Red squirrels are listed as a priority species for protection under Section 41 of the Natural Environment & Rural Communities Act 2006, as well as being protected from reckless or intentional acts of damage or disturbance under the Wildlife & Countryside Act 1981 and the Wild Mammals (Protection) Act 1996.

1.1.1.1. Diet and Caching Behaviour

Red squirrels have a broad diet mostly consisting of energy-rich seeds, along with fungi, fruits and berries, tree shoots, bark, lichen, insects, and occasionally songbird eggs and chicks (Moller 1983). Squirrels have also been seen gnawing on antlers and bones, potentially to trim their teeth or to obtain calcium in the case of breeding females (Carlson 1940, Lurz 2010). Red squirrels are 'scatter hoarders', meaning that they cache food items in different locations when resources are abundant in order to retrieve those items when resources become scarce. This method of foraging aids their chances of over-winter survival and reproductive success the following spring (Wauters et al. 1995), and plays a vital role in the woodland ecology by dispersing seeds and fungal spores (Zong et al. 2010).

1.1.1.2. Body Form and Activity Patterns

Red squirrels (200 – 480 g) are typically smaller than grey squirrels (350 – 800 g) with less seasonal bodyweight gain at approximately 10% increase from spring to winter, compared to approximately 20% increase for grey squirrels (Kenward & Tonkin 1986, Bosch & Lurz 2012). Red squirrels also spend much more of their active time in the canopy (67% on average throughout the year) than grey squirrels (14%), but they will forage on the ground particularly when scatter-hoarding during autumn (Kenward & Tonkin 1986). Although red squirrels do not hibernate during the winter, they are less active with only a short period of activity in the morning (Tonkin 1983, Bosch & Lurz 2012). As the days become longer and warmer through spring and summer, they typically have two periods of activity with one immediately after dawn and another in the afternoon (Tonkin 1983).

1.1.1.3. Breeding and Reproduction

Red squirrels have a polygynous-promiscuous mating system (Gurnell et al. 2008), in that both sexes will often mate with more than one individual and there are no long-term pair-bonds. Red squirrels become sexually active when they are between nine to 11 months old (Bosch & Lurz 2012). The breeding season can vary between years depending on food availability but generally occurs from late December until the following August, with two reproductive peaks in winter and spring leading to spring-born and summer-born litters respectively (Gurnell et al. 2008). Although females are capable of producing two litters per year, successful reproduction relies on good body condition as females can only come into oestrus once they attain a minimum body mass of approximately 300 g (Wauters & Dhondt 1989a). The average red squirrel litter size is four, but can range from one to six kittens (Lurz et al. 2005, Bosch & Lurz 2012). Mortality is high during the first year and less than a quarter of juveniles will survive their first winter, but the average life expectancy for those that do survive this initial period is approximately three years (Lurz et al. 2005).

1.1.1.4. Population Genetics

The genetic structure of the red squirrel population in the UK is complex. There have been significant past population fluctuations and local extinctions due to widespread deforestation, followed by reintroductions between British populations and from mainland Europe (summarised in Barratt et al. 1999). For example, red squirrel populations in northern (northern Northumberland and Scottish Borders) and eastern England (southern Northumberland and County Durham) were determined to be of continental ancestry and more genetically similar to red squirrels from Sweden than those from remnant British populations in Cumbria in western England (Hale et al. 2004). Hale et al. (2004) suggested that the introduced Scandinavian red squirrels may have already been adapted to the artificially planted spruce (genus *Picea*) forests found in northern England. This may have conferred a selective advantage to those individuals over those with a British or southern European ancestry, leading to introgression of the Scandinavian haplotype into the British red squirrel populations.

There is evidence to suggest that population fluctuations, leading to small and isolated populations, have resulted in genetic bottlenecks in some cases (Barratt et al. 1999, Ogden et al. 2005, Finnegan et al. 2008). Several studies have found that red squirrel populations in the UK have less genetic diversity than continental European populations (Barratt et al. 1999, Hale et al. 2004, Ballingall et al. 2016, Hardouin et al. 2019). As genetic diversity is key to population survival, for example by providing resistance to novel diseases, this loss of diversity may have contributed to the red squirrels' decline in the UK (Ballingall et al. 2016).

There are discrepancies between researchers over the exact number of red squirrel subspecies, ranging from 17 to 40 (Lurz et al. 2005), although genetic studies have found no substantiated evidence for the presence of a British haplotype representing the *Sciurus vulgaris leucourus* subspecies (Hale et al. 2004, Hardouin et al. 2019). However mitochondrial DNA (mtDNA) studies have identified unique haplotypes in British red squirrel populations, for example in Clocaenog in Wales (Ogden et al. 2005), Cumbria in England (Hale et al. 2001), and on Brownsea Island, Furzey Island, and the Isle of Wight off the southern coast of England (Hardouin et al. 2019). In addition, mtDNA studies have indicated that the Irish population includes haplotypes that are now rare or non-existent in Great Britain and elsewhere in Europe (Finnegan et al. 2008, O'Meara et al. 2018). Therefore, these populations should be preserved to maintain population genetic diversity, as they could be derived from ancestral British lineages.

1.1.1.5. Predators of Red Squirrels

Red squirrels are known to be predated by a range of species, often when they venture down to the ground to feed and cache food, including red foxes (*Vulpes vulpes* Linnaeus 1758), feral and domestic cats (*Felis catus* Linnaeus 1758), mustelids such as pine martens (*Martes martes* Linnaeus 1758), and raptors such as goshawks (*Accipiter gentilis* Linnaeus 1758) and buzzards (*Buteo buteo* Linnaeus 1758; Halliwell 1997, Kenward & Hodder 1998, Magris & Gurnell 2002, Petty et al. 2003, Sheehy & Lawton 2015). Red squirrels typically constitute only a small proportion of predators' diets (Petty et al. 2003) and there is no evidence to suggest that predation has significantly contributed to their national decline, but the additional pressure of predation could have localised impacts in areas where populations are already fragile (Halliwell 1997).

1.1.2. Introduction of Grey Squirrels

Grey squirrels are listed as one of the 100 World's Worst Invasive Species on the IUCN's Global Invasive Species Database (2021). Almost 80% of documented global grey squirrel introductions have been successful (Bertolino 2009) and in many cases the founder populations consisted of less than ten individuals (Wood et al. 2007, Lawton et al. 2010). Grey squirrels seem to have inherently high problem-solving capabilities, which may enhance their invasive success (Chow et al. 2018).

The first recorded case of grey squirrels being released into the UK was in Cheshire in 1876, followed by over 30 documented introductions and translocations in the next 50 years (Middleton 1930). Between 1930 and 1945, the grey squirrel population swiftly grew, causing extensive timber damage due to bark-stripping, which led to efforts to control their numbers (Sheail 1999, Bosch & Lurz 2012). Despite a bounty scheme removing over a million grey squirrels by 1958, it was unsuccessful in significantly reducing the overall population (Sheail 1999). Consequently, grey squirrels are now established in the UK with an estimated population of 2.7 million (Mathews et al. 2018) and have been listed as a Schedule 9 species under the Wildlife & Countryside Act 1981, which prohibits their release (either an intentional introduction or by allowing them to escape) into the wild. In addition, some populations of grey squirrels have been found to be most closely related to geographically distant populations, for instance the populations in Aberdeen and the New Forest in Hampshire (Signorile et al. 2016). This cannot be explained by natural expansion processes, which suggests that undetected accidental or deliberate human-mediated translocations have aided the grey squirrels' spread across the UK (Signorile et al. 2016).

Grey squirrels have also been deliberately introduced into Italy on at least three separate occasions since 1948 (Bertolino & Genovesi 2005). As most of Europe consists of potentially ideal habitat for grey squirrels, it has been predicted that grey squirrels will only take 30 to 40 years to start encroaching into the Alps and approximately 70 years to spread across the Italian border into France, even in the best-case scenario (Bertolino et al. 2008). A similar pattern and rate of displacement of red squirrels by grey squirrels as occurred in the UK (approximately 18 km^2 per year) has already been observed in northern Italy (17.2 km^2 per year; Okubo et al. 1989, Bertolino & Genovesi 2003). Grey squirrels could eventually colonise the whole of mainland Europe, indicating that grey squirrels are not only a threat to red squirrels in the UK but potentially their entire global distribution (Bertolino & Genovesi 2003, Nie et al. 2023).

1.1.2.1. Decline of Red Squirrels in the UK

Historical data show that red squirrels have suffered from population fluctuations in the UK even prior to the arrival of the grey squirrel, particularly in the 15th and 16th centuries due to extensive deforestation for agriculture and timber (Bosch & Lurz 2012). Red squirrel populations gradually increased as a result of reintroductions (see section 1.1.1.4, p. 3), large-scale planting of woodlands, and predator control by gamekeepers. They recovered to such a degree that they were considered a pest species by the late 19th century and controlled in some areas. In the early 20th century, there was another population decline, thought to be due to increased war-time demand for timber, by which time the grey squirrel had been introduced and contributed to the red squirrel population being unable to recover.

Consequently, red squirrel populations have become increasingly isolated across the UK (Fig. 1.1). Approximately 75% of the population remains in Scotland with a few populations scattered across England, Wales, Ireland, and some offshore islands including the Isle of Wight and Brownsea Island (Harris et al. 1995). Many of these areas have been classified as red squirrel strongholds, where long-term viable populations can be isolated from surrounding grey squirrel populations by buffer zones.

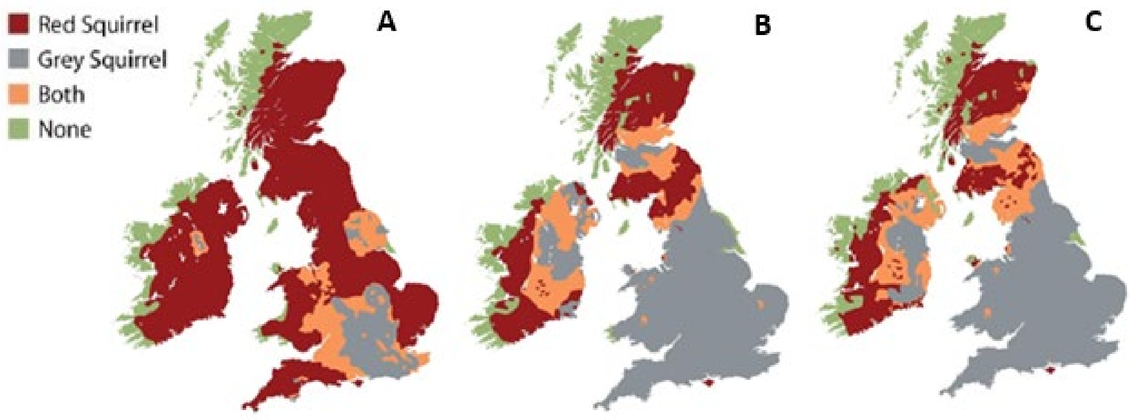


Figure 1.1. Historical distributions of red and grey squirrel populations in (A) 1945, (B) 2000, and (C) 2010 in the UK (Shuttleworth & Red Squirrel Survival Trust 2012). Permission to reproduce this figure has been granted by C Shuttleworth.

1.1.2.1.1. Competitive Exclusion

Red and grey squirrels occupy similar ecological niches, with overlap estimated to be over 70% (Wauters et al. 2002a). Red squirrel populations can occasionally persist in the presence of grey squirrels, in one case for up to 30 years (Bryce et al. 2002), but this seems to be a rare occurrence and replacement has taken place in a time-scale as small as five years (Skelcher 1994, as cited in Skelcher 1997). There is little evidence to suggest that the displacement of red squirrels is due to interference competition by grey squirrels, either through direct aggressive interactions or by interrupting mating behaviour (Wauters & Gurnell 1999). Instead, it is thought to be exploitative competition for resources due to a lack of niche partitioning, leading to competitive exclusion (Wauters et al. 2002a), as well as disease-mediated replacement that will be discussed in more detail in section 1.1.2.1.2.

Grey squirrels are more efficient than red squirrels at exploiting certain natural food resources, particularly those found in broadleaf woodlands. For example, grey squirrels are better adapted to digest the polyphenols found in acorns and can feed on them earlier than the red squirrels, providing an advantage in oak-dominated habitats (Kenward & Holm 1993). Kenward and Holm (1993) demonstrated that red squirrels were unable to persist alongside grey squirrels in woodlands with an oak canopy greater than 14%.

Research suggests that the interspecific competition primarily impacts juvenile and sub-adult red squirrels, resulting in reduced growth rates and smaller body sizes (Wauters et al. 2000, 2001). A considerable proportion of juvenile red squirrel mortality is attributable to starvation even in the absence of grey squirrels (LaRose et al. 2010), which is exacerbated in the presence of grey squirrels that consume the immature nuts before they ripen (Huxley 2003). Therefore, juvenile recruitment in the red squirrel population is decreased. In addition, grey squirrels are known to steal the red squirrels' scatter-hoarded food caches, diminishing the energy intake of red squirrels with a high extent of interspecific core-area overlap in their home ranges (Wauters et al. 2002b). This leads to a significant reduction in body mass in adult red squirrels causing reduced fecundity, as females cannot attain the minimum mass to enter oestrus (Wauters & Dhondt 1989a, Wauters et al. 2002b, Gurnell et al. 2004). It is highly likely that the impacts of interspecific competition increase as grey squirrel density increases, particularly in broadleaf woodlands, causing red squirrel populations to decline and eventually become extinct (Wauters et al. 2002b).

1.1.2.1.2. Disease-Mediated Replacement

Grey squirrels can replace native red squirrels through competitive exclusion alone, but it has been shown that red squirrel populations decline at a rate 17 to 25 times faster in areas where squirrelpox virus (SQPV) is present in the grey squirrel population compared to areas where SQPV is absent (Rushton et al. 2006). Grey squirrels are asymptomatic carriers of SQPV, whereas the infectious disease is fatal to red squirrels within two to three weeks (Sainsbury et al. 2000, Tompkins et al.

2002, Rushton et al. 2006). SQPV can cause a high mortality rate in red squirrel populations, as demonstrated during the outbreak in Merseyside in 2007/08 where the population crashed by 87% in just over a year (Chantrey et al. 2014). The resultant rapid population decline typically leads to disease 'fade out' where the remaining population is at risk of competitive exclusion by grey squirrels or stochastic events that can lead to extinction, but can also recover to pre-outbreak levels if competitive impacts are reduced through grey squirrel control (see section 1.1.2.2, p. 11; White et al. 2014).

SQPV has been confirmed in red squirrel populations in England (Carroll et al. 2009, Chantrey et al. 2014), Wales (Everest et al. 2017), Ireland (McInnes et al. 2013, Naulty et al. 2013), and even Scotland where the majority of the British red squirrel population persists (McInnes et al. 2009). There is currently no evidence that SQPV is present in Italy (Romeo et al. 2019). Although the origin of SQPV in the UK is unknown, it is suspected to have been introduced from the USA with the grey squirrel. McInnes et al. (2006) found that serum samples from grey squirrels in Wisconsin tested positive for SQPV antibodies, which suggests that this may have been the case.

Following infection, red squirrels typically develop painful haemorrhagic ulcers and lesions, often located around the eyes, mouth, nose, and genital region (Bruemmer et al. 2010). Although the transmission route is not fully understood, there is strong evidence to suggest that is it transmitted via environmental contamination through bodily fluids or potentially ectoparasites (Bruemmer et al. 2010, Collins et al. 2014, Fiegna et al. 2016). Infected individuals with exudative skin lesions can survive for an average of 10 days and therefore continue to expose conspecifics to the disease during that time (Carroll et al. 2009). Social behaviours and interactions, including scent marking, grooming, or fighting, may play a key role in the propagation of the disease throughout a population (Fiegna et al. 2016). This supports the theory that, once SQPV is introduced to a population by a grey squirrel, the main driver of infections in red squirrels is between conspecifics (Tompkins et al. 2002, McInnes et al. 2013, Chantrey et al. 2014).

Despite the high mortality rate, seropositive red squirrels have been identified in Merseyside (Chantrey et al. 2014) and Northern Ireland (McGowan et al. 2018). This suggests that a small proportion of a red squirrel population, 8% in the case of the SQPV outbreak in Merseyside in 2007/08, are able to survive exposure to the disease due to an effective immune response as indicated by the presence of SQPV antibodies (Chantrey et al. 2014). The findings also suggest that the development of a vaccine could be a feasible management technique (Sainsbury et al. 2008), although progress has been hampered by the associated financial costs (McInnes & Nettleton 2005) and lethal control of grey squirrels would need to continue due to the impact of interspecific competition.

In addition to SQPV, there is emerging evidence of apparent macroparasite-mediated competition between grey and red squirrels in northern Italy (Santicchia et al. 2020). Grey squirrels can carry the gastro-intestinal helminth *Strongyloides robustus*, whilst red squirrels can carry *Trypanoxyuris sciuri* (Cameron 1932) but can also acquire *S. robustus* through parasitic spillover from grey squirrels (Romeo et al. 2013, 2015). Santicchia et al. (2020) found that *S. robustus*-infected red squirrels showed reduced activity compared to non-infected individuals.

1.1.2.1.3. Habitat Loss and Fragmentation

Habitat loss and fragmentation due to widespread deforestation have resulted in past red squirrel population fluctuations (Bosch & Lurz 2012). Habitat fragmentation may even confer a competitive advantage to the grey squirrel over the red squirrel, as suggested by Jessen et al. (2017) who found that the invasive Eastern grey squirrel had a competitive advantage over the native Western grey squirrel (*Sciurus griseus* Ord 1818) in areas of central California with high canopy fragmentation.

Multiple studies have demonstrated that the occupancy and density of a red squirrel population in a woodland fragment depends on (1) its size, (2) the habitat quality in terms of the availability of suitable natural resources or access to supplemental food, and (3) the degree of isolation from surrounding patches (Verboom & Apeldoorn 1990, Celada et al. 1994, Delin & Andrén 1999, Rodríguez & Andrén 1999, Verbeylen et al. 2003a, b). For example, Rodríguez and Andrén (1999) found that, despite variations between woodlots in terms of habitat quality and spatial layout within the landscape, there was a high probability (at least 90%) of occupancy when the fragments were larger than 10 ha and less than 600 m away from the nearest source population. When the woodland fragments became smaller than 10 ha and further away than 600 m from the nearest source population, then the probability of occupancy fell to only 17%.

However, the effects of habitat loss and fragmentation can be difficult to disentangle as they are closely linked. In several of the studies, the supposedly isolated woodlots were within easy dispersal distance for a red squirrel and therefore could be viewed as functionally continuous habitat (Andrén & Delin 1994, Delin & Andrén 1999, Verbeylen et al. 2003b). Other studies found that the matrix surrounding the woodland fragments was a significant factor influencing connectivity, either due to unsuitable habitat (e.g. dense urban developments or rivers) preventing movement or the presence of habitat corridors (e.g. hedgerows) allowing movement between patches (Verboom & Apeldoorn 1990, Celada et al. 1994, Verbeylen et al. 2003a). Therefore, it is important to consider the wider landscape in terms of both the distance between woodlots and the surrounding matrix when assessing the degree of isolation between woodland fragments. In addition, several studies argued that the only effect of habitat fragmentation appeared to be related to pure habitat loss rather than the fragmentation itself, suggesting that red squirrel population distributions are mainly affected by habitat loss (Delin & Andrén 1999, Mortelliti et al. 2011).

1.1.2.2. Grey Squirrel Control

It has been argued that it is unfeasible to eliminate the grey squirrel from the British mainland due to their extensive numbers, widespread distribution, public opinion, and the expense involved (Gurnell & Pepper 1993, Macdonald & Burnham 2010). The grey squirrel is often one of the only mammals that people, particularly urban inhabitants, encounter frequently in their gardens and so many people feel positively towards them (Forestry Commission 2006, Baker & Harris 2007). Therefore, culling of grey squirrels remains a controversial and complex issue.

Nonetheless, culling is a widespread and well-utilised tool in red squirrel conservation to reduce grey squirrel numbers and even achieve local eradications, reducing competitive impacts and the risk of disease transmission (Lurz et al. 2015). For instance, following the onset of lethal control in 1998, Anglesey in North Wales was declared grey squirrel-free in 2013 (Schuchert et al. 2014, Jones et al. 2016). Red squirrels, which were once almost extinct on Anglesey, have since recolonised many areas of the island and have even dispersed over to mainland Wales (Shuttleworth et al. 2015). Control efforts are often more successful on islands or in coastal regions, due to the protection provided by geographical boundaries and a more targeted management area.

Non-lethal alternatives are now being developed as a more acceptable and humane form of control, such as immunocontraception (Barr et al. 2002, Dunn & Marzano 2015) and gene drive technology (Faber et al. 2021). In addition, grey squirrel populations have been observed to fall to low densities in areas where pine marten populations have recovered, whereas local red squirrel populations have increased to normal densities and have even been found in regions where they have been absent for over 30 years (Sheehy & Lawton 2014, Sheehy et al. 2018, Twining et al. 2020a). The findings suggest that the recovery of the endangered native pine marten may act as a form of biological control for the invasive grey squirrel through predation. This well-publicised research appears to have substantially raised public awareness of both red squirrel and pine marten conservation (MacPherson 2014).

Part B: A Systematic Review into the Suitability of Urban Refugia for the Red Squirrel

1.2. INTRODUCTION

Currently, 55% of the global human population inhabits urban areas; the percentage is projected to increase to 68% by 2050 (United Nations 2019). The resultant urban growth and intensification are dramatic forms of habitat alteration, which present substantial challenges to wildlife conservation (McCleery et al. 2014). For instance, urban areas can lack sufficiently large and connected greenspaces to provide foraging resources, nesting sites, and dispersal for wild animals (Marzluff & Ewing 2008). The presence of roads has been identified as a mortality risk and a potential barrier to movement (Rondinini & Doncaster 2002). The urban environment may also support an increased abundance of predators, particularly free-ranging companion animals such as cats and dogs (*Canis lupus familiaris* Linnaeus 1758), associated with higher numbers of human inhabitants (Baker & Harris 2007).

Urban developments have historically been ignored as potential wildlife habitat (McCleery et al. 2014). However, it has been demonstrated that urban areas can be biodiverse and, in some cases, support populations of endangered species (Alvey 2006). Environmental action plans now include the development of urban areas, particularly identifying the importance of greenspaces (e.g. DEFRA 2018), which highlights the urgent need to advance our understanding of urban wildlife ecology to inform appropriate management. Resources can be plentiful in urban habitats, resulting in higher population densities of wild animals than in rural locations. For example, peregrine falcons (*Falco peregrinus* Tunstall 1771) have significantly higher clutch sizes, brood sizes, and fledging success in urban areas (Kettel et al. 2018). Some species have the behavioural flexibility to adapt to the urban environment, resulting in urban populations having adaptations that are not shared by their rural counterparts. For instance, some urban mammals have been shown to alter their foraging patterns temporally and spatially, to avoid periods of peak human activity (Lowry et al. 2013).

This study focusses on the red squirrel, which is a diurnal, arboreal rodent that currently remains widespread throughout most of Eurasia. In the UK, the population has declined following extensive habitat loss and the introduction of the grey squirrel from North America in the late 19^{th} Century (Bosch & Lurz 2012). Grey squirrels outcompete the red squirrel for resources (Wauters et al. 2002a) and spread SQPV, which they carry asymptomatically but is often fatal to red squirrels (Rushton et al.

2006). Following the more recent introduction and subsequent spread of grey squirrels in Italy, the continental population of red squirrels is now also threatened (Bertolino et al. 2008, 2014).

In the UK, red squirrels are part of the natural heritage and an iconic species, which tourists specifically travel to reserves to see (Shuttleworth 2001). Red squirrels are highly charismatic and interactions with them can encourage people to connect with nature and develop a wider interest in wildlife. This is particularly important in urban areas, where access to greenspaces may be limited, as contact with local wildlife has been shown to provide environmental education and improve mental well-being (Dearborn & Kark 2009, Irvine et al. 2010). People who live alongside red squirrels tend to have positive feelings towards the species and are aware of the conservation issues affecting the squirrels (Rotherham & Boardman 2006). Therefore, red squirrels have a high cultural value (Gurnell & Pepper 1991), but need conservation management in the UK and in other countries where populations are declining (e.g. Turkia et al. 2018). Towns and cities could potentially act as refugia for red squirrels, so understanding the species' urban ecology would help to inform effective management.

1.2.1. Review Objective

Systematic reviews using strict methodologies were pioneered in the field of medicine to overcome the selection and interpretation biases that can occur in traditional literature reviews (Haddaway et al. 2015). In recent years, systematic reviews have been promoted within conservation biology, due to the associated benefits of increased transparency and objectivity, in order to inform evidence-based management decisions (Cook et al. 2013).

This systematic review aims to evaluate the current published literature regarding the urban ecology of red squirrels, in order to determine whether urban areas are suitable refugia for red squirrels. The initial broad literature search aimed to ensure an objective approach to the review and to overcome any potential selection bias. Following the screening process and based on the final dataset of articles, this review aims to identify and synthesise key topics regarding the urban ecology of red squirrels.

1.3. METHODS

1.3.1. Definitions

Interpretation of the term 'urban' is complicated, with definitions based upon different factors (e.g. density of buildings or the human population) and varying between scientific disciplines (McIntyre et al. 2000). Therefore, for the purpose of this review, 'urban' is broadly classified as any area characterised by a collection of buildings, including houses and shops, and associated infrastructure, such as gardens, roads, and parkland (Baker & Harris 2007).

In the context of this study, we define anthropogenic food sources as food provided by humans for wild animals; this includes supplemental feeding, which is the deliberate provision of food (e.g. through bird feeders or squirrel boxes), as well as the accidental provision of food (e.g. through garden allotments or rubbish, which can be scavenged). In addition, we define natural food sources as food that would be available without human intervention, regardless of whether those items are available through artificial planting and management by humans. For example, in the context of squirrels, this would include a range of coniferous and broadleaf tree species growing naturally in rural woodlands, as well as those being managed by humans in forestry plantations and urban areas.

1.3.2. Literature Search

A systematic literature review was undertaken in December 2019 following the PRISMA protocols (Moher et al. 2009). The PRISMA protocols aim to improve reporting of systematic reviews by following a process of screening and assessing the identified literature using clearly defined inclusion and exclusion criteria.

A literature search was conducted on Scopus, Web of Science, and Google Scholar using the following search terms: (‘red squirrel*’ OR ‘Eurasian red squirrel*’ OR ‘European red squirrel*’ OR

‘*Sciurus vulgaris*’) AND (urban* OR town OR city). All the results listed on Scopus and Web of Science were collected. Only the first 200 articles of Google Scholar were collected, following the threshold used in other reviews (e.g. Lisón et al. 2020); after this point the returned results become less relevant. There were no restrictions regarding the year of publication.

1.3.3. Inclusion Criteria

Articles were excluded if they were not published in a peer-reviewed journal or not written in English. The articles were then screened for inclusion using the following criteria: (1) the title, abstract, or keywords specified ‘(Eurasian) red squirrel’ or ‘*Sciurus vulgaris*’ AND either (2) the keywords included e.g. ‘urban ecology’ or ‘urbanisation/urbanization’, OR (3) the abstract specified that the study was conducted in an urban environment, including comparisons with rural areas, OR (4) the abstract specified that the study investigated the impact of an anthropogenic activity (e.g. road traffic mortality, supplemental feeding). Any articles that did not meet the screening criteria were excluded from the review.

For the articles that passed the initial screening process, the full text was then assessed for eligibility. The articles were required to meet all three of the following inclusion criteria, otherwise they were excluded: (1) Eurasian red squirrels were the focal study species; (2) the research had been conducted on an urban population, including if compared with a rural population; and (3) the study investigated at least one aspect of red squirrel biology (e.g. behavioural ecology, genetics, population mortality) or conservation (e.g. reintroductions). Assessment of the scientific quality (e.g. identification of causal relationships, empirical evidence, replication, and wider application) was not used to exclude articles at this stage, but instead informed later evaluation in this systematic review. Only the key findings from the final dataset of articles were collated and reported.

1.4. RESULTS

The initial literature search returned 226 articles once duplicates were removed (Fig. 1.2). During the screening process and eligibility assessment, 201 articles were excluded in total, resulting in a final dataset of 25 articles describing 25 studies (Appendix II). Of the 25 articles, 76% were published since 2014, whilst the remaining articles were published between 1986 and 2009, often with large gaps between years (Fig. 1.3). The majority of the published research was conducted in mainland Europe (*n* = 16); five were conducted in the UK and Republic of Ireland, three in Japan, and one was carried out in both Poland and the UK (Appendix II).

Of the 25 articles identified for this review, 84% provided evidence that urban areas can be suitable habitat for red squirrels, whilst the remaining 16% highlighted the potential risks of urban environments, such as mortality threats (Appendix II). Five broad topics regarding the urban ecology of red squirrels were categorised from the studies, with each article evaluating at least one of the identified topics (Fig. 1.4).

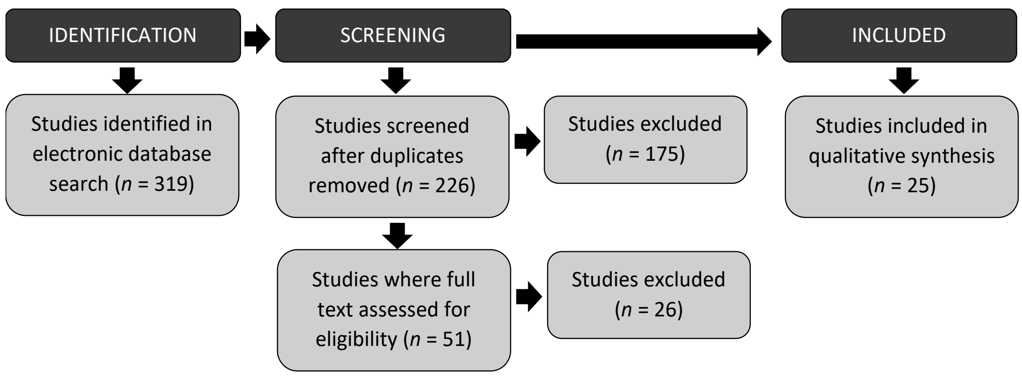


Figure 1.2. PRISMA literature search and screening flow diagram of articles included and excluded from systematic analysis.

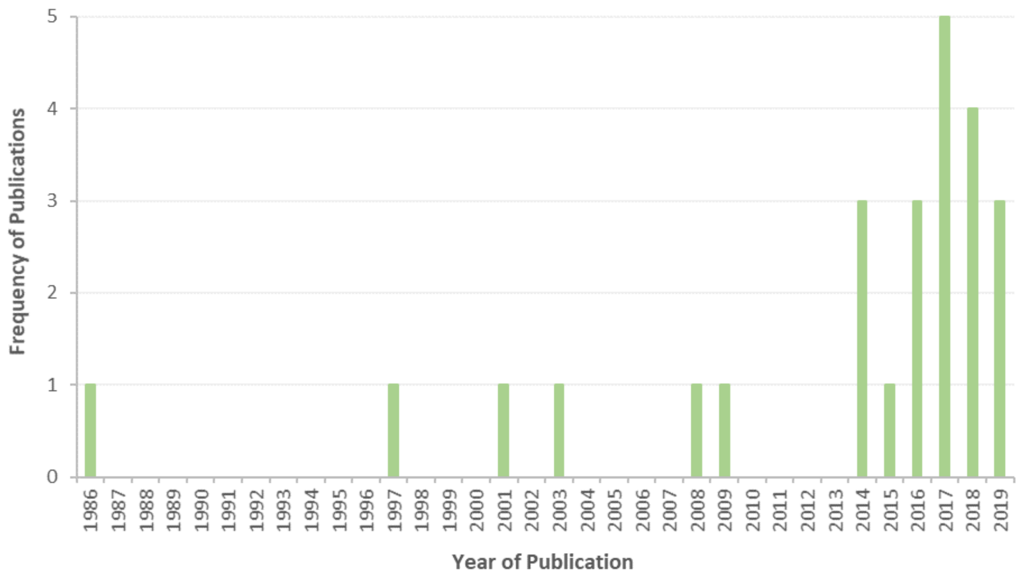


Figure 1.3. The number of red squirrel urban ecology studies, based on the final dataset of articles (n = 25), published each year from 1986 to 2019.

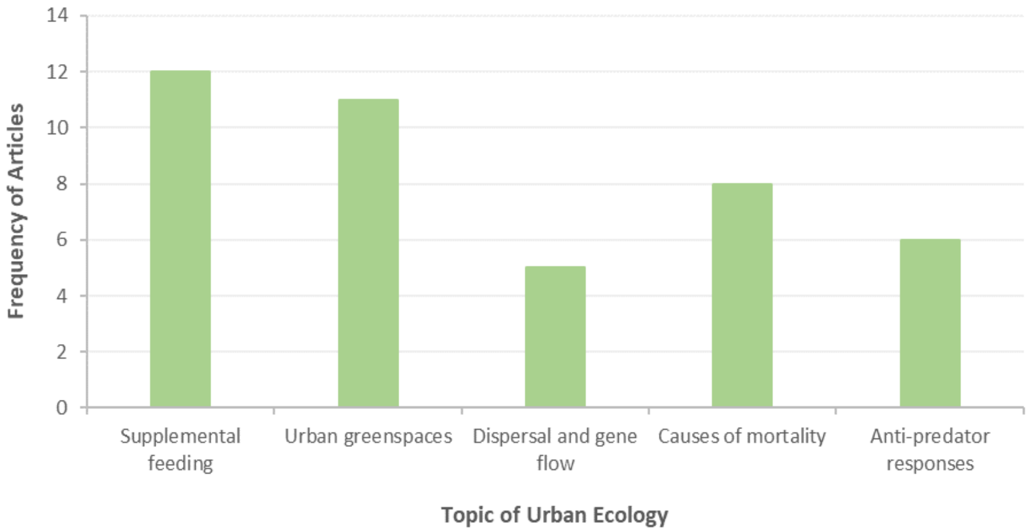


Figure 1.4. The number of articles evaluating each topic regarding the urban ecology of red squirrels, as identified and categorised by this review, where each article can include information on more than one topic.

1.4.1. Supplemental Feeding

Supplemental feeding appears to be widespread in urban areas throughout the red squirrels' geographic range (Appendix II) and can help to increase the viability of small, isolated populations (Bertram & Moltu 1986, Rézouki et al. 2014, Vieira et al. 2015). Reher et al. (2016) found that red squirrels' home ranges in a cemetery in Hamburg, Germany, always incorporated stable natural food sources, but squirrels responded to seasonal changes in the provision of supplemental food by relocating their core areas. Core areas were shifted closer to reliable supplemental food sources in the autumn, when availability was highest, and moved to locations with natural food supplies in spring and summer when supplemental food became limited. Therefore anthropogenic food sources had a measurable effect on patterns of space use, despite the abundance of natural food (Reher et al. 2016).

Supplemental feeding can support high red squirrel population densities in urban areas (Babińska-Werka & Żółw 2008, Kopij 2014, Jokimäki et al. 2017), as urban home ranges were found to be smaller with a greater degree of overlap, than those in more rural populations (Reher et al. 2016, Thomas et al. 2018). Thomas et al. (2018) also reported that the urban squirrels in Hamburg city centre were less active throughout the day (mean activity rate of 25% per one-hour time slot) than the individuals inhabiting a nearby semi-natural site (mean activity rate of 58%). Furthermore, Turner et al. (2017) found that the red squirrels' body mass in the same cemetery site in Hamburg was relatively constant throughout the year, with only minimal physiological changes (e.g. metabolic rate) in response to seasonal fluctuations. These studies indicate that the widespread and abundant supplemental food sources available in urban environments allow red squirrels to satisfy their energy requirements whilst minimising energy expenditure, by reducing the need to travel as far or as often for foraging excursions (Reher et al. 2016, Turner et al. 2017, Thomas et al. 2018). Supplemental feeding may further increase population sizes, by allowing females to start breeding at the earliest opportunity and enhancing juvenile survival (Rézouki et al. 2014).

However, there are also risks associated with encouraging squirrels to travel between gardens whilst foraging and caching human-provided food items. Red squirrels have been shown to spend more time foraging on the ground when supplemental food is available, which may lead to more encounters with road traffic or predators; red squirrels usually spend 67 – 90% of their active time foraging for natural food in the canopy, but this is reduced to 50 – 53% when foraging for supplemental food (Shuttleworth 2001). Reproductive rates are density-dependent, so artificially high population densities can reduce fecundity in some females and therefore decrease breeding success (Kopij 2014, Stirkė 2019). Excessive consumption of supplemental food such as peanuts (*Arachis hypogaea* Linnaeus 1758) can also cause nutritional deficiencies, which may have health implications in highly urbanised environments where squirrels may not be able to compensate for such malnutrition if there is limited availability of natural food sources (Thomas et al. 2018). Finally, feeders may facilitate the spread of parasites and diseases, such as SQPV, by encouraging interactions both between red squirrels and with grey squirrels (Chantrey et al. 2014, Kopij 2014, Stirkė 2019).

1.4.2. Size, Quality, and Connectivity of Urban Greenspaces

Of the studies identified for this review, 44% highlighted the importance of size, quality, and/or connectivity of urban greenspaces for the persistence of red squirrel populations (Appendix II). For example, Verbeylen et al. (2003a) found that woodland patches within an urban landscape had a higher chance of red squirrel occupation if they were larger (>5 ha), higher quality, and well-connected to nearby populations.

Using visual transects, Babińska-Werka and Żółw (2008) and Kopij (2014) recorded higher densities of red squirrels in city parks (1.8 and 0.26 individuals/ha, respectively) than in woodlands located on the outskirts of the cities (0.004 – 0.033 and 0.01 individuals/ha, respectively). Similarly, Jokimäki et al. (2017) found that relative squirrel abundance was higher in urban habitats (4.24 individuals/10 km transect) than in forests (1.43 individuals/10 km transect).

Babińska-Werka and Żółw (2008) and Kopij (2014) also found a significant positive correlation between the density of red squirrels and the size of the urban park, with higher densities in the larger parks, where diverse and mature trees provided ample natural food sources and which were connected to other nearby parks via habitat corridors. Conversely, smaller and more isolated urban parks with immature and few tree species had lower squirrel densities. For example, Kopij (2014) reported densities of 0.31 – 1.0 individuals/ha in the larger urban parks of 15 to 75 ha, compared to 0.0 – 0.4 individuals/ha in the parks less than 12 ha in size. Kopij (2014) also reported that parks that were close to allotment gardens, which provided anthropogenic food sources, supported higher densities of squirrels (0.55 individuals/ha) than similar-sized parks that were not bordered by allotments (0.1 individuals/ha).

The importance of high-quality habitat, specifically the availability and diversity of natural food sources, was further emphasised by Wauters et al. (1997), Vieira et al. (2015), and Jokimäki et al. (2017). Wauters et al. (1997) found that female reproductive rates and subsequent juvenile recruitment improved as the tree seed crop abundance increased. Jokimäki et al. (2017) also found that squirrel abundance increased as the spruce cone crop increased. Vieira et al. (2015) reported that two red squirrel populations, which had been reintroduced into urban parks, were suffering from long-term declines due to the poor-quality habitat. Tree species composition in urban greenspaces is also important for providing suitable nesting sites (Kopij 2009, Stirkė 2019).

Although red squirrels seem to prefer areas with more trees available than in the surrounding urban landscape, they have been observed using very small groups or even lone trees, encircled by buildings and with no connectivity to other greenspaces, for dispersal and exploration (Hämäläinen et al. 2018). Urban red squirrels were found to travel in closer proximity to buildings than would be expected at random, with some instances of squirrels travelling along roofs and using buildings as nesting sites. This indicates that they are not confined to using only the available greenspaces (Hämäläinen et al. 2018, 2019).

1.4.3. Dispersal and Gene Flow

Fey et al. (2016) found that dispersing and non-dispersing red squirrels responded differently to the presence of roads. Non-dispersing individuals seemed to avoid roads during routine daily activities (e.g. foraging) within their home ranges, and perceived large roads (average daily traffic of 2000 - 7000 vehicles) as more dangerous than smaller roads (average daily traffic of 500 - 2000 vehicles).

Conversely, roads did not act as barriers for dispersing individuals, who crossed them regardless of their size. Hämäläinen et al. (2019) also found that the landscape structure typically did not affect red squirrels' final straight-line dispersal distances, although it did alter their movement paths, with individuals favouring woodland patches whilst attempting to avoid more unsuitable habitats (e.g. open fields or buildings). In other words, individuals were likely to bypass barriers by travelling longer distances, but this did not impact how far they settled from their natal site. However, the overall dispersal distances of urban individuals were shorter than those of rural individuals (Fey et al. 2016, Selonen et al. 2018, Hämäläinen et al. 2019).

Despite shorter dispersal distances in urban populations, Fey et al. (2016) and Hämäläinen et al. (2019) suggest that the dispersal process still maintains the potential for gene flow at the population level. This is supported by Rézouki et al. (2014), who found that a red squirrel population in an urban park had relatively high levels of genetic diversity and minimal levels of inbreeding, despite being comparatively small, due to immigration from the surrounding woodlands. Similarly, Selonen et al. (2018) found no evidence to suggest that squirrels in their urban study site had been genetically isolated from the adjacent rural population.

1.4.4. Causes of Mortality

Of the articles identified for this review, 24% discussed causes of recorded deaths. Instances of road traffic accidents were reported in 16% of the articles, ranging from 20% (*n* = 10; Bertram & Moltu 1986) and 33.3% (*n* = 12; Wauters et al. 1997) to 50.7% (*n* = 337; Blackett et al. 2018) and 65% (*n* = 188; Shuttleworth 2001) of recorded deaths. In some individual habitats, 88% of recorded deaths were attributed to road traffic (Shuttleworth 2001). Further in-depth analysis of road traffic mortality by Shuttleworth (2001) determined that there was a distinct seasonal pattern, with a clear peak in the autumn months accounting for 53.7% of the total number of recorded deaths. Although there was no overall significant difference in the sex ratio of road traffic casualties, breeding adult males were more likely to be killed during the winter months.

Predation incidents were reported in 20% of the articles, ranging from 5.3% (*n* = 188; Shuttleworth 2001), 7.1% (*n* = 337; Blackett et al. 2018), and 10% (*n* = 10; Bertram & Moltu 1986) to 21.9% (*n* = 32; Fey et al. 2016) and 25% (*n* = 12; Wauters et al. 1997) of recorded deaths. Many of the mortality events were attributable to predation by free-ranging domestic or feral cat attacks (Bertram & Moltu 1986: 10%, Wauters et al. 1997: 8.3%, Blackett et al. 2018: 5%). Shuttleworth (2001) and Fey et al. (2016) did not specify the predator species, although the latter suggested they were most likely to be free-ranging cats and red foxes.

Only two of the studies discussed the potential impact of accidental anthropogenic poisoning on red squirrel populations. Blackett et al. (2018) reported that 1.2% of red squirrels found dead displayed signs of poisoning, most likely by anticoagulant rodenticides. Lurz et al. (2017) conducted a pilot study investigating levels of industrially produced mercury in an urban red squirrel population in the city of Warsaw, Poland, and in two rural populations on the islands of Arran and Brownsea, UK. The results indicated that red squirrels have high levels of mercury, even individuals from rural UK islands where industrial activities are minimal, with currently unknown health implications. However, there is little evidence to suggest that accidental anthropogenic poisoning is a limiting factor for urban red squirrel populations.

Finally, Blackett et al. (2018) reported that 34.4% of recorded casualties on Jersey in the Channel Islands, UK, were attributed to diseases, and that many individual red squirrels found dead were suffering concurrently with multiple conditions. The confirmed diseases included: amyloidosis (19.3%), where deposits of an abnormal protein can result in renal or hepatic failure; fatal exudative dermatitis (FED) associated with *Staphylococcus aureus* (Rosenbach 1884) infection (14.5%); and parasitic infections of *Capillaria hepatica* (Bancroft 1893) (33.5%), *Hepatozoon* (Miller 1908) species (16.1%), and *Toxoplasma gondii* (Nicolle & Manceaux, 1908) (2.1%). Amyloidosis and FED were determined to be major contributors to red squirrel mortality on the island. Amyloid deposits were often found alongside co-existing FED or *C. hepatica* infections, suggesting there may be some association between the diseases. In squirrels on Jersey, there was no evidence of SQPV, which is visually similar to and often confused with FED (Blackett et al. 2018). SQPV can be a significant cause of red squirrel mortality, as occurred in Merseyside, UK, where the red squirrel population was reduced by over 80% (Chantrey et al. 2014). Despite this, Chantrey et al. (2014) found that 8.4% of red squirrels exposed during an SQPV outbreak survived the infection. Analyses supported the prediction that grey squirrels were responsible for initiating the outbreak but, once the disease had been introduced, the main driver of SQPV infections were red squirrels transmitting the disease to each other (Chantrey et al. 2014).

1.4.5. Anti-Predator Responses

Urban red squirrels had significantly shorter flight initiation distances (FID) and vertical escape distances (VED) than their rural conspecifics when approached by humans, which indicates strong habituation (Uchida et al. 2016, 2017). It is not clear whether urban squirrels are better at assessing risk (i.e. they tolerate human presence, until humans are in close proximity) or have reduced vigilance levels; if the latter, then they may not respond appropriately to predation threats (Uchida et al. 2016). As described by Uchida et al. (2017), alert distance (AD; the distance at which an animal first detects a potential threat) represents vigilance, whilst FID and VED represent risk assessment. Uchida et al. (2019) found that both AD and FID were significantly shorter in urban squirrels than in their rural conspecifics, which implies that, although urban individuals have reduced vigilance, they are also able to evaluate risk levels.

When reintroduced into an urban park, red squirrels that were used to human disturbance had a higher probability of survival and breeding than individuals initially taken from rural woodlands (Wauters et al. 1997). Wauters et al. (1997) suggested that squirrels that are familiar with receiving food from humans might adapt better to supplemental feeding, although red squirrels translocated from rural Fife in Scotland to Regent's Park in London still habituated to human disturbance and successfully adapted to using supplemental food hoppers (Bertram & Moltu 1986). However, these individuals had a period of captivity before release (Bertram & Moltu 1986), whereas the individuals described by Wauters et al. (1997) study were released on the day of capture.

Another study monitored the impact of visitors to Fota Wildlife Park, Ireland, on the red squirrel population and found temporal avoidance of public areas (Haigh et al. 2017a). The authors observed that, even though large numbers of squirrels continued to use the park, the squirrels only moved into the public areas when the park was closed, and instead were significantly more active in the non-public areas of the park when it was open. Despite this, there was no significant association between faecal cortisol metabolite levels (commonly used as a measure of stress) and visitor abundance.

1.5. DISCUSSION

The literature search highlighted the gradual increase in articles regarding urban ecology of red squirrels since 2014, which suggests that this is an area of growing research interest and most likely reflects the wider realisation of the need to understand the impact of increasing urbanisation on wildlife ecology (McCleery et al. 2014). Red squirrels have successfully adapted to urban environments, where their behavioural flexibility allows them to exploit the resources available whilst avoiding or adapting to the risks present.

1.5.1. Risks in the Urban Environment

Of the reviewed studies that reported instances of road traffic mortality, only Blackett et al. (2018) compared different causes of death. However, other comparative studies have also found that road traffic accounted for 41.7% (n = 163; Simpson et al. 2013b), 42.9% (n = 245; LaRose et al. 2010), and 48% (n = 119; Shuttleworth et al. 2015) of recorded red squirrel deaths, comparable to the 50.7% reported by Blackett et al. (2018). This suggests that road traffic is a significant cause of mortality in some urban red squirrel populations, although there are opposing arguments regarding whether records tend to be over- or under-estimated (Shuttleworth 2001). Roadkill tends to be more conspicuous, and may be more likely to be reported, than other causes of mortality (e.g. predation). On the other hand, cases could go unreported due to the carcasses being disposed of by roadkill removal services, eaten by scavengers, or degraded by road traffic and the weather. In some instances, squirrels injured by road traffic shelter under nearby vegetation or in dreys before dying.

The seasonal pattern in road traffic mortality may be due to a combination of reasons. Red squirrels spend more of their active time engaging in foraging and scatter-hoarding behaviours on the ground, increasing from 32% in the summer months to 44% in the autumn due to the increased food availability, which could result in squirrels crossing roads more often as they travel to find and cache food items (Shuttleworth 2000, 2001). There are also typically lower red squirrel numbers in late spring and early summer, followed by a post-breeding increase in numbers in autumn and early winter, when the young are weaned (Bosch & Lurz 2012). As the juveniles then disperse, they are potentially more likely to encounter road traffic, although Shuttleworth (2001) found that the majority of autumnal road traffic casualties were adults. Similarly, Blackett et al. (2018) reported that only 2.9% of the 171 identified road traffic casualties were juveniles and 8.2% were sub-adults, although there was no further investigation into seasonal patterns of mortality. The male-biased winter mortality peak may be due to the breeding males searching for sexually active females (Shuttleworth 2001), as the start of the reproductive period tends to fall in December or early January (Bosch & Lurz 2012).

The presence of predators in urban environments appears to vary depending on the species and potentially the extent of urbanisation. Some studies suggest that predator abundance is decreased compared to rural areas, whereas others have suggested higher densities of predators in urban areas (e.g. Shochat et al. 2006, Bateman & Fleming 2012, Jokimäki et al. 2017). Similarly, predation risk varies between studies, ranging from less than 10% (Shuttleworth 2001, Blackett et al. 2018) to over 20% of recorded deaths (Wauters et al. 1997, Fey et al. 2016). When predation of red squirrels in urban environments does occur, it appears that free-ranging domestic and feral cats are generally responsible (Fey et al. 2016, Jokimäki et al. 2017, Blackett et al. 2018).

From the reviewed articles, there is currently little evidence to suggest that predation has significantly contributed to the decline of the red squirrel; this is consistent with other published studies (e.g. Petty et al. 2003, Turkia et al. 2018). However, the additional mortality pressure could have localised impacts in areas where predator densities are particularly high, as is the case with cats in urban environments, especially where red squirrel populations are already vulnerable. In addition, predation events may be under-estimated as they can be difficult to identify, for instance if the squirrel carcass is mostly consumed or carried away by the predator. This implies that the impact of predation, particularly by free-ranging domestic and feral cats, on urban red squirrel populations may be greater than previously estimated, and so may benefit from further investigation.

Red squirrels are susceptible to a range of diseases, some of which could potentially limit populations, such as SQPV (Chantrey et al. 2014). High concentrations of viral DNA have been found in the oral mucosa and exudative skin lesions of SQPV-infected individuals, which suggests that red squirrels may transmit the disease amongst themselves via social interactions (e.g. fighting, grooming, drey sharing) and scent marking (Fiegna et al. 2016). Local disease outbreaks may be exacerbated in urban environments, where red squirrel populations can often reach higher densities and the presence of supplemental feeders may act as sources for the spread of infection. Therefore, there is a need to identify any infectious individuals rapidly (Fiegna et al. 2016), ideally using non-invasive surveillance strategies such as those developed by Everest et al. (2019), to remove those individuals and reduce transmission of the disease.

There has been growing evidence that adenovirus may present a significant threat to red squirrel populations (e.g. Everest et al. 2014, 2018). Blackett et al. (2018) did not fully explore the potential for adenovirus infections within the Jersey population, having only tested 12 out of 337 individuals; however, 41.7% of those 12 tested positive for adenovirus. Another emerging disease is squirrel leprosy (*Mycobacterium lepromatosis* and *Mycobacterium leprae* Hansen 1874), for which clinical cases have been diagnosed across the UK (e.g. Simpson et al. 2015, Avanzi et al. 2016), but which was not identified on Jersey (Blackett et al. 2018). In addition, there was no evidence of SQPV on Jersey (Blackett et al. 2018), which is likely due to the absence of grey squirrels on the island (McInnes et al. 2009), but the disease is likely to be a significant cause of red squirrel mortality where grey squirrels are present (Chantrey et al. 2014).

Blackett et al. (2018) highlighted FED and amyloidosis as causes of concern on Jersey. FED has also been identified as a potential cause of concern on the Isle of Wight (Simpson et al. 2010), although to a lesser extent than on Jersey, and there has been at least one possible case on Anglesey in North Wales (Shuttleworth et al. 2015b). There have been no other published reports of amyloidosis, apart from one potential case on the Isle of Wight (V Simpson, unpublished observations, as cited in Blackett et al. 2018) and occasional cases in Lancashire, UK (J Chantrey, *pers. comm.*, as cited in Blackett et al. 2018). The origins and development of both FED and amyloidosis are currently unclear, although some evidence suggests that genetic predisposition (i.e. a heritable allele that increases an individual's susceptibility to a disease) and stress may be factors for amyloidosis (Caughey & Baron 2008, Simpson et al. 2013a). This may explain why the disease is so prevalent on Jersey, where the red squirrel population is small and isolated, and stresses associated with traffic, pets, and high local densities of squirrels lead to agonistic interactions at food sources (Blackett et al. 2018). As urban populations of red squirrels are often isolated and exposed to similar stressors, the impact of FED and amyloidosis requires further investigation in other locations.

1.5.2. Resources in the Urban Environment

Urban wildlife populations are reported to have higher numbers of bold individuals than rural populations (e.g. Møller 2012, Díaz et al. 2013, Lowry et al. 2013), as demonstrated in urban red squirrel populations (Uchida et al. 2016, 2017, 2019), allowing them to exploit the available resources. Boldness is thought to result from repeated exposure to non-lethal stimuli from frequent human disturbance, resulting in a 'transfer of habituation' to other predator stimuli and an overall reduction in anti-predator response (McCleery 2009). This adaptive response helps to prevent urban animals repeatedly fleeing and expending energy unnecessarily, instead allowing them to spend more time foraging (Sol et al. 2013). Furthermore, Uchida et al. (2019) suggested that the capacity to assess varying risk levels effectively and respond accordingly may reflect higher cognitive abilities, which supports the proposition that learning in urban animals may be improved by the environmental complexity and unpredictability associated with urban areas (Griffin et al. 2017).

Urban environments appear to support higher population densities of red squirrels than rural areas (e.g. Babińska-Werka & Żółw 2008, Kopij 2014, Jokimäki et al. 2017). However, as highlighted by Jokimäki et al. (2017), the use of visual transects to count red squirrels in urban environments and woodlands may be affected by differences in habitat and habituation to people. For example, detectability may be higher in urban areas due to the increased visibility in more open greenspaces (e.g. parks) and more bold individuals (e.g. Uchida et al. 2016). On the other hand, detectability may be reduced due to obstructions by buildings (Jokimäki et al. 2017). The use of capture-mark-recapture can provide more accurate and reliable estimates of abundance (e.g. Turlure et al. 2018), but this invasive method requires time, resources, and experience. Alternatively, the use of baited visual transects may be a more effective method in future studies to improve detectability whilst maintaining the benefits of this non-invasive technique (Gurnell et al. 2011).

Many of the studies in this review have emphasised the importance of the availability and quality of urban greenspaces for red squirrel populations. For example, it is crucial to manage the tree species composition in urban greenspaces to ensure the availability of natural food sources (Vieira et al. 2015, Reher et al. 2016, Jokimäki et al. 2017) and the provision of suitable nesting sites (Kopij 2009, Stirkė 2019). However, it is difficult to unpick the relative importance of natural and supplemental food sources and habitat quality, as there often tends to be supplemental feeding wherever red squirrels are present in urban areas, as well as the presence of other anthropogenic food sources.

For example, city parks with diverse and mature tree species had high densities of red squirrels but, of those parks, those that bordered allotments with anthropogenic food sources had the highest population densities (Kopij 2014). Clearly, high-quality natural food sources can support higher population densities, but it appears that the availability of supplemental food further increases the habitat quality. This is supported by the fact that supplemental feeding directly impacts patterns of space use, even when natural food sources are abundant (Reher et al. 2016).

Supplemental food is also typically available throughout the year, and so can be relied upon when natural food is seasonally scarce, although supplemental food may not be nutritionally ideal: many people provide peanuts, which are high in fat and desirable to the squirrels, but can result in calcium deficiencies (Bosch & Lurz 2012). Natural food sources can help to provide a more nutritionally balanced diet and compensate for any malnutrition caused by eating supplemental food (Thomas et al. 2018).

Thomas et al. (2018) suggested that the smaller and over-lapping home ranges observed in urban individuals may be because suitable habitat is sparse and movement between fragments is energetically costly, so red squirrels may simply occupy the remaining habitat fragments with available food sources. This is supported by Wauters et al. (1994), who found that the core areas of red squirrel home ranges in fragmented woodlands were smaller than those in larger, continuous woodlands, which suggests that home range size can be limited by the size of the woodland fragment.

The higher population densities in urban areas are likely to be due to the distribution and abundance of supplemental food, combined with the availability of natural food sources in the remaining greenspaces throughout the urban landscape, resulting in smaller home ranges with a greater extent of overlap between individuals (Reher et al. 2016, Thomas et al. 2018). This is supported by other studies that suggest that food availability is the main factor limiting abundance (Petty et al. 2003), and that squirrels alter their patterns of space use in response to changes in the distribution and abundance of food in rural woodlands (Wauters et al. 2005). The increased food availability also means that urban individuals can quickly attain the minimum body weight required to come into oestrus (Wauters & Dhondt 1989a), further increasing population sizes by allowing females to start breeding at the earliest opportunity (Rézouki et al. 2014), and can maintain stable body masses throughout the year (Turner et al. 2017). This differs in comparison with rural populations where bodyweight is at a minimum in late summer and reaches a maximum in late winter, due to the limited provision or absence of supplemental food and the large seasonal changes in ambient temperature that affect natural food availability (Wauters & Dhondt 1989b).

Wauters et al. (2010) suggested that increasing habitat fragmentation when fragments are surrounded by an unsuitable or hostile matrix (e.g. barriers or urbanised areas) may inhibit dispersal, whereas, when the matrix is not hostile (e.g. farmland or rural villages that are unsuitable for settling but not for movement), different degrees of habitat fragmentation appear not to affect dispersal behaviour. Contrary to the suggestion that urban areas could be considered a hostile matrix, the findings from this review indicate that movement ability does not appear to be restricted by built structures within the urban landscape. Instead, the shorter dispersal distances of urban individuals appear to be due to the stable resource availability reducing the need to disperse further (Fey et al. 2016, Selonen et al. 2018, Hämäläinen et al. 2019). This is supported by the fact that urban red squirrels travel in closer proximity to buildings than would be expected at random, as they move through the urban landscape to exploit supplemental food (Jokimäki et al. 2017, Hämäläinen et al. 2019).

Habitat loss and fragmentation are closely correlated with urbanisation (Liu et al. 2016), so it can be difficult to distinguish between the impacts of urbanisation and habitat fragmentation on red squirrel populations, as both can result in similar behavioural patterns. For example, similar effects of habitat fragmentation on spatial organisation and dispersal have been identified in both urban and rural woodland squirrel populations (Wauters et al. 1994, 2010, Thomas et al. 2018, Hämäläinen et al. 2019). However, the findings from this review suggest that habitat loss due to urbanisation has a greater impact on red squirrel populations than fragmentation, which is supported by Fahrig (1997). Therefore, increasing the availability and quality of greenspaces would be of most benefit to red squirrel conservation in urban environments, rather than improving connectivity between greenspaces.

1.6. CONCLUSION

The findings from this systematic literature review indicate that urban habitats can be suitable refugia for red squirrels, as their behavioural flexibility has allowed them to adapt successfully to the urban environment. However, it is desirable to manage urban habitats more effectively for both wildlife and people. For instance, the management of sufficiently large greenspaces and their tree species composition would ensure the availability of high quality, reliable natural food sources and appropriate nesting sites for red squirrels, as well as benefitting the mental and physical well-being of the human residents. Furthermore, the provision of supplemental food can have substantial benefits, although mitigation measures may be required to minimise any negative impacts. For example, public engagement regarding suitable food items and appropriate hygiene practices may help to reduce nutritional deficiencies in the squirrels' diets and prevent disease outbreaks from sharing feeders. As is the case with existing red squirrel reserves, grey squirrel control within and around urban refugia would help to reduce SQPV outbreaks.

Mitigation measures may also help to reduce mortality; for instance, the use of non-invasive surveillance strategies could help to identify potential disease outbreaks, or rope bridges could be constructed to provide crossing points over busy roads to reduce road traffic casualties. In addition, the current evidence regarding the impact of predation by free-ranging domestic and feral cats is limited but would benefit from further research to evaluate whether it has been previously under-estimated. Mitigation measures may help to reduce instances of predation by pets, for example owners could keep their cats inside during early morning when squirrels are most active and likely to be predated, but this would not address any potential impact of feral cats.

Red squirrels are well-adapted to inhabiting urban areas, becoming strongly habituated to human presence and being able to move through the built landscape to exploit the available resources. Therefore, urban refugia, if appropriately managed, may aid conservation efforts for this declining, native species whilst simultaneously benefitting the human inhabitants.

Part C: Research Aims and Objectives

By systematically reviewing the current published literature, this chapter has highlighted that red squirrels are an urban-adaptable species and that urban environments can provide a suitable habitat for the species, if appropriately managed. The overall aim of this research project is to understand how red squirrels use urban environments, using the town of Formby in Merseyside as a study site, including how the associated resources and risks affect their behavioural ecology. By understanding this, conservation management strategies for red squirrels will be recommended, both to enhance the study site's suitability as a long-term stronghold for this endangered native species and to benefit other populations across their distribution range. Urban management is also likely to benefit some other urban wildlife species, as well as the human inhabitants.

The objectives are:

1. To examine the current red squirrel population in the study site.
2. To evaluate the resources and risks present in the urban environment, focussing on supplemental feeding, habitat quality, and mortality threats in the study site as key topics identified through the systematic literature review.
3. To analyse the home ranges of the red squirrels in the study site, in relation to the resources and risks present in the urban environment as evaluated in objective two.

4. To formulate conservation management recommendations to benefit the red squirrels in the study site and other strongholds, through collating and evaluating the data analysed across the previous objectives.

2.0. Chapter Two: An Evaluation of the Red Squirrel Study Population in Formby, Merseyside

This chapter examines the current red squirrel population in the study site of Formby, Merseyside. The findings will be incorporated into the data analyses and discussions of subsequent chapters.

2.1. INTRODUCTION

Understanding wildlife population dynamics is key to developing wildlife conservation management plans. For example, it can help to determine how populations may change in response to variations in resource availability (e.g. Anderies et al. 2007, Wauters et al. 2008), the landscape matrix (e.g. Wiegand et al. 1999, Verbeylen et al. 2003b), habitat management (e.g. Lurz et al. 2003, Bro et al. 2004), or the interaction between native and invasive species (e.g. Gurnell et al. 2004, Carlsson et al. 2010). For instance, Bro et al. (2004) investigated the impact of a widely used habitat management scheme (i.e. wildlife cover strips on intensively cultivated farmland) on grey partridge (*Perdix perdix* Linnaeus 1758) populations, through monitoring population density, reproductive success, and over-winter mortality. They found that population density and reproductive success were not increased in the managed sites compared to the control sites, but also that there was higher over-winter mortality as predators targeted the cover strips for hunting. Therefore, this habitat management scheme may not be appropriate for grey partridge conservation efforts. In addition, Lurz et al. (2003) used a spatially explicit population model to predict the impact of a proposed forest felling and restocking plan on a red squirrel population, from which it was determined that there was a risk of extirpation. The findings were then used to inform a revised forest management plan to ensure the persistence of the red squirrel population in the study site.

As discussed in Chapter One and will be further explored in Chapter Three, the urban ecosystem has different environmental characteristics and therefore urban wildlife population dynamics can often differ from their rural counterparts, such as densities, demographics, reproduction, survival rates, and causes of mortality (Rodewald & Gehrt 2014). For example, body size is often greater in urban environments compared to conspecifics in rural areas, which can be associated with increased reproductive success (Wright et al. 2012, Hall & Warner 2017). Parasite burdens between urban and rural populations appear to differ depending on the host species and parasite type, with some studies reporting higher parasite prevalence in urban areas whilst others reported lower compared with rural populations (Delgado-V & French 2012). The spatial distribution of wildlife species in urban environments is strongly associated with habitat suitability, in terms of its availability, connectivity, and quality for the target species in the study site (Turner et al. 2022, Nelli et al. 2022). Mortality due to predation and hunting/trapping (in the case of mammals) tends to decline with increasing urbanisation, whereas collisions with vehicles and structures (e.g. buildings, in the case of birds) and disease-related mortality tends to increase (Rodewald & Gehrt 2014). Despite this, studies suggest that overall survival rates may be higher in urban wildlife populations (Prange et al. 2003, McCleery et al. 2008). As a result, potentially due to the availability of reliable and abundant resources, urban areas can often support higher wildlife population densities compared to rural areas (Prange et al. 2003, Fitzgibbon et al. 2011).

As highlighted by these examples, urban wildlife population dynamics can vary compared to rural populations. Therefore, further research is required to identify trends and different drivers of population dynamics in the urban environment, to inform future conservation management.

2.1.1. Chapter Aim and Objectives

The aim of this chapter is to examine the current red squirrel population in the study site of Formby, Merseyside, specifically the population demographics, distribution, and abundance/density. The data that are analysed in this chapter will provide baseline information that will also be incorporated into Chapters Three to Five.

The objectives are:

1. To compare the population demographics between the urban area and the adjacent woodlands, including sex ratios, breeding condition, and age structure.

2. To determine the population distribution across the study site.
3. To calculate the population abundance and density and compare changes between years (from 2012 to 2019).

2.2. METHODS

All the necessary research licences (Appendix III), landowner permissions (Appendix III), and ethical approval were obtained prior to commencing data collection and renewed annually as required. Ethical approval was granted by the Nottingham Trent University (NTU) School of ARES Research Ethics Committee (ARE595/ARE666) and by the Animal Welfare & Ethical Review Body (AWERB). Fieldwork protocols (Appendix IV) were developed based on pre-existing protocols provided by the Lancashire Wildlife Trust (LWT) and refined through consultations with the Animals (Scientific Procedures) Act 1986 Home Office Inspector and various squirrel experts, including the LWT Red Squirrel Officer, the Director of Red Squirrels Trust Wales, and a postgraduate researcher from Newcastle University. The fieldwork protocols were approved by NTU's AWERB.

2.2.1. Study Site

Formby is a coastal town of approximately 17 km^2 within the Metropolitan Borough of Sefton in Merseyside, Lancashire (Ordinance Survey grid reference SD293074; latitude 53.558438, longitude -3.0687685; Fig. 2.1), with a population of around 22,500 (Office for National Statistics 2015). Formby is an affluent area because it is a commuter town for the adjacent city of Liverpool (One NorthEast 2009). Therefore, many of the residential properties are a considerable size (0.08 – 0.3 ha), particularly those within approximately 800 m of the coastal woodlands, with large gardens bordered by a mixture of coniferous and broadleaf, often ornamental, tree species (Shuttleworth 2001).

Formby contains a network of roads with a maximum legal speed limit of either 20 or 30 miles per hour (mph), as well as the bypass to the east with a maximum legal speed limit of 60 mph that may act as a barrier to dispersing wildlife. The town is also bisected by the commuter trainline, which may act as a barrier to wildlife dispersal between the east and west sides of the town (Fig. 2.1).

The land between the town and the coast consists of a variety of habitats, including coniferous and broadleaf plantation woodlands, sand dunes, and seasonal ponds. This area is designated a Site of Special Scientific Interest (SSSI), which is protected and managed by the National Trust, as well as being part of the Sefton Coast's Special Area of Conservation (SAC). Formby is a popular tourist destination due to the beaches, with the National Trust reserve receiving approximately 250,000 visitors each year (Shuttleworth 2001), which leads to increased levels of road traffic during the summer months.

Formby and its residents have a strong connection with the local red squirrel population (*pers. obvs.*). Throughout the town, there are numerous examples of houses and roads, businesses, pubs, and even locally made beverages incorporating red squirrels into their names or logos.

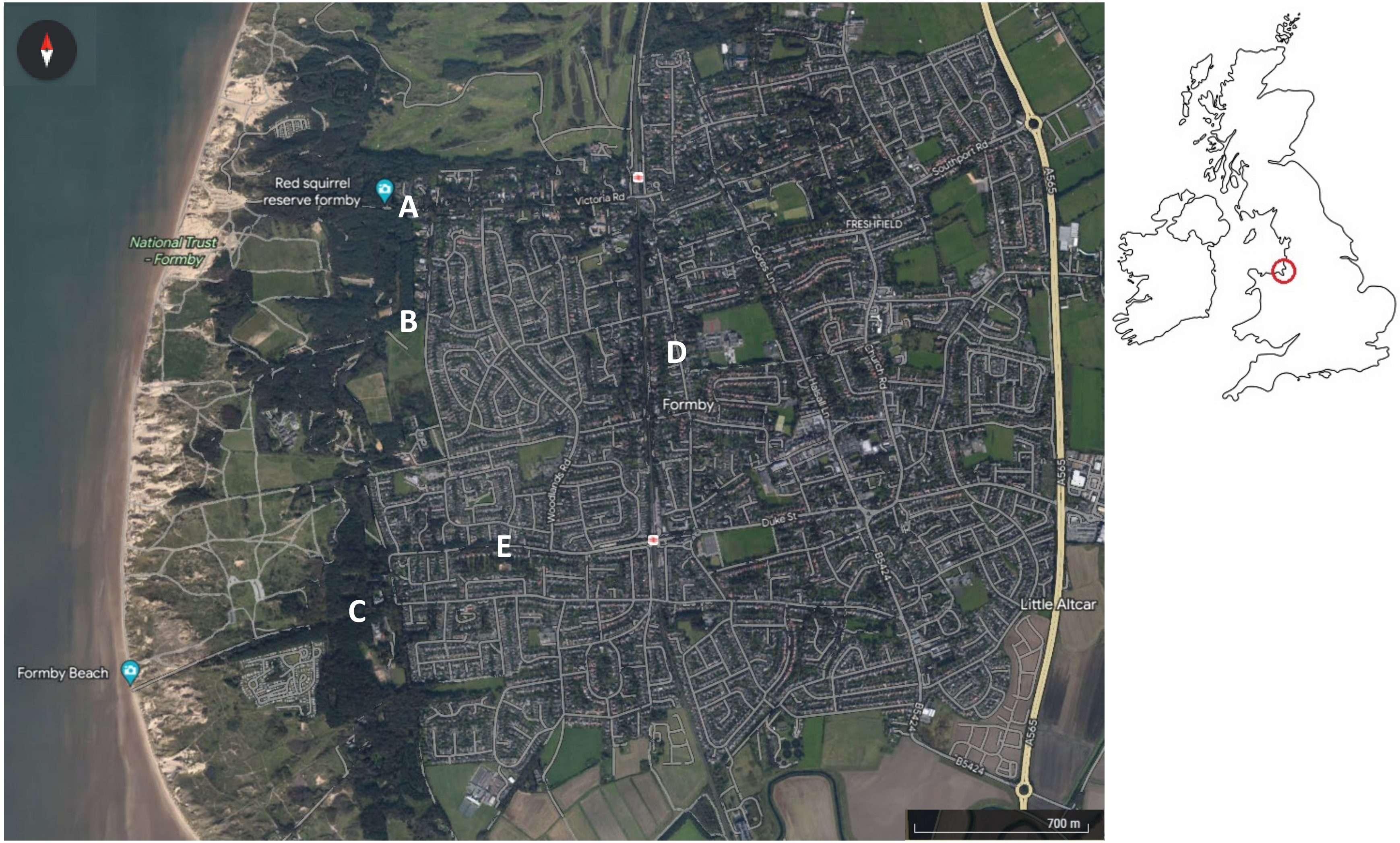


Figure 2.1. Satellite overview of the study site of Formby, Merseyside, with the A565 bypass to the east of the town and the coastal woodlands to the west. The three roads providing access to the woodlands are labelled: (A) Victoria Road, which extends eastwards to the railway line, (B) Blundell Avenue, and (C) Lifeboat Road. Other key roads are also labelled: (D) Freshfield Road, which runs north-south parallel with the railway line, and (E) Kirklake Road (©Google Earth 2023).

2.2.1.1. Red Squirrel Reserve

Formby is part of the Merseyside red squirrel stronghold (Fig. 2.2), one of the seven designated strongholds made up of 17 reserves located in Northern England. The red squirrel population is thought to have originated from individuals introduced into the nearby Ainsdale Sand Dunes National Nature Reserve (NNR) from Europe approximately 80 years ago (M Garbett, *pers. comm.*, as cited in Gurnell and Pepper 1993). In order to protect the red-only core stronghold centred around Formby and the Ainsdale NNR, ongoing culling of grey squirrels is carried out in the surrounding buffer zone by volunteers and the LWT Red Squirrel Project team. The town is ideally situated as it is protected from invading grey squirrels by the sea on the western side, so culling efforts can be focussed on the inland side. The stronghold suffered from a SQPV outbreak in 2007/08, which killed almost 90% of the red squirrel population (Chantrey et al. 2014). Another SQPV outbreak occurred in 2018/19 (*pers. obvs.*), which will be discussed in more detail throughout this thesis.

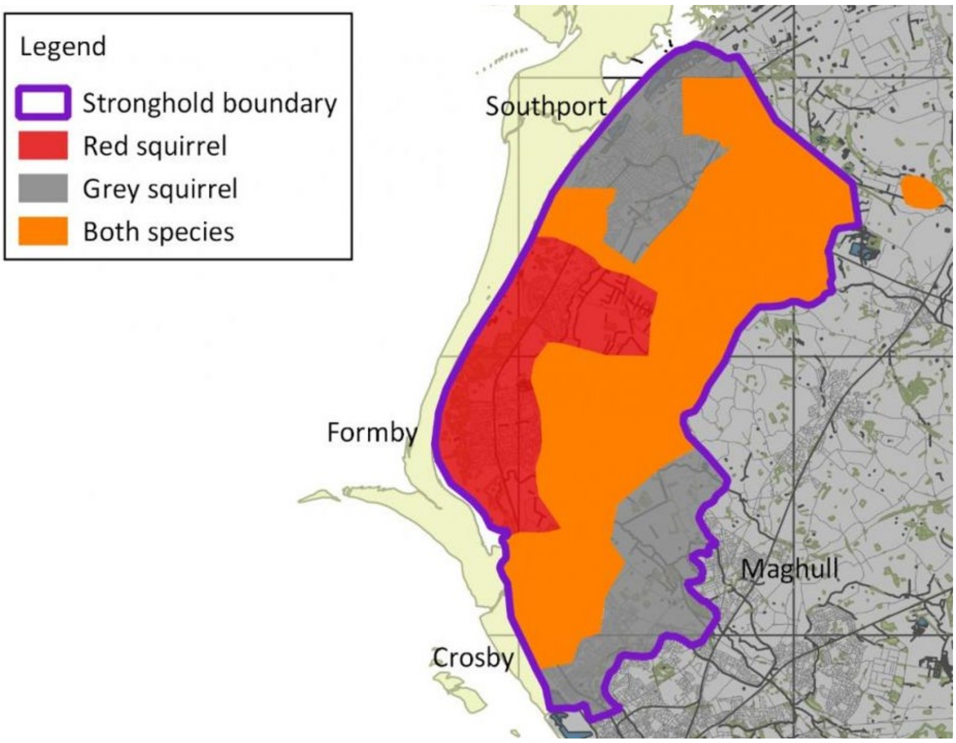


Figure 2.2. Overview of the Merseyside red squirrel stronghold, and the distributions of red and grey squirrels within the stronghold (The Lancashire Wildlife Trust 2020).

The woodlands are immediately adjacent to the urban area (Fig. 2.1) and therefore should be classified as 'peri-urban', rather than rural or 'non-urban' (Kowarik 2005). The National Trust reserve consists of approximately 70 ha of mixed coastal, sand dune woodlands that are dominated by around 40 ha of mature Scots pine (*Pinus sylvestris* Linnaeus 1758) and Corsican pine (*Pinus nigra* subsp. *laricio* Maire 1928) interspersed with stands of sycamore (*Acer pseudoplatanus* Linnaeus 1758), common beech (*Fagus sylvatica* Linnaeus 1758), and other deciduous tree species (Gurnell & Pepper 1993, Shuttleworth 2001). The Red Squirrel Reserve is in the northern part of the woodlands adjacent to Formby and accessed by Victoria Road (Fig. 2.1). The Red Squirrel Reserve is primarily focussed around a short (approximately 300 m) looped walk immediately south of Victoria Road (labelled A in Fig. 2.1), accessed straight from the car park, known as 'Squirrel Walk'. This part of the woodland, situated south of Victoria Road down to Blundell Avenue (labelled B in Fig. 2.1), is generally the busiest and the main thoroughfare for tourists walking to the beach, whilst the area north of Victoria Road up to the golf course is typically much quieter and predominantly used by locals (*pers. obvs.*).

Historically, visitors were able to purchase peanuts from the reserve office to feed the squirrels, of which more than 2500 kg was sold annually (Shuttleworth 2001), although the sale of peanuts was halted in 2008 due to the SQPV outbreak. Supplemental food was still provided by National Trust volunteers once a day (at approximately 10:00) at designated feeders on 'Squirrel Walk' and had been ongoing since the mid-1980's (Shuttleworth 2000), but was permanently stopped in mid-2018 following the onset of another SQPV outbreak. Many visitors continue to bring their own peanuts (*pers. obvs.*), although this is not encouraged. Due to the long-term provision of supplemental food, it is thought that the red squirrel population density is very high; a previous study estimated the red squirrel population to be 400 individuals in the reserve area alone (Harris et al. 1995).

The southern part of the woodlands, which is situated adjacent to Lifeboat Road (labelled C in Fig. 2.1), was previously owned by Sefton County Council and received minimal management until ownership was passed to the National Trust in 2018. As with Victoria Road, this area can become very busy during the summer months with visitors parking to access the beach. The part of the woodlands north of Lifeboat Road is also dominated by pine species interspersed with stands of deciduous tree species, primarily sycamore, whilst the area south of Lifeboat Road is more mixed with stands of sycamore, common beech, pedunculate oak (*Quercus robur* Linnaeus 1758), and other deciduous tree species.

2.2.2. Live-Capture Trapping

Training in the correct procedures for live-capture trapping and handling squirrels was undertaken with the South Lakes Red Squirrel Group (April 2016) and the Director of Red Squirrels Trust Wales (August 2016 and February 2017). Live-capture traps (Trapman 'squirrel model', 15 x 17.5 x 54.5 cm; Elgeeco 'Tree-mounted squirrel trap', 40 x 20 x 20 cm) were placed in the National Trust woodlands, in areas with limited public access as recommended by the National Trust Property Manager, and in the urban gardens of consenting volunteers (Fig. 2.3). Therefore, traps could not be set in a grid across the study site, but instead were located more opportunistically in secluded areas of the woodland and where volunteers consented for the use of their gardens.

In the woodlands, the trap sites were primarily selected based on the presence of feeding signs, specifically the scales and stripped cores of pine cones, as well as ensuring that the traps were hidden from view and placed in the shade. Within the confines of the available secluded areas, the trap sites were spaced apart as much as possible, so that ideally the same squirrels would not be caught in multiple traps. In the urban gardens, the traps were typically placed in the shade near any existing feeders or, if the volunteer did not provide supplemental food, where signs of feeding were located or there was a potential corridor for movement (e.g. hedgerow or fence). The volunteers were asked to remove any supplemental food from their feeders for the duration of trapping, to encourage the squirrels to enter the traps rather than continuing to use the feeders.

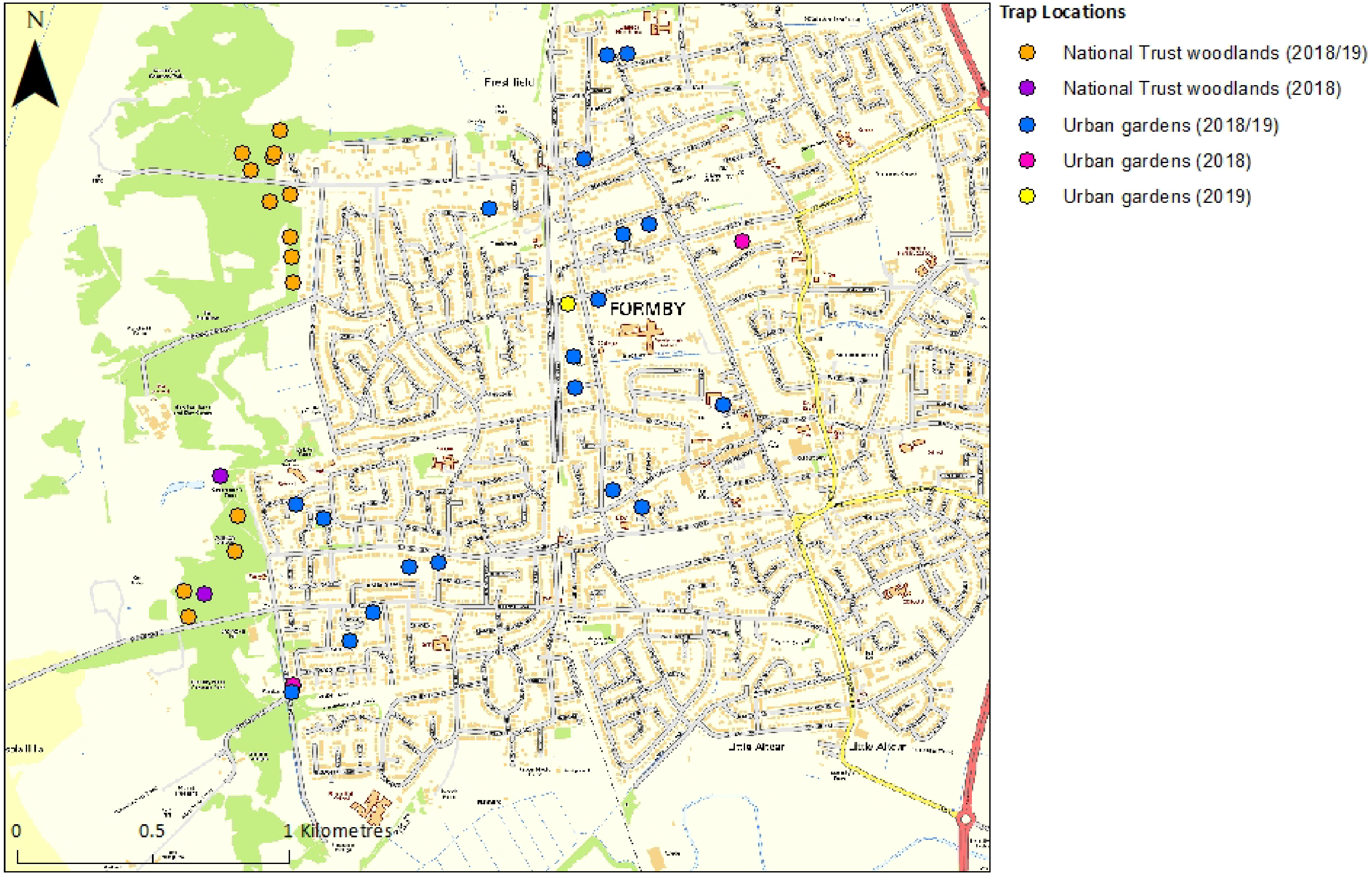


Figure 2.3. Trap locations in the National Trust woodlands and urban gardens, highlighting where traps were used in both years (2018/19) and where additional traps were used in either 2018 or 2019 (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

Live-capture traps were placed either on the ground (Wauters et al. 2002a, Santicchia et al. 2018) or in trees (e.g. Magris and Gurnell 2002), although it has been suggested that trapping success for red squirrels may not substantially differ between these methods (D Brady, University of Newcastle, *pers. comm.*). In this study, ground traps (Trapman 'squirrel model', 15 x 17.5 x 54.5 cm) were used in the northern woodland and urban gardens, where the risk of interference from the public was minimal. The traps were placed on flat ground so as not to deter the squirrels from entering, which can occur if the trap moves due to uneven ground, and to prevent any injuries caused by their paws getting stuck through the mesh, which is a potential risk if there is a gap present under the trap. All the ground traps were covered with roofing membrane and foliage to provide shelter against the weather and minimise stress of any squirrels whilst in the trap, as well as to camouflage the trap from the public (Fig. 2.4). The ground traps were also padlocked to a nearby tree trunk with a metal cable in case of interference by the public, although this was not necessary in the private urban gardens. The locations of the traps were marked using a GPS device and the co-ordinates recorded. In addition, signs were attached to the top of the traps to explain their purpose and provide contact details in case any members of the public found a trap and had some concerns or queries.

Figure 2.4. A ground trap in the northern woodland, with the information sign visible at the front, the blue roofing membrane sheltering the back two-thirds, and covered with foliage (photo: K Hamill).

Ten ground traps were placed in the northern woodland, with five in the area north of Victoria Road and five behind 'Squirrel Walk' in the area parallel with Larkhill Lane (Fig. 2.3). Approximately 20 ground traps were placed in the gardens, although this number fluctuated as residents either volunteered, withdrew, or were excluded from the study. In 2018, initially there were 23 resident volunteers' gardens. Volunteers were recruited via the LWT Red Squirrel Officer, who circulated an email around existing volunteers of the LWT Red Squirrel Project and the local golf club. Prior to commencing fieldwork, two withdrew, one was excluded due to the risk of their dog interfering with the trap, and one passed away, resulting in 19 urban gardens being used for the Phase 1 trapping period (see section 2.2.1.1). Two additional volunteers were also recruited for the Phase 2 trapping period (see section 2.2.1.2), one via an existing volunteer and the other opportunistically through meeting while conducting the fieldwork. In 2019, in addition to the same 21 volunteers from 2018, another resident volunteered but one withdrew and one was excluded as they were unwilling to halt providing supplemental food, initially resulting in 20 urban gardens. However, two more volunteers' gardens were excluded during the first week of trapping, one due to brown rats (*Rattus norvegicus* Berkenhout 1769*)* repeatedly triggering the trap and another as they were unable to provide ongoing access to the trap, resulting in 18 urban gardens being used for the remainder of the fieldwork.

Elgeeco traps ('Tree-mounted squirrel trap', 40 x 20 x 20 cm) housed in wooden boxes (Fig. 2.5) were trialled in 2018 in six locations in the southern woodland, in the area north of Lifeboat Road, which is more open and easily accessible by the public. Using an extendable boat hook, the traps were hung on metal T-shaped brackets that were screwed into the tree trunks. These traps help to attract less attention from the public, as they are camouflaged at height (approximately 3 – 3.5 m) in the trees (European Squirrel Initiative 2015). Unfortunately, the Elgeeco traps were unsuccessful in capturing any red squirrels during Phase 1 of trapping. Ground traps were then trialled alongside the Elgeeco traps in four of the six locations, as the other two locations were too accessible to the public, and were found to be more successful. Subsequently, the Elgeeco traps were removed and the four ground traps were used for the remainder of the fieldwork in 2018 and 2019.

Figure 2.5. An Elgeeco trap housed in a wooden box, hanging *in situ* on a metal T-shaped bracket in the southern woodland (photo: K Hamill).

Two phases of trapping were undertaken from mid-May to mid-August in 2018 and 2019 (Fig. 2.6). Phase 1 was the initial trapping period, which was conducted from mid-May until mid-June, to record the population demographics and attach radio collars (see Chapter Four for details regarding radio-collaring and tracking). At the end of the fieldwork season, Phase 2 was conducted from the end of July to mid-August, where a focussed trapping effort was made to re-trap specific individuals in order to remove their radio collars.

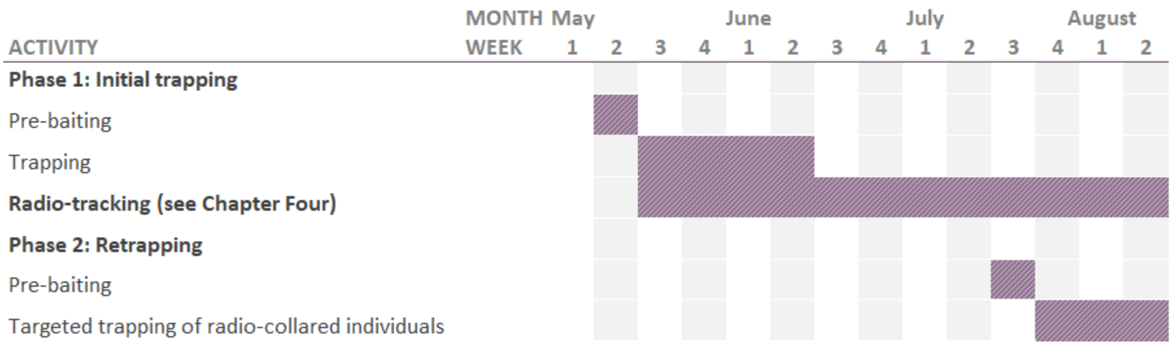


Figure 2.6. Timeline of Phase 1 and Phase 2 trapping during data collection from mid-May to mid-August in 2018 and 2019.

2.2.2.1. Phase 1 of Trapping

In May, the traps were locked open and pre-baited for at least three days with sunflower seeds (*Helianthus annuus* Linnaeus 1758), shelled peanuts, unshelled peanuts (i.e. ‘monkey nuts’), and apple to provide a source of moisture. In the woodlands, the traps were locked open with keyed-alike padlocks (i.e. only one key was required for all the padlocks) to prevent any members of the public from setting the traps, whereas a carabiner was used in the gardens and the volunteers were asked not to interact with the traps. The traps were then set each morning at 07:00 and locked open at the end of each day, at approximately 16:00. Although it would have been preferred to open the traps at dawn (approximately 04:00) when the squirrels are most active (Tonkin 1983), these timings ensured that the local veterinary practice was open in case of an injured squirrel. The traps were locked open during the pre-baiting period and overnight during the trapping period, so that the squirrels continued to become habituated to entering the traps.

Trapping took place for up to four weeks until either no new squirrels were caught for three consecutive days or the four weeks of trapping were complete, which was a condition of the Natural England research licence. Trapping only took place from Monday to Friday, excluding any public holidays. This was to minimise interference from members of the public, as the woodlands are adjacent to a popular beach that can be busy on the weekends and during holidays. The traps were checked at regular intervals (every two to three hours) throughout the day, which was recommended as a suitable period to allow the squirrels to enter the traps without being restrained for too long (C Shuttleworth, *pers. comm.*).

Trapping did not occur or was halted early on days when the weather was poor (i.e. heavy rain and/or strong winds), as squirrels can quickly become hypothermic in the trap in cold, wet weather and there were safety concerns for the researchers due to the risk of falling branches/trees. As the fieldwork was conducted during the summer months, the weather was generally suitable and therefore the risk of any weather-related injuries, for both the squirrels and researchers, was minimised. However, if the weather was very hot (above 25 ^{o}C) for an extended period, trapping either started early (from 05:00 to approximately 12:00) or late (from 13:00 to approximately 20:00), to prevent squirrels being captured in the hottest part of the day.

In 2018, initially half of the woodland (i.e. five in the north and three in the south) and half of the urban (i.e. ten) traps were set each day, with a view to alternating days for the remaining traps at each site. However, due to the high trapping success, particularly in the woodlands, it took too long to process all the squirrels whilst maintaining checks at the required regular intervals of two to three hours. Subsequently, half of the woodland traps were set for one day followed by half of the urban traps the next day, alternating for the remainder of weeks one and two during Phase 1. Trapping success continued to be high in the woodlands, however trapping success in the urban gardens was much lower. In week three, trapping continued to alternate between half of the woodland traps and half of the urban traps each day, but the number of days was weighted in favour of the urban gardens to increase capture rate, with three days compared to two days in the woodlands (although one day was lost due to poor weather). It was then decided that more traps could be opened each day, whilst still maintaining the required regular checks. Therefore, during the final week of Phase 1 in 2018, trapping alternated between all the urban traps set one day and all the woodland traps the following day, again with three days in the urban gardens compared to two days in the woodlands. Any traps that were not in use for the day were padlocked open and pre-baited to ensure that the squirrels continued to habituate to entering the traps.

In 2019, Phase 1 trapping continued to alternate between the urban and woodland traps (as identified as the optimal trapping method during fieldwork in 2018), but on a weekly basis to streamline access permissions, particularly to the urban gardens, whilst maintaining a similar trapping effort. Therefore, all the urban traps were set during weeks one and three, whilst the woodland traps were opened during weeks two (excluding the public holiday) and four (although two days were lost due to poor weather). In addition, the order in which the traps were opened each day was alternated, which allowed different traps to be opened earliest in the morning when the squirrels would be most active.

On 31st May 2019, an individual squirrel with suspected SQPV was trapped in the southern woodland and had to be euthanised at the local veterinary practice. As the bait can encourage interactions between squirrels, the traps in the southern woodland were removed to reduce the risk of spreading SQPV. Subsequently, only the 10 traps in the northern woodland were used for the remainder of the fieldwork in 2019, alongside the traps in the urban gardens.

In both 2018 and 2019, all four weeks of Phase 1 trapping were conducted as new squirrels continued to be captured. Any squirrels that were trapped were processed as detailed in section 2.2.2.3, after which the traps were thoroughly disinfected *in situ* using Anigene HLD4V before being reset or locked open. Anigene has been recommended as a safer alternative to Virkon™, as it is environmentally friendly and approved for use in veterinary practices. At the end of Phase 1, the traps were collected, thoroughly disinfected using Anigene, and securely stored away until needed for Phase 2.

2.2.2.2. Phase 2 of Trapping

Phase 2 consisted of up to three weeks of trapping effort following similar methods as in Phase 1, until either all the radio collars were retrieved or the three weeks of trapping were complete as required under the Natural England research licence. As with Phase 1, trapping occurred from 07:00 to approximately 16:00 from Monday to Friday, but the home ranges of the collared squirrels were targeted with two traps in each location. Generally, the same trap sites from Phase 1, where the squirrels were originally caught, could be used for Phase 2. However, some individuals shifted their home ranges during the fieldwork season. In these cases, often other nearby woodland or urban trap sites could be used instead, but in two instances in 2018 additional volunteers had to be recruited to establish new urban trap sites.

Once individuals were recaptured and their collars retrieved, the traps in those locations were removed and disinfected before storage for the winter. During Phase 2, processing of new and recaptured individuals continued as per Phase 1 (see section 2.2.2.3). At the end of Phase 2, all the remaining traps were collected, thoroughly disinfected using Anigene, and securely stored away. A checklist was used throughout both trapping phases to ensure no traps were accidentally left unchecked.

Phase 2 in 2019 was delayed to May 2020 due to the ongoing SQPV outbreak, in order to prevent the spread of the disease between squirrels at the baited traps. Due to the COVID-19 pandemic, Phase 2 was further postponed to June 2020, condensed into 15 days of continuous daily trapping, and the methods refined to focus on the removal of the radio collars to address the requirements of the Natural England research licence. As such, the additional data (e.g. body mass, breeding condition, etc.) were not collected and non-collared squirrels were immediately released at the site of capture, whilst radio-collared squirrels had collars removed and were briefly checked over for any injuries before release. All three weeks of Phase 2 trapping were conducted for both fieldwork seasons as some radio-collared individuals were not recaptured.

2.2.2.3. Processing of Trapped Squirrels

The live-capture trapping was always conducted by two people, where one processed the squirrels whilst the other recorded the relevant information on the datasheets, to minimise the amount of time the squirrels were restrained. During the first season of fieldwork, handling and processing were conducted under supervision of either the LWT Red Squirrel Officer or an experienced vet. Once the author was approved as competent in the required procedures, other volunteers (e.g. master’s students) could assist with recording the datasheets whilst the author processed the squirrels in place of the LWT Red Squirrel Officer or vet.

Any trapped squirrels were visually checked for signs of injuries or disease whilst in the trap to determine whether veterinary treatment was required. If an individual showed signs of disease (e.g.

SQPV lesions) or major injury (e.g. heavy bleeding or broken limbs), the protocols for 'Capture of a suspected injured or sick red squirrel' (Appendix IV) would be followed. Minor injuries, such as small cuts from the environment or fights with conspecifics, are relatively common for red squirrels and so these individuals were still included in the study. In addition, any captured grey squirrels were humanely dispatched, as legally required under Schedule 9 of the Wildlife & Countryside Act 1981, following the protocols for 'Capture of a grey squirrel' (Appendix IV).

The individuals that were deemed suitable for inclusion in the study were flushed from the trap into a handling sack and then into a handling cone for processing. Hessian bags were initially used as handling sacks but were prone to developing holes that allowed the squirrels to escape, so pillowcases were used for the remainder of fieldwork. The handling sacks and cones were provided on loan by the LWT. The squirrels were weighed to the nearest five grams (Pesola spring scales, Switzerland), sexed, and their breeding condition assessed using the presence of descended testes in males and visible nipples in females (therefore both pregnant and lactating females were classified as in breeding condition). Individuals were classified as adults, sub-adults, or juveniles primarily based on their mass (adults: 270 g or over, sub-adults: 230 to 265 g, juveniles: 225 g or less; Bosch and Lurz 2012), but also a visual inspection (e.g. juveniles have short and dense tail fur, a 'static' look to body fur, and often slight 'blonde' colouration to fur particularly on the face). Initially, the length of the right hind foot was going to be measured as an indication of body size, but this was difficult to achieve with the individual positioned in the handling cone. Instead, the length of the right shin was recorded (mm) by measuring from the heel to the top of the bent knee using callipers. The squirrels were also visually inspected to assess ectoparasite burden, recording both the level and type of parasite (Fig. 2.7).

		Number of fleas				
		0	1 – 2	3 – 5	6 – 9	10+
Presence of lice and/or harvest mites	Absent					
	Present					

Figure 2.7 Criteria for assessing parasite burden (grey: none, dark green: very low, light green: low, orange: moderate, red: high). No ticks were recorded on squirrels and so have not been included.

Finally, a passive integrated transponder (PIT) tag was implanted into the nape of the neck for long-term identification of individuals (Unique RFID, 12.4 x 2.1 mm). PIT tags were recommended as an alternative to ear tags (C Shuttleworth, *pers. comm.*), in order to minimise tag loss and minimise the risk of injury caused by ear tags being pulled out during movement through the canopy (Schooley et al. 1993). The PIT tags were determined to be the optimal size, being as small as possible but providing an appropriate range for the PIT tag reader, through consultations with the suppliers and various researchers. Before release, a small amount of fur was trimmed from the tail as a short-term indicator that the individual had been PIT-tagged. This allowed for quick identification of whether an individual was a new capture or a recapture, therefore making the processing procedure more efficient and to reduce the handling time of the squirrels. Once processing was completed, all individuals were immediately released at the site of capture. As permitted under the Natural England licence, up to 75 individuals could be PIT tagged in 2018 and this was increased to 100 individuals in 2019. When the number of trapped squirrels reached this limit, new individuals (identified from the absence of trimmed tail hair) were immediately released at the site of capture whilst recaptures were scanned before release.

2.2.2.4. Data Analysis

Descriptive statistics and qualitative analyses were conducted in Microsoft Excel, mapping and spatial analyses were conducted in ArcGIS (v10.5.1, ESRI 2017), and statistical tests were conducted in R Statistical Software (RStudio Team 2022). All results graphs were produced in Microsoft Excel.

Trapping success was calculated in Microsoft Excel, using the total number of squirrels captured and the total number of trap days. The number of trap days, or trapping effort, was calculated by the number of traps multiplied by the number of operational days. The kernel density analysis was conducted by importing the co-ordinates of the trap locations multiplied by the number of successful captures at each trap (e.g. if a trap captured two squirrels then the co-ordinates of that trap were included twice in the imported database) to create a shapefile, from which a raster layer was generated using the Kernel Density tool available in the ArcToolbox in ArcGIS.

Due to the small sample sizes, data for sub-adults and juveniles were summed for comparison with adults. Comparative analyses of population demographics were conducted using Chi-Square Tests of Independence, or Fishers Exact tests where the expected cell counts were small (< 5), from the 'stats' package that is included in RStudio. These included associations between: (1) sex and location (i.e. woodlands or urban gardens), (2) breeding condition and location, (3) sex and breeding condition, (4) age and sex, (5) age and location, (6) parasite burden and age, and (7) parasite burden and location. Comparative analyses of adult body masses between (1) males and females and (2) locations (woodlands and urban gardens) were conducted using unpaired t-tests, as the parametric assumptions (i.e. normal distributions and homogeneity of variances) were met, from the 'stats' package in RStudio. Comparative analyses of adult shin length (as an indicator of body length) between (1) males and females and (2) locations were also conducted using unpaired t-tests, again from the 'stats' package. Unpaired t-tests were used as, although the parametric assumption of homogeneity of variances was met, the parametric assumption of normality was not met but t-tests are relatively robust to violations of the normality assumption (Skovlund & Fenstad 2001, as cited in Fagerland 2012).

2.2.3. Population Distribution and Abundance

The red squirrel population distribution and abundance in the study site were evaluated using long-term datasets of squirrel sightings reported by the public (see section 2.2.3.1) and distance transect data collected by volunteers (see section 2.2.3.2) provided by the LWT.

2.2.3.1. Population Distribution

Opportunistic sightings of both alive and dead red and grey squirrels have been recorded across the stronghold since the late 1980's, originally by the volunteer-led Red Alert Group and later by the LWT. Sightings were reported by the public either by contacting the LWT Red Squirrel Project Officer via phone or email, or via the project website. For each sighting, the following details were recorded: species, number of individuals, date, whether alive or dead, location (both the address and grid reference), habitat, and any additional useful information (e.g. signs of injuries or disease, observed being hit by a car).

In order to visualise the red squirrel population distribution in the study site, the collated sightings from 2015 to 2017 were used in a kernel density analysis. As the LWT red squirrel mortality dataset was also available from 2015 to 2019 (see section 3.2.3, p. 96), any missing records of dead squirrels in the population distribution dataset were cross-checked and included. Only red squirrel sightings within Formby were included in the analysis, so any sightings of grey squirrels and sightings of red squirrels in the wider stronghold were excluded.

2.2.3.2. Population Abundance and Density

The LWT has been conducting distance transects, with assistance from volunteers, in woodlands across the Merseyside stronghold to monitor the red squirrel population since 2002. Transects have been carried out twice each year, in spring (typically during March) and autumn (typically during October), and repeated three times during both seasons over the course of a week or two. Initially 26 transects were established in woodlands throughout the Merseyside stronghold and additional transects were added in later years, resulting in 33 transects in total. In order to assess the population abundance and density in the study site alone, seven transects situated through the National Trust woodlands were included in the analysis (Fig. 2.8), which were 500 m to 1200 m in length. Due to the limited availability of distances recorded prior to 2012, the analysis focussed on the period from 2012 to 2019, incorporating the duration of this study.

Some of the transects (1: Victoria Road, 2: Caravan Park, 5: Lifeboat Road, and 7: Ravenmeols) were walked by the same volunteer each time, whilst the remaining transects (3: Asparagus Fields, 4: Blundell Avenue, and 6: Shorrocks Hill) were sometimes carried out by different individuals. The transects were started between approximately 06:30 and 08:00, and walked at a slow, steady pace. When a squirrel was sighted, the perpendicular distance from the transect to the squirrel was estimated in metres and recorded. Following a sighting, the volunteers were careful to avoid double counting individuals by being aware of which direction the previously sighted squirrel was travelling in and by walking a short distance (approximately 25 m) further along the transect before commencing monitoring again.

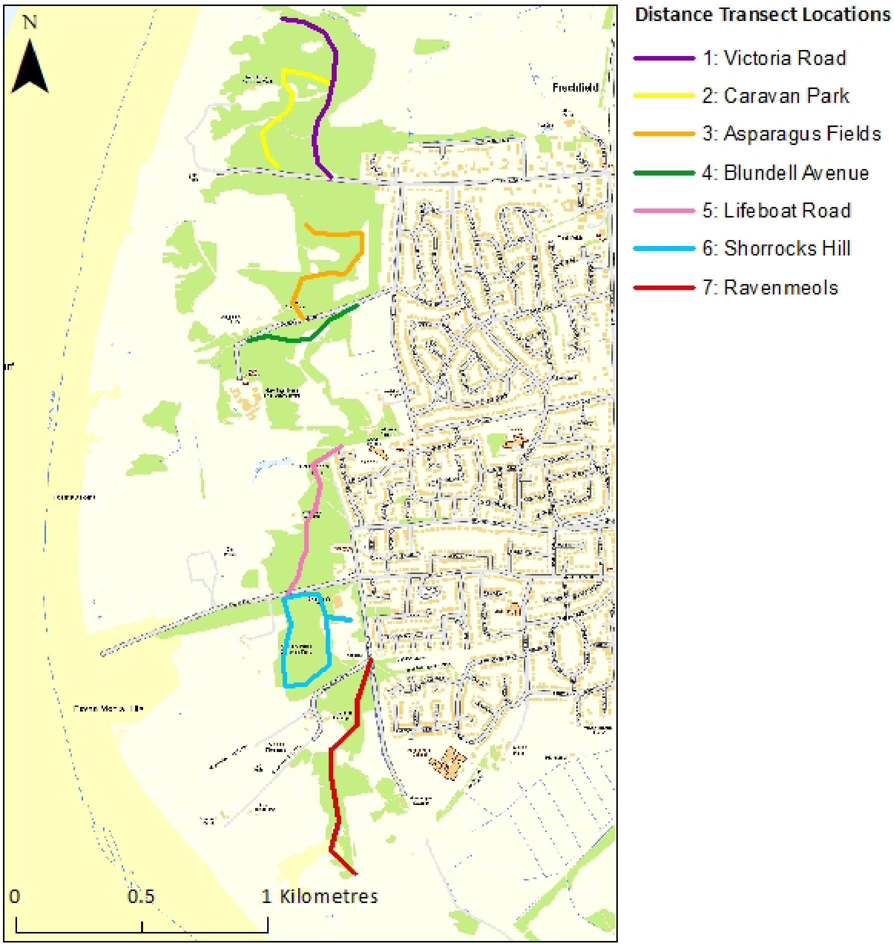


Figure 2.8. Locations of the red squirrel distance monitoring transects in the National Trust woodlands in Formby (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

2.2.3.3. Data Analysis

For the population distribution, the kernel density analysis was conducted by importing the co-ordinates of the sightings data (487 sightings from within Formby, from 2015 to 2017) to create a shapefile, from which a raster layer was generated using the Kernel Density tool available in the ArcToolbox in ArcGIS.

To obtain annual population abundance and density estimates, the distance transect data were analysed separately for each year, from 2012 to 2019, using the 'Distance' package in R (Miller et al. 2019) and following similar methods to Miller et al. (2019). The approximate area of the woodland surveyed via the transects was estimated by creating a polygon of the woodland area in ArcGIS and using the 'calculate geometry' option in the attribute table. There were nine instances where a transect was not conducted for a season and one instance where one transect was only walked twice, rather than three times, in a season, so these transects were not included in the analyses.

A significant Goodness of Fit test result ($p < 0.05$) suggests that the model does not fit the data well and is therefore not 'plausible', according to Miller et al. (2019). Through exploratory analyses, only hazard rate models had non-significant Cramér-von Mises Goodness of Fit test statistics ($p > 0.05$) and were therefore deemed 'plausible', whereas half-normal and uniform models were not 'plausible' and so were discarded. There were two covariates, 'season' and 'observer', resulting in four potential models: (1) hazard rate model with no covariates, (2) hazard rate model with season as a covariate, (3) hazard rate model with observer as a covariate, and (4) hazard rate model with season + observer as covariates. As covariates were included, no adjustment terms were included in the models (Miller et al. 2019). All models were truncated to 100 m to remove potential outliers, as it is unlikely that an observer would realistically be able to detect a squirrel more than 100 m away. With the set of 'plausible' models, Akaike's Information Criterion (AIC) can then be used to select between models. The models that were selected (Table 2.1) were used to determine the population abundance and density for each year.

In addition, the LWT has been using the distance transect survey data to monitor changes in the red squirrel population since 2002, by comparing the mean numbers of squirrels counted in the spring and autumn transects for each year to the initial survey year (i.e. 2002). In this way, the population index in 2002 is 1.0 and the indices calculated for the subsequent years could be < 1.0 (where the population was lower in comparison with 2002) or > 1.0 (where the population was higher in comparison with 2002). For this study, the population indices were calculated for each year from 2002 to 2019, by dividing the mean number of squirrels from both seasons for transects 1 – 7 (see Fig 2.8 in section 2.2.3.2) in each year by the mean number of squirrels from both seasons for transects 1 – 7 in 2002.

Table 2.1. Hazard rate model results for the distance transect analysis, where the bold AIC values indicate the selected model used to calculate the population abundance for 2012 to 2019. Any models with significant Cramér-von Mises Goodness of Fit test results were deemed 'not plausible' and were discarded.
**Models with similar AICs (where the difference is < 2) have similar estimated probabilities of detection and therefore little difference between the models, so the simplest AIC models have been selected in these cases as recommended by Miller et al. (2019).*

Year	Sample Size (*n*)	Covariate(s)	AIC
2012	179	None	1377.563
		Season	1379.555
		Observer	**1319.424***
		Season + Observer	1321.418*
2013	228	None	1715.904
		Season	**1708.573**
		Observer	Not plausible
		Season + Observer	Not plausible
2014	185	None	Not plausible
		Season	Not plausible
		Observer	**1282.849***
		Season + Observer	1284.525*
2015	295	None	2137.531
		Season	2136.209
		Observer	**2093.979***
		Season + Observer	2094.067*
2016	341	None	2536.215
		Season	2519.092
		Observer	2460.243
		Season + Observer	**2451.914**
2017	327	None	Not plausible
		Season	2194.537
		Observer	2169.914
		Season + Observer	**2166.983**
2018	259	None	Not plausible
		Season	1971.952
		Observer	**1935.263***
		Season + Observer	1937.129*
2019	190	None	1411.432
		Season	**1391.495***
		Observer	1412.375
		Season + Observer	1390.052*

2.3. RESULTS

Overall, 198 squirrels were live-capture trapped in 2018 and 2019, of which 142 were new captures and 45 were recaptures. Some individuals were not processed (*n* = 28) and so have been excluded from the data analyses. These individuals either escaped during handling (*n* = 7), were immediately released at the site of capture due to being in the trap with another individual (*n* = 4), or were immediately released as the PIT tagging limit stipulated in the Natural England licence had been reached (*n* = 17). In June 2020, 88 squirrels were also live-capture trapped but, apart from their estimated ages, no additional data were collected due to the restrictions of COVID-19. Therefore, only data collected during 2018 and Phase 1 of 2019 were used, which resulted in a sample size of 125 individuals for inclusion in the data analyses.

2.3.1. Trapping Success

There were 396 trap days in the woodlands (323 in the north woods and 73 in the south woods) and 497 in the urban gardens. Considering Phase 1 and 2 for both fieldwork seasons and considering 'captures' as both new individuals and recaptures, overall trapping success was 32.03 captures/100 trap days. Trapping success was much higher in the northern woodlands (69.35 captures/100 trap days) compared with the southern woodlands (9.59 captures/100 trap days) and the urban gardens (11.07 captures/100 trap days). Out of the 22 urban trap sites, only 11 captured any squirrels, which were generally located near either Victoria Road, Freshfield Road, Kirklake Road, or in roads adjacent to the woodlands (Fig. 2.9). The maximum number of captures at a single urban trap site was 12, which was located in a cul-de-sac off Freshfield Road (as highlighted in Fig. 2.9). Out of the 16 woodland trap sites, four did not capture any squirrels, which were all located in the southern woodlands. The maximum number of captures at a single woodland trap site was 57, which was located in the northern woodlands (as highlighted in Fig. 2.9) immediately adjacent to 'Squirrel Walk' in a patch of mixed beech and pine. The northern woodlands accounted for 78.32% of all captures, whereas the urban gardens accounted for 19.23% and the southern woodlands only 2.45% of captures.

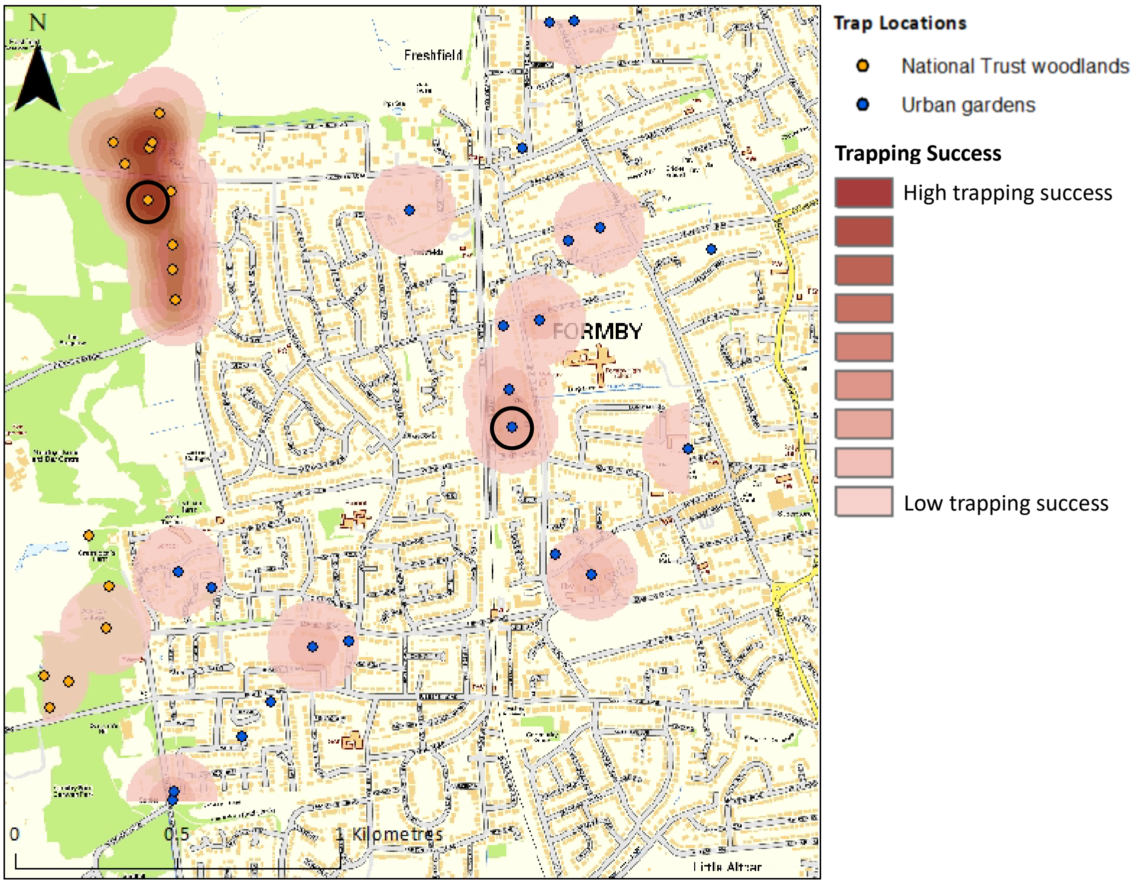


Figure 2.9. Trapping success in the National Trust woodlands and urban gardens, with trap locations highlighted as points. The trap sites with the highest number of captures in the woodlands and urban gardens are circled (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

2.3.1.1. Live-Capture Trapping

In 2018, 132 red squirrels were live-capture trapped, with 64 in Phase 1 and 68 in Phase 2. During Phase 1, there were 51 new captures, eight recaptures, and five individuals that escaped during handling. During Phase 2, there were 42 new captures and 26 recaptures, including one juvenile that was processed but not PIT tagged due to concerns regarding its high parasite burden. In accordance with the Natural England licence, 75 individuals were PIT tagged, 51 in Phase 1 and 24 in Phase 2, after which any new captures were immediately released at the site of capture.

In 2019, 66 red squirrels were live-capture trapped during Phase 1, of which 49 were new captures and 11 were recaptures. In addition to the 60 individuals that were processed, two escaped during handling and four were immediately released at the site of capture without processing as they were in the trap with another individual and there were concerns about over-stressing them during processing.

2.3.1.2. Population Demographics

Of the 125 individuals that were processed, 28% were trapped in the urban gardens and 72% in the woodlands. Overall, there were 59 males, of which 20.3% were in breeding condition and all were captured during Phase 1 of trapping, and 66 females, of which 53% were in breeding condition (Fig. 2.10).

There was no statistically significant association between the location and either sex (Chi-Square Test of Independence: $X^2 = 1.01$, df = 1, $p = 0.31$) or breeding condition (Chi-Square Test of Independence: $X^2 = 0.57$, df = 1, $p = 0.45$). However, there was a statistically significant association between sex and breeding condition (Chi-Square Test of Independence: $X^2 = 14.19$, df = 1, $p = 0.0002$). A *post hoc* pairwise comparison with false discovery rate (FDR) correction (Benjamini & Hochberg 1995) found that females were more likely to be in breeding condition than males, whereas males were more likely to be non-breeding than in breeding condition.

Of the 125 individuals that were processed, 85.6% were adults, 4.8% were sub-adults, and 9.6% were juveniles (Fig. 2.11). There was no statistically significant association between age and sex (Chi-Square Test of Independence: $X^2 = 1.62$, df = 1, $p = 0.20$). However, there was a statistically significant association between age and location (Chi-Square Test of Independence: $X^2 = 11.44$, df = 1, $p = 0.0007$). A *post hoc* pairwise comparison with FDR correction found that adults were more likely to be located in the woodlands compared to sub-adults/juveniles and compared to the urban gardens.

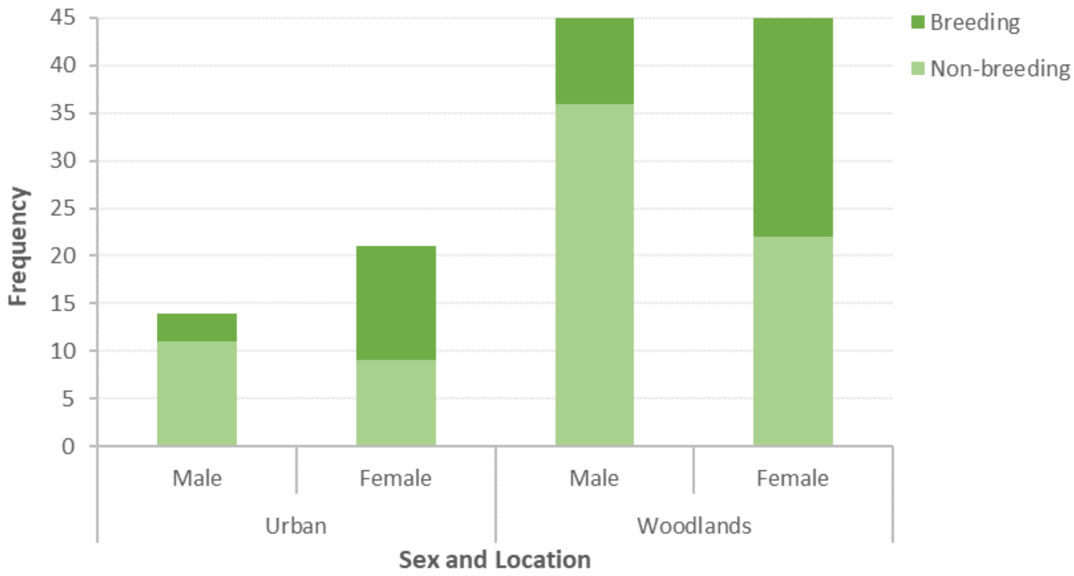


Figure 2.10. Frequencies of breeding and non-breeding males and females, which were live-capture trapped in the urban gardens and woodlands.

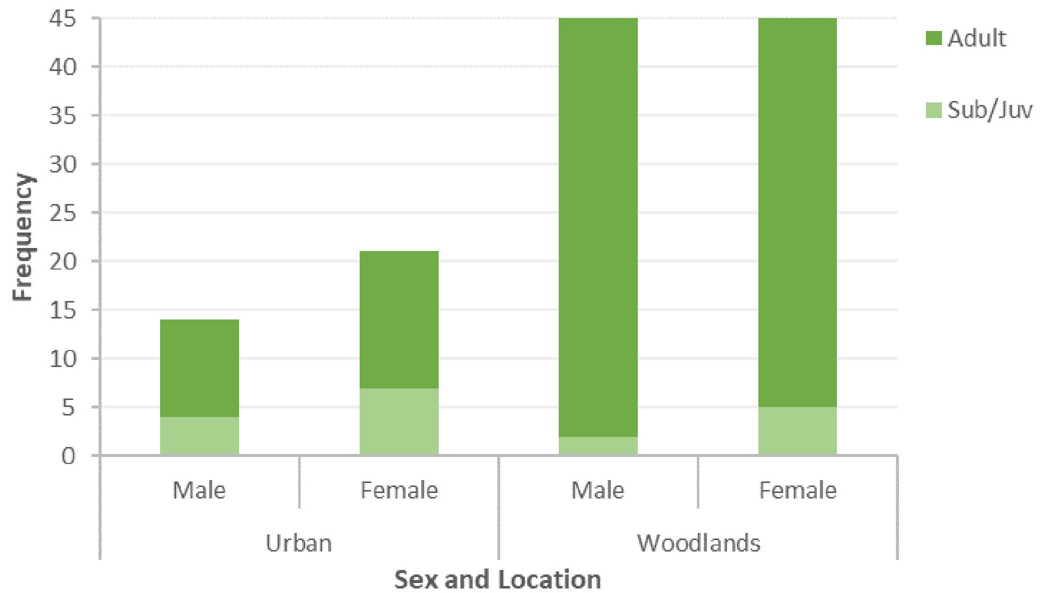


Figure 2.11. Frequencies of adult and sub-adult/juvenile males and females, which were live-capture trapped in the urban gardens and woodlands.

Excluding sub-adults/juveniles, there was no significant difference in the shin length (as an indicator of body length; t = 1.16, df = 101.63, p = 0.25) between adult males ($\bar{x} \pm$ SD = 708.89 ± 31.99 mm, n = 53) and females ($\bar{x} \pm$ SD = 715.58 ± 27.43 mm, n = 53). There was also no significant difference in the shin length (t = 0.06, df = 33.65, p = 0.95) of individuals from the woodlands ($\bar{x} \pm$ SD = 712.13 ± 28.91 mm, n = 82) and the urban area ($\bar{x} \pm$ SD = 712.58 ± 33.52 mm, n = 24).

In addition, excluding breeding females and sub-adults/juveniles, there was no significant difference between adult male ($\bar{x} \pm SD = 347.45 \pm 27.45$ g, $n = 53$) and female ($\bar{x} \pm SD = 359.21 \pm 24.57$ g, $n = 19$) body mass ($t = 1.73$, $df = 35.27$, $p = 0.09$). However, there was a significant difference in adult body mass (again, excluding breeding females and sub-adults/juveniles) between locations ($t = -3.88$, $df = 15.25$, $p = 0.001$), with heavier individuals located in the woodlands ($\bar{x} \pm SD = 355.75 \pm 24.37$ g, $n = 60$) compared to those located in the urban gardens ($\bar{x} \pm SD = 324.58 \pm 25.62$ g, $n = 12$).

Three individuals were not assessed for parasite burden when they were live-capture trapped ($n = 122$). There was a statistically significant association of parasite burden with age (Fisher's Exact test: $p = 0.00014$). A *post hoc* pairwise comparison with FDR correction found that adults were more likely to have low/very low parasite burdens compared with sub-adults/juveniles, whilst a higher proportion of sub-adults/juveniles had high parasite burdens compared with adults (33% compared to 1%; Fig. 2.12). However, there was no statistically significant association between parasite burden and location (Fisher's Exact test: $p = 0.76$).

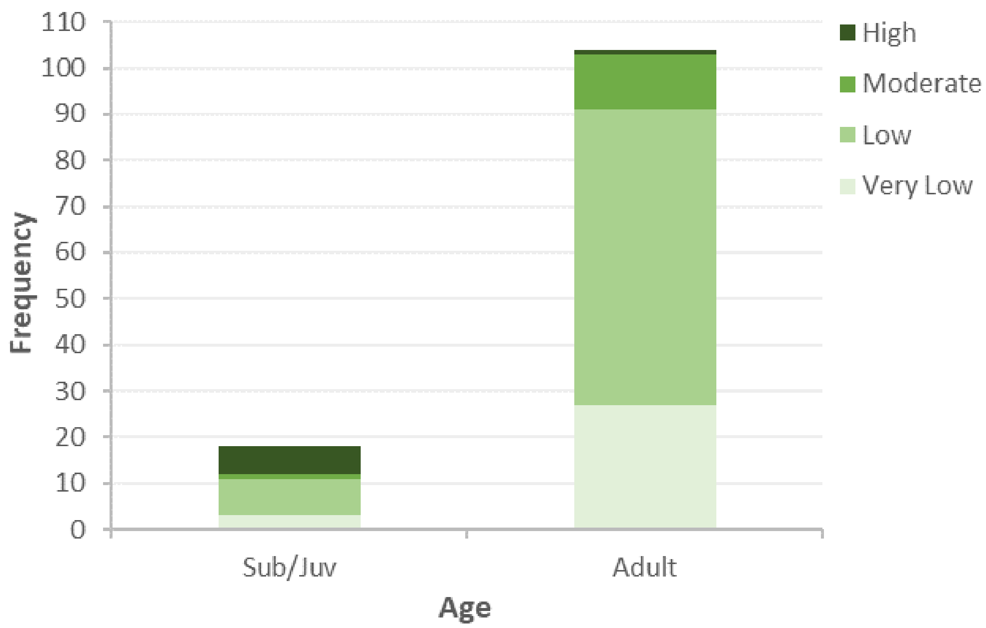


Figure 2.12. Frequencies of sub-adults/juveniles and adults in each parasite burden category.

2.3.2. Population Distribution

Of the 1454 squirrel sightings reported in total from 2015 to 2017, 33.5% (n = 487) were of red squirrels from within Formby, of which 70.8% (*n* = 345) were of live squirrels and the remaining reports (*n* = 142) were of dead squirrels. Kernel density analysis shows that the highest number of red squirrel sightings were reported along Kirklake Road in the south-western area of the town (box A in Fig. 2.13). There is a second cluster of sightings along Freshfield Road, primarily in the vicinity of Formby High School, which runs parallel with the railway line through the centre of Formby (box B). There appears to be occasional sightings of red squirrels throughout most of Formby, across to the bypass on the eastern edge of the town. However, there are some clear areas where no sightings were reported from 2015 to 2017; for example, there is a noticeable gap adjacent to the woodlands (box C) even though it is surrounded by high numbers of sightings on Freshfield Road to the east and Kirklake Road to the south, as well as sightings along Victoria Road to the north and in the woodlands. It is also worth noting that there appears to be a cluster of sightings on 'Squirrel Walk' (box D) but a lack of sightings in other parts of the woodlands, particularly north of Victoria Road, immediately south of Blundell Avenue, and in the far south-western corner of the woodlands.

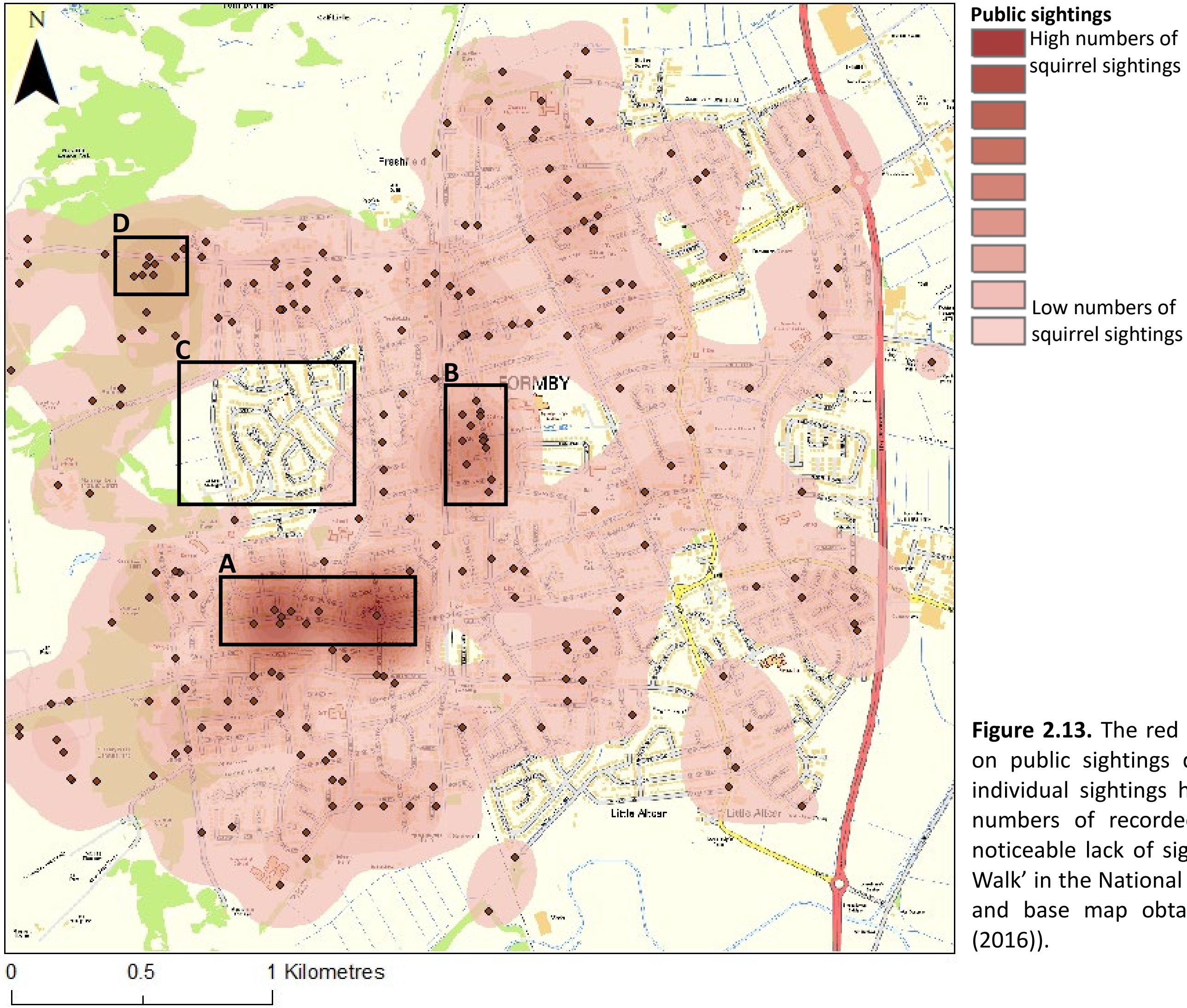


Figure 2.13. The red squirrel population distribution in the study site based on public sightings data recorded from 2015 to 2017 by the LWT, with individual sightings highlighted as points. The two areas with the highest numbers of recorded sightings are marked by boxes A and B, with a noticeable lack of sightings highlighted by box C. Box D highlights 'Squirrel Walk' in the National Trust woodlands (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

2.3.3. Population Abundance and Density

Although the numbers of squirrels sighted during the distance transects have been recorded since 2002, recordings of the distance estimates were more sporadic up until 2012. Hence, the population indices could be calculated from 2002 – 2019 but the annual population abundance and density, based on the distance data, could only be calculated from 2012 – 2019.

The estimated area of the woodland surveyed via the transects was 98.47 ha. The total number of recorded observations varied between 179 and 341 for each year. Transect 3: Asparagus Fields had the highest mean number of squirrels recorded, whereas transect 1: Victoria Road had the lowest (Fig. 2.14).

The estimated red squirrel population abundance in the National Trust woodlands was found to increase from 213 in 2012 to a peak of 605 in 2017, before declining to 244 in 2019 (Fig. 2.15). The population density was estimated to be 2.16 individuals/ha in 2012 and 6.14 individuals/ha at the peak in 2017.

From the population indices, the red squirrel population in the National Trust woodlands was found to increase by approximately 50% from 2002 to a peak in 2006, prior to a SQPV outbreak in 2007/08 where the population declined by approximately 75% (Fig. 2.16). The population then gradually recovered between 2009 and 2017, returning to almost the same level as 2006, before another SQPV outbreak in 2018/19 that reduced the population again by approximately 50%.

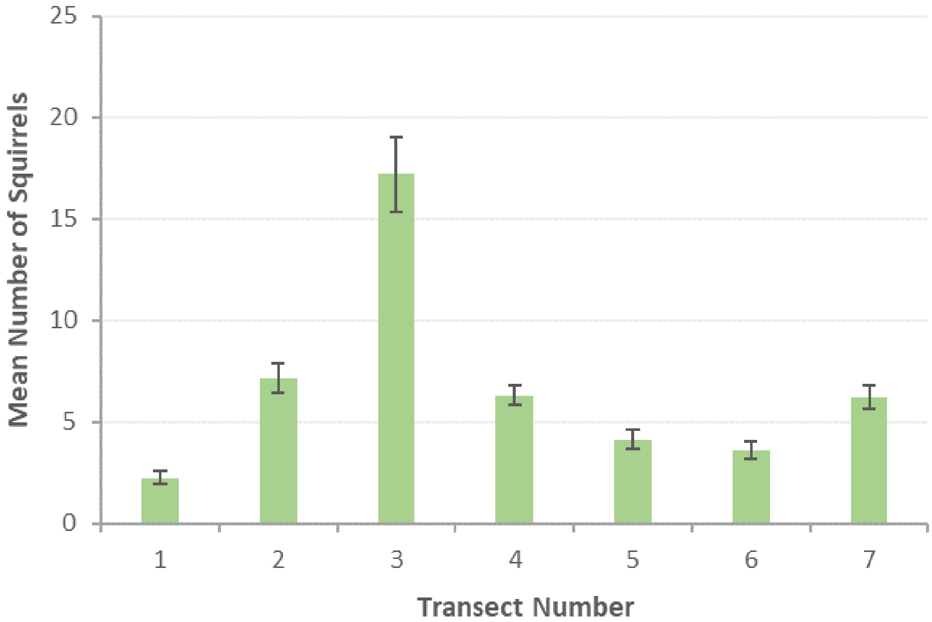


Figure 2.14. Mean number (± SE) of red squirrels recorded on each distance transect in the National Trust woodlands, across both seasons (spring and autumn) and all years (2012 – 2019).

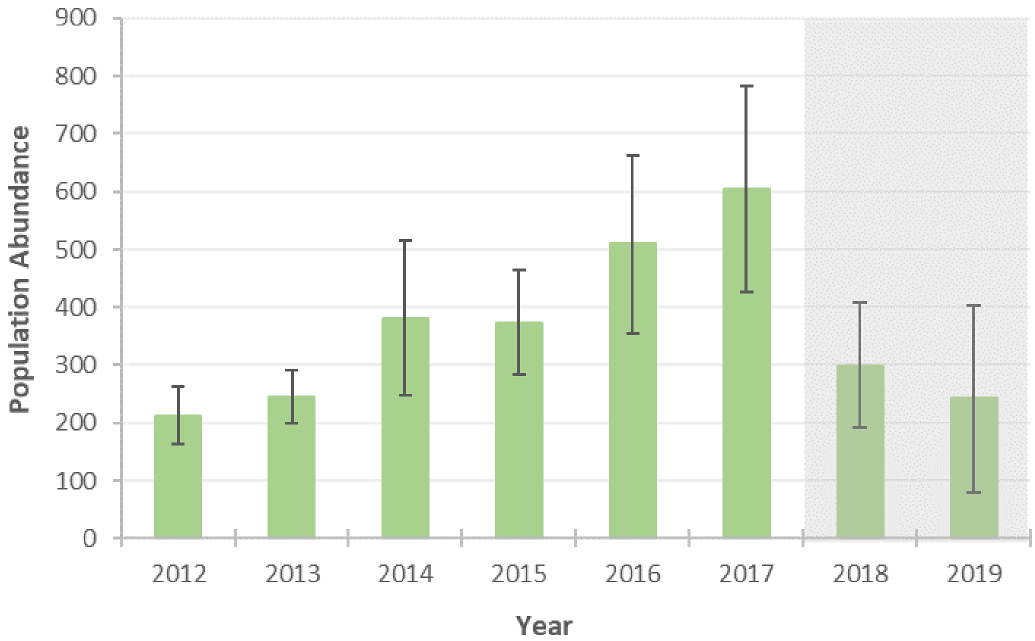


Figure 2.15. Estimated red squirrel population abundance (± SE) in the National Trust woodlands in Formby from 2012 to 2019, where the shaded area represents the period of the SQPV outbreak.

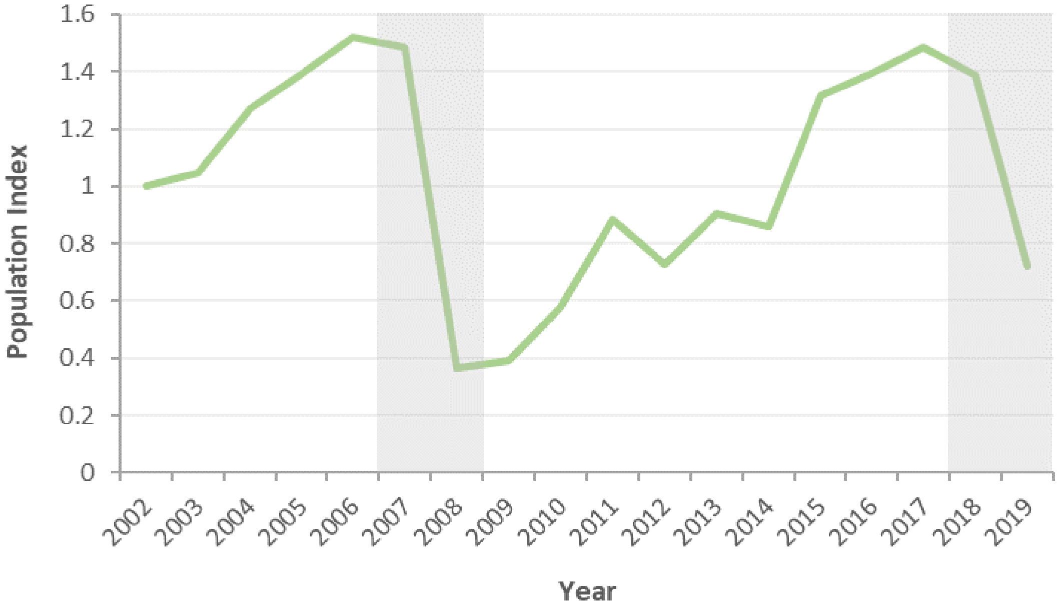


Figure 2.16. Red squirrel population indices in the National Trust woodlands in Formby from 2002 to 2019, where the shaded areas represent the periods of SQPV outbreaks.

2.4. DISCUSSION

Key findings from each topic of the results section (trapping success, population demographics, population distribution, and population abundance and density) have been summarised below (Table 2.2).

Table 2.2. Summary of key findings from Chapter Two.

Topic	Key Findings
Trapping success	• Overall trapping success was 32.03 captures/100 trap days (northern woodlands: 69.35 captures/100 trap days, southern woodlands: 9.59 captures/100 trap days), urban gardens: 11.07 captures/100 trap days) • Only 50% of urban trap sites captured any squirrels, with the maximum number of captures at a single urban trap site being 12 squirrels (in Freshfield Road) • 25% of woodland trap sites did not capture any squirrels, all of which were in southern woodlands • Maximum number of captures at a single woodland trap site was 57 squirrels (in northern woodlands next to 'Squirrel Walk')
Population demographics	• Of 125 processed individuals, 72% were trapped in woodlands and 28% in urban gardens • 47.2% were males (of which 20.3% were in breeding

	condition) and 52.8% were females (of which 53% were in breeding condition) • 85.6% were adults, 4.8% were sub-adults, and 9.6% were juveniles • No significant association between location and sex • No significant association between location and breeding condition • Significantly more females were in breeding condition than males ($p < 0.001$) • No significant association between age and sex • Significantly more adults were located in the woodlands than sub-adults/juveniles ($p < 0.001$) • No significant difference in body length between adult males and females • No significant difference in body length between adults from woodlands and those from urban gardens • No significant difference in body mass between adult males and females • Individuals from woodlands were significantly heavier than those from urban gardens ($p < 0.001$) • Significantly more adults had low/very low parasite burdens ($p < 0.001$)
Population distribution	• Highest numbers of sightings were reported along Kirklake Road and Freshfield Road, although there are occasional sightings throughout most of Formby • Noticeable gaps in reported sightings in some locations, including in residential areas adjacent to the woodlands and in parts of the woodlands themselves
Population abundance and density	• Transect 3: Asparagus Fields had the highest mean number of squirrels recorded • Population abundance increased from 213 in 2012 to a peak of 605 in 2017, before declining to 244 in 2019 • Population density was estimated to be 2.16 individuals/ha in 2012 and 6.14 individuals/ha in 2017 • Population indices showed an increase of ~50% from 2002 to 2006, followed by a ~75% decline coinciding with a SQPV outbreak in 2007/08, then a gradual recovery from 2009 to 2017, before another decline of ~50% coinciding with a second SQPV outbreak in 2018/19

2.4.1. Trapping Success

Although the published literature states that trapping success of tree squirrels is generally high (Bertolino et al. 2009), it is difficult to compare between studies as a standardised format for reporting trapping success (e.g. number of captures per 100 trap days) is often not included. Therefore, it is difficult to establish whether the trapping success reported in this study is particularly high or comparable to other research studies. However, as discussed in the Methods, trapping effort in the northern woodland had to be reduced as it was taking too long to process all the captured squirrels within the pre-determined time limit, which suggests that trapping success in this area was very high. It would be beneficial to report trapping success in future publications to allow for comparisons between studies, including evaluating the impact of any methodological variations in trapping on the number of captures, as trapping success can be useful as an indicator of population density for monitoring wildlife. In addition, optimising trapping success is important for ensuring the accuracy of any data collected for further analyses.

Trapping success was substantially higher in the northern woodland compared to the southern woodland and urban gardens, which was likely to be due to a higher abundance of squirrels in the vicinity of 'Squirrel Walk' as highlighted by transect 3: Asparagus Fields of the distance transect surveys. In addition, the most suitable trap sites could be selected in the woodlands, as there were more options to locate traps where they could be hidden from public view. The most successful trap site, both in the woodlands and overall, was located immediately adjacent to 'Squirrel Walk' in a patch of mixed beech and pine, which was an area highly likely to be occupied by squirrels due to the availability of both supplemental and natural food resources.

The lower trapping success in the southern woodland compared to the northern woodland was likely due to a combination of factors, including a lower abundance of squirrels (as highlighted by transect 5: Lifeboat Road compared to transect 3: Asparagus Fields), the initial use of the Elgeeco traps instead of ground traps, the area being more open and accessible to the public, and the capture of a suspected SQPV squirrel. The Elgeeco traps that were trialled in the southern woodland were unfortunately unsuccessful in trapping any red squirrels in this study, despite being used successfully to trap grey squirrels in urban areas of Scotland. The treadle needed to be relatively stiff so that the trap did not trigger when being lifted onto the bracket, but it appeared that the red squirrels were either too light to trigger the trap or were avoiding standing on the treadle. Due to the southern woodland being more open and accessible, trap sites were very constrained to locations hidden from the public, particularly after switching to the ground traps, so only four trap sites could be used compared to ten trap sites in the northern woodland. Following the capture of the suspected SQPV squirrel in May 2019, no further trapping could be conducted in the southern woodland to reduce the risk of spreading SQPV, resulting in a reduced trapping effort.

Trapping success in the urban gardens was similar to the southern woodland, despite an increased trapping effort in the former. In addition, half of the urban traps did not capture any squirrels, despite the homeowners reportedly seeing squirrels in their gardens. This may be due to a lower abundance of squirrels, particularly in comparison with the northern woodland, although this is difficult to determine as no distance transect surveys have been conducted in the urban area. It may also have been due to the widespread availability of supplemental food, so the squirrels may have instead used any feeders in adjacent gardens rather than entering the trap whereas the bait may have been considered a higher value food item in the woodlands, particularly if natural food sources were scarce as they would have been during the summer months.

The successful urban trap sites appear to align with the findings of the kernel density analysis for the population distribution and the highest numbers of reported squirrel sightings, which suggests that there may be a higher abundance of squirrels in these locations or that these areas may be more heavily exploited by the squirrels inhabiting the town (e.g. due to the presence of supplemental or natural food resources, or habitat corridors for movement).

2.4.2. Population Demographics

2.4.2.1. Age, Sex, and Breeding Condition

There is a tendency for equal sex ratios across most sexually reproducing species according to Fisher's principle, so the approximately 1:1 ratio in this study was expected. There were also no significant associations between location and sex, location and breeding condition, and age and sex, which suggests that the population is generally equally balanced in these demographics across the study site.

Overall there were significantly more females in breeding condition than males, which may have been due to the timings of the trapping phases being in May/June (Phase 1) and July/August (Phase 2). Shuttleworth (1996) found that the majority of males with large and visible testes were trapped between February and June after which the mass of the testes declines during July and August (Bosch & Lurz 2012), which may be reflected in the fact that all the males identified as in breeding condition were captured during Phase 1 of trapping. On the other hand, females can be sexually active from late December through to August (Bosch & Lurz 2012) and so were detected in breeding condition during both phases of trapping. However, Phase 2 of trapping was targeted to specific locations and so captured far fewer individuals than in Phase 1, which may be why no breeding males were captured during this phase. Furthermore, males just entering breeding condition can have very small testes and so may have been misidentified as non-breeding.

Although only a small number of juveniles were trapped, a greater proportion were located in the urban gardens compared to the woodlands. The lower overall trapping success for juveniles may be because they do not stray far from the drey during exploratory forays, so a trap may need to be in the vicinity of a drey in order to capture the exploring juveniles. In addition, once weaned, juveniles undertake natal dispersal (Wauters & Dhondt 1993, Fey et al. 2016, Hämäläinen et al. 2018, 2020), so they may be less likely to encounter a trap by chance whilst dispersing. For instance, the most successful urban trap, which was in a cul-de-sac off Freshfield Road, predominantly captured juveniles and it was suspected that the individuals were littermates from a nearby drey. It may be that the juveniles are being driven out of the higher quality woodland habitat into the urban area by dominant adults, as significantly more adults were located in the woodlands. Alternatively, as discussed by Hämäläinen et al. (2020), decision-making by dispersing juveniles may be driven by food availability and conspecific density, and so more juveniles may be dispersing into the urban area with predictable resource availability and a lower population density compared with the woodlands.

Beliniak et al. (2022) also found a higher number of juveniles in an urban park compared with a peri-urban forest in Warsaw, Poland. Furthermore, they found that the forest squirrels were heavier than those from the urban park, similarly to this study (which will be further discussed in section 2.4.2.2), and there was a higher number of breeding females in the urban park compared with the forest. Beliniak et al. (2022) suggested that their findings reflect a higher reproductive rate in the urban park, potentially due to the abundance of supplemental food, allowing the squirrels to breed without the typical required fat reserves (as female red squirrels can only come into oestrus once they attain a minimum body mass of approximately 300 g; Wauters & Dhondt 1989a), which may also be the case in Formby.

2.4.2.2. Body Length and Mass

Similarly to the published literature (e.g. Wauters & Dhondt 1989b, Wauters et al. 2000, 2007, Reher et al. 2016), this study found no sexual dimorphism in adult body length or mass. However, despite breeding females being excluded from the analyses, mean female body mass was still >10 g more than mean male body mass. As this study was conducted during the summer and therefore the breeding season, it may have been that some females were in the early stages of pregnancy but not yet showing outward signs (i.e. visible nipples), and so were incorrectly classified as non-breeding and included in the analyses. Alternatively, Wauters et al. (2007) also found that females averaged

>10 g heavier than males in one of their study sites in the central Alps, which may suggest possible sexual dimorphism in some populations.

Adult squirrels inhabiting the peri-urban woodlands were found to be significantly heavier than those in the town, although there was no significant difference in body lengths between the two locations. This suggests that the woodland squirrels had greater fat reserves, rather than being larger in terms of body size, than the urban squirrels. These findings contrast with the Thomas et al. (2018) study that similarly compared non-breeding red squirrels inhabiting the central urban area of Hamburg city, Germany, and the Ohlsdorf Cemetery in the city outskirts, and found no difference in mean body mass. However, the findings support the Beliniak et al. (2022) study that similarly compared red squirrels in an urban park with a peri-urban forest in Warsaw, Poland, and found that the forest squirrels were heavier. These differences may be due to the degree of supplemental feeding (as this study and the Thomas et al. (2018) study had supplemental feeding in both the urban and peri-urban areas, whereas the Beliniak et al. (2022) study appeared to only have supplemental feeding in the urban park) and/or the availability and quality of natural food resources.

Therefore, in addition to comparative research between urban and rural wildlife populations, it would also be beneficial to further investigate the potential impact of different degrees of urbanisation on wildlife populations (e.g. comparisons between the urban core area, isolated woodlands embedded within the urban landscape, and peri-urban woodlands on the city outskirts). These studies suggest there are differences between squirrels inhabiting urban areas compared with peri-urban woodlands, which may have implications for conservation management. For example, in this study, the squirrels inhabiting the woodlands may have more widespread and reliable access to both natural and supplemental food resources, resulting in a higher body mass, whereas individuals inhabiting the urban area may be more restricted to only supplemental food sources, which may not provide an entirely suitable diet and therefore results in a lower body mass. In other words, the woodland squirrels may have the ‘best of both worlds’, in terms of occupying their preferred habitat whilst still maintaining access to the benefits that the urban area has to offer. On the other hand, Beliniak et al. (2022) suggest that the lower body mass of urban squirrels is a result of the reduced requirement to store fat reserves due to abundant and reliable supplemental food resources.

When comparing this study and the Thomas et al. (2018) study with research conducted in rural woodlands (e.g. Wauters et al. 2000, 2007, Gurnell et al. 2004), adult red squirrels inhabiting peri-urban/urban habitats appear to be heavier (351 – 372.5 g) than those in coniferous or mixed rural woodlands (304 – 326 g). This supports the findings of other studies, across several different mammalian species, that have found that urban individuals often have greater body masses than their rural counterparts due to the reliability of food resources (McCleery 2010), which may consequently impact, for instance, breeding success and over-winter survival.

2.4.2.3. Parasite Burden

There were no individuals without any fleas, lice, or harvest mites, which demonstrates that ecto-parasites are widespread throughout the population. It may be that some parasites should be expected in a wild animal population or that ecto-parasites are able to spread more easily amongst the high-density population, particularly with individuals sharing supplemental feeders. This could be determined through comparison with a lower density rural population without access to supplemental feeders, however most published studies on parasite burdens in red squirrel populations have been determined by necropsies on dead animals (e.g. LaRose et al. 2010, Simpson et al. 2013). As ecto-parasites will tend to abandon a deceased host, this does not provide an accurate assessment of parasite burden in a living population.

The greater proportion of juveniles with high parasite burdens may be due to the heavy parasite loads present in the dreys, as the kittens remain in the drey with exploratory forays outside from six weeks old until they are weaned at approximately ten weeks old (Bosch & Lurz 2012). It is likely that the juveniles being trapped were newly weaned and therefore still had high parasite loads built up from occupying the drey. The few adults with a high/moderate parasite burden appeared to have other health issues; for example, one individual had a large abscess at the base of its tail, most likely as a result of a bite from another individual. As highlighted by LaRose et al. (2010), there is an association between poor body condition and a higher ecto-parasite burden, although it is not clear whether the poor body condition is as a result of the infestation or whether a weakened immune system due to injury/illness allows the infestation to become established.

2.4.3. Population Distribution

Almost half of the reported sightings were of grey squirrels, which highlights the public's enthusiasm for protecting the red squirrels as these sightings help to inform the LWT conservation project's grey squirrel control strategy. For example, if a grey squirrel is sighted in a private garden, the reporter is typically contacted to request that they join the project's trap loan scheme (i.e. where they host and monitor a live-capture trap to attempt to remove the grey squirrel, whilst being able to immediately release any red squirrels captured). In addition, if a grey squirrel is sighted in a woodland, these areas can then be targeted for control either by volunteers or project staff. This demonstrates the importance of public sightings for conservation management, by allowing for a rapid and proactive response to any reports of grey squirrels, as well as providing informative data for monitoring long-term population distributions for both species.

The fact that almost two-thirds of the overall reported red squirrel sightings were from within Formby most likely reflects the town being situated within the red-only core of the stronghold (see Fig. 2.2 in section 2.2.1.1), so there are likely to be higher densities of red squirrels compared to the wider stronghold and therefore the most sightings. Residents may also be more engaged with the LWT red squirrel conservation project, particularly with the well-known National Trust reserve being located immediately adjacent to the town, and so may be more likely to report any sightings. From the other perspective, this may suggest that the red squirrels are not dispersing out of the core area into the wider stronghold, or that the public within the wider stronghold are not as engaged or aware that they should be reporting sightings. Both may have implications for conservation efforts, as the LWT project aims for the red squirrel population to recover across North Merseyside and West Lancashire, not just to maintain the current core area (The Lancashire Wildlife Trust 2020).

The clear cluster of sightings around 'Squirrel Walk' in the National Trust woodlands is to be expected as it is the busiest area (being adjacent to the Victoria Road car park and the main thoroughfare to the beach), it is specifically visited by people wishing to see the red squirrels, and supplemental feeding occurred here until 2018 so it is likely that there was a higher density of squirrels in this area of the woodland. However, there are fewer sightings than in comparison with some areas of the town, such as Kirklake Road and Freshfield Road, despite higher numbers of squirrels in the northern woodland compared with the urban gardens as highlighted by the trapping success. In addition, the sparsity or complete lack of sightings elsewhere in the woodland, despite the presence of squirrels as highlighted by the distance transect surveys, may be due to fewer people accessing certain areas of the woodland (*pers. obvs.*), or the perception that the red squirrels are 'common' in the National Trust woodlands and therefore sightings do not need to be reported. Sampling biases in citizen science data, such as these, have already been widely reported and discussed in the published literature (e.g. Dennis & Thomas 2000, Bird et al. 2014, Fraisl et al. 2022), so it is highly likely that the red squirrel distribution has been under-estimated in the woodlands. Therefore, the public sightings data may be more beneficial for assessing the population distribution in the urban area, where it may be difficult to conduct other surveying methods, such as transects, which can be carried out in the woodlands.

The spatial distribution of reported sightings in the urban area may reflect the provision of supplemental food in residential gardens, the availability of natural food resources, and/or the availability of habitat corridors for movement. For example, there is an absence of sightings on the western side of Formby (see box C in Fig. 2.13 in section 2.3.2), despite being immediately adjacent to the woodlands and surrounded by areas with high numbers of sightings, so it could be argued that squirrels are expected to be present here. However, there may be a lack of habitat corridors allowing the squirrels to disperse out of the woodlands, resulting in their absence from this area. Similarly, this may be the reason for more gaps and scarcer numbers of reported sightings on the eastern side of the town. On the other hand, Kirklake Road, which has the highest number of reported sightings (see box A in Fig. 2.13), is lined with mature trees (*pers. obvs.*) that the squirrels may use as corridors for movement.

2.4.4. Population Abundance and Density

Unsurprisingly, the transect immediately adjacent to 'Squirrel Walk' (3: Asparagus Fields) has more than double the mean number of squirrels recorded compared with the other transects, which is most likely due to the supplemental feeding that took place until 2018. This analysis could be expanded to include additional years post-2018 to evaluate the impact of halting the supplemental feeding on 'Squirrel Walk'. Unfortunately, this was not feasible in this study as the distance transect surveys were not conducted in 2020/21 due to COVID-19.

The mean number of squirrels recorded on the other transects are more comparable to each other and any variations may either be due to access to supplemental feeding or the habitat quality. For example, transects 5 (Lifeboat Road) and 6 (Shorrocks Hill) were conducted in mature coniferous-dominated woodland, whereas transect 7 (Ravenmeols) was conducted in mixed woodland where there is likely to be higher quality natural food resources (e.g. beech nuts). These areas in the southern woodland have no supplemental feeding, but the squirrels would have access to feeders in adjacent urban gardens. The availability of supplemental and natural food resources across the study site will be investigated in more detail in Chapter Three.

Following the previous SQPV outbreak in 2007/08, which reduced the population by over 80% (Chantrey et al. 2014), the population appears to recover and return to pre-outbreak levels by 2017. At this point, another SQPV outbreak occurred in 2018/19 that reduced the population by approximately 50%. This suggests that there may be a population density threshold at which the population may be prone to future disease outbreaks. The second SQPV outbreak appears to be less severe than the first, which may be due to the rapid response of the LWT and local people following their experience of the 2007/08 outbreak. However, it would be interesting to repeat the Chantrey et al. (2014) study to investigate whether there is now a higher proportion of SQPV-resistant individuals in the population (compared to the 8% from their study of the 2007/08 outbreak) that may have helped to reduce the impact of the 2018/19 outbreak.

Red squirrel population densities are typically higher in more urbanised habitats compared to rural, including when comparing city centres with peri-urban greenspaces (Babińska-Werka & Żółw 2008, Kopij 2014, Jokimäki et al. 2017, Krauze-Gryz et al. 2021). This is further supported by the high population densities found in this study when compared with rural coniferous and deciduous woodlands (e.g. Lurz et al. 1995, Verbeylen et al. 2003a, Wauters et al. 2004). However, population densities could not be compared within the study site between the peri-urban woodland and the urban area, as the distance transect surveys were not conducted in the latter.

As urban wildlife ecology is a relatively new area of research and there are limited published studies of urban red squirrel populations, it is difficult to compare population densities between different urban sites. However it seems that the population densities calculated in this study are generally higher, particularly at the peak in 2017, compared with another urban population in Łazienki Park in Warsaw, Poland, where the density was estimated to be approximately 2 individuals/ha (Krauze-Gryz et al. 2021, Beliniak et al. 2022). Both the National Trust reserve in Formby and Łazienki Park in Warsaw are similar in size (approximately 70 ha), have (or previously had) supplemental feeding, and have high numbers of visitors, although the National Trust reserve has around 250,000 visitors compared to 2 million in Łazienki Park (Shuttleworth 2001, Krauze-Gryz et al. 2021). However there are various differences between the study sites, for example Łazienki Park is predominately deciduous woodland whereas the National Trust reserve is largely coniferous woodland with some mixed-deciduous stands, which makes it difficult to identify why the population density in this study is higher compared with the Krauze-Gryz et al. (2021) and Beliniak et al. (2022) studies. It would be beneficial to compare population densities between other urban red squirrel populations, as more research is published.

From the models used for the distance transect analysis to determine the annual population abundance and density, the 'season + observer' covariate often had the lowest AIC or a very similar AIC (with a difference of < 2) to the selected simplest models. As the volunteers were asked to estimate the perpendicular distance to the sighted squirrels, there will be differences in their ability to accurately estimate these distances. Therefore, it would be beneficial for the volunteers to use a rangefinder for future distance transect surveys, to improve the accuracy of the data and the subsequent population estimates. Furthermore, it is understandable that there are seasonal differences, as the spring transects typically take place in March when the population is at its lowest following winter mortality and prior to the spring breeding season, whilst the autumn transects take place when the population is at its highest following the breeding season (Wauters & Lens 1995, Gurnell et al. 2008).

2.5. CONCLUSION

These findings indicate that the study site supports a high population density of red squirrels (between 2 – 6 individuals/ha), most likely due to widespread and reliable food resources, both supplemental and natural, throughout Formby. There is a particularly high density around 'Squirrel Walk' in the northern woodland due to historic supplemental feeding alongside natural food resources, but this may change following the cessation of supplemental feeding in 2018 due to the SQPV outbreak. There is also evidence for higher numbers of red squirrels in some parts of the urban area (Kirklake Road and Freshfield Road), which may be due to the availability of habitat corridors for movement and/or the availability of supplemental food in residential gardens. However, such high levels of food availability appear to be discouraging the red squirrels from dispersing out of Formby into the wider stronghold, which may be also exacerbated by the presence of the bypass acting as a barrier to dispersal, and leaves the population vulnerable to disease outbreaks if the proposed density threshold is reached.

It is currently unclear whether the higher quality habitat is the urban area, with abundant and reliable supplemental food resources allowing for higher reproductive success without the need for higher body masses, or the peri-urban woodlands, which are acting as a source population with less dominant adults and juveniles dispersing out into the urban area. The impacts of supplemental feeding, and habitat availability and quality on the red squirrel population and their spatial ecology will be further investigated in Chapters Three and Four, incorporating the data and findings from this chapter.

3.0. Chapter Three: Resources and Risks for Red Squirrels in an Urban Environment

Chapter One identified that human-provided supplemental food, the availability and quality of greenspaces with natural food sources, and mortality threats are important topics within urban ecology research of red squirrels. This chapter further investigates these potential resources and risks for the red squirrel population in the study site of Formby, Merseyside, incorporating the findings from Chapter Two. The results from both Chapters Two and Three will then be incorporated into the data analyses for Chapter Four.

3.1. INTRODUCTION

3.1.1. Urban Ecosystems

With 68% of the global human population projected to inhabit urban areas by 2050 (United Nations 2019), the subsequent urban growth and intensification substantially alters the available habitat for wildlife, as the biotic and abiotic characteristics of the urban ecosystem can be considerably different to the natural landscape (Gehrt 2010). For example, the heat island effect can be significant in larger cities, with temperatures in the inner urban core being 6 – 12 ^{o}C higher compared with the surrounding landscape (Kaye et al. 2006), along with other microclimatic changes such as decreased mean wind speeds, higher levels of precipitation, and more cloud cover (Berry 2008). This can impact, for instance, vegetation phenology with plant species flowering earlier and abscission occurring later (Neil & Wu 2006), which can affect the wildlife relying on those species for food or nesting sites. There have also been numerous studies published regarding light pollution (e.g. Longcore & Rich 2004, Poot et al. 2008, Haddock et al. 2019), anthropogenic noise pollution (e.g. Warren et al. 2006, Duquette et al. 2021), changes to biogeochemical processes such as hydrological and nutrient cycles (e.g. Groffman et al. 2002, Kaye et al. 2006, McGrane 2016), and other impacts of urbanisation on wildlife.

Increasing urbanisation typically results in a decline in mammalian diversity and certain species, including habitat specialists and species with large home range requirements, appear to be more sensitive (McCleery 2010). However smaller-sized, omnivorous, and generalist species, like the red squirrel, have been shown to be able to adapt to the urban environment and exploit the available resources.

3.1.1.1. Connection with Nature in Urban Areas

There is increasing evidence that contact with nature and wildlife are beneficial for human physical and mental well-being, as well as environmental awareness and education (e.g. Otto & Pensini 2017, Richardson & McEwan 2018). This is particularly important in urban areas, where access to greenspaces may be more limited and there are concerns of a growing disconnection from the natural world, which can result in public disinvestment in the natural world as they no longer view nature as valuable or relevant to their lives (Miller 2005). Therefore, there is increasing interest in improving urban green infrastructure and its natural capital that would benefit both human and wildlife inhabitants (Alvey 2006, Goddard et al. 2010). Many environmental action plans now include the development of urban areas (e.g. DEFRA 2018). Charismatic urban-adaptable species, such as red squirrels, may act as a flagship species and encourage conservation efforts by inspiring an appreciation through positive encounters with the public (Cypher et al. 2010).

3.1.2. Behavioural Adaptations of Urban Red Squirrels

Urban red squirrels have been shown to assess risk and habituate to human disturbance (Uchida et al. 2016, 2017, 2019) most likely as a result of repeated non-lethal exposure to people (McCleery 2009), which allows them to spend more time foraging rather than fleeing unnecessarily (Sol et al. 2013). It has been observed that some urban red squirrels will even approach people to beg for food (Uchida et al. 2016, Dagny et al. 2021). This appears to be linked to a variation in individual personalities, with those of a bolder temperament spending more time on the ground and approaching people for food, whereas those of a more shy temperament were more arboreal and more likely to react to people with an alert or escape response (Dagny et al. 2021).

Other studies have found that urban individuals differ in their temporal activity patterns and overall activity levels compared to more rural conspecifics, spending less time active and with activity onset later in the day (Thomas et al. 2018, Beliniak et al. 2021). This is likely due to the widespread availability and reliability of supplemental food resources in the urban environment, either allowing for a reduced foraging effort (Turner et al. 2017, Thomas et al. 2018) or due to shifting their activity patterns to coincide with higher visitor numbers with associated food provisioning to the urban parks (Beliniak et al. 2021). In addition, urban individuals have demonstrated dietary plasticity compared to rural conspecifics, allowing them to exploit a wider variety of food sources, including anthropogenic food items such as biscuits, and shift their diet in response to low availability of natural food sources (Wist et al. 2022). This behavioural flexibility is a crucial trait for urban-adaptive species to successfully exploit the available resources and cope with any challenges in the urban environment (Lowry et al. 2013).

3.1.3. Resources and Risks in the Urban Environment

3.1.3.1. Supplemental Feeding

Supplemental feeding of urban red squirrels is widespread throughout their distribution range and research has shown that it can have a significant impact on their behavioural ecology. For instance, supplemental feeding has been shown to alter patterns of space use, with smaller and more overlapping home ranges (Reher et al. 2016, Thomas et al. 2018), and therefore higher population densities (Babińska-Werka & Żółw 2008, Kopij 2014, Jokimäki et al. 2017). In addition, the availability and reliability of supplemental food allows urban individuals to maintain stable body masses above the minimum mass required for oestrus throughout the year (Wauters & Dhondt 1989a, Turner et al. 2017), leading to higher reproductive rates and reduced overwinter mortality. Red squirrels have shown to have an awareness of and be attracted to newly-installed feeders, which suggests that supplemental feeding could be used in conservation management to encourage individuals to disperse into neighbouring habitats (Starkey & delBarco-Trillo 2019).

On the other hand, supplemental feeding may contribute to population mortality by facilitating the spread of diseases through individuals sharing feeders, particularly in high density populations, and by encouraging increased terrestrial foraging, leading to a higher risk of encountering road traffic (Shuttleworth 2001, Chantrey et al. 2014). Furthermore, excessive consumption of human-provided food, especially peanuts that are low in calcium or anthropogenic food items that are high in sugar, may result in malnutrition or other health impacts, particularly if natural food sources are scarce as is often the case in highly urbanised environments (Bosch & Lurz 2012, Thomas et al. 2018, Wist et al. 2022). In fact, osteopenia has been reported in free-living red squirrels in the UK, with possible suggested causes including malnutrition and calcium deficiency (Garriga et al. 2004). These studies highlight the importance of managing urban greenspaces to ensure the availability of diverse natural food sources and therefore a nutritionally balanced diet for the red squirrels.

3.1.3.2. Habitat Availability and Quality

Studies have shown a positive correlation between red squirrel population density with both the size and quality of urban greenspaces (Verbeylen et al. 2003a, Babińska-Werka & Żółw 2008, Kopij 2014), but also that their dispersal behaviour is not inhibited by built structures within the urban landscape (Fey et al. 2016, Hämäläinen et al. 2018). This suggests that improving the availability and quality of urban greenspaces would be more beneficial for red squirrel populations than increasing connectivity.

A study by Pauleit et al. (2005) assessed changes in land use and cover across residential areas in Merseyside from 1975 to 2000. They found a loss of greenspaces in all 11 study sites and that the amount lost was associated with socio-economic status, with more affluent, low-density areas losing more tree cover, predominantly due to infill densification (i.e. existing gardens being developed into new housing or car parking). Although Formby was not one of the study sites, it is an affluent area with large residential properties and gardens and has similarly been undergoing urban intensification, leading to a loss of greenspaces and tree cover (Fig. 3.1). This ongoing decline in the availability and quality of greenspaces may have implications for the long-term conservation of the red squirrel population, unless appropriately managed.

3.1.3.3. Causes of Mortality

Causes of mortality for mammalian species are often different for rural and urban populations, with disease outbreaks in high density populations and road traffic being identified as common causes of mortality in the latter (McCleery 2010).

3.1.3.3.1. Road Traffic

Arguably roads are one of the most pervasive anthropogenic alterations to the natural landscape, with the expanse of roads increasing worldwide leading to further fragmentation of remaining habitats and the creation of boundaries. This can impact wildlife populations either directly as a source of mortality, or indirectly through fragmentation and genetic isolation (Rondinini & Doncaster 2002, Shepard et al. 2008). It is not only the road itself that needs to be considered, but also the 'road-effect zone' where the ecological impacts extend further outwards (Forman and Deblinger, 2000). For example, Slater (2002) reported that grey squirrel mortality was greatest on roads that were bordered by trees compared to roads with a wider grass verge.

Road traffic appears to be a significant cause of death in red squirrel populations, accounting for between 41.7 to 65% of recorded casualties (Shuttleworth 2001, LaRose et al. 2010, Simpson et al. 2013c, Shuttleworth et al. 2015b, Blackett et al. 2018). Roads in suburban areas may be a higher risk for squirrels compared with more heavily urbanised areas, since interactions with vehicles are more sporadic and unpredictable (Fey et al. 2016). However, it is difficult to determine whether roadkill records are accurate (Shuttleworth 2001). Counts are typically opportunistic, so do not consider injured animals moving away from the road before dying or carcasses being removed (e.g. eaten by

Figure 3.1. Satellite imagery of a section of Victoria Road in the north of the study site of Formby, Merseyside, highlighting several examples of urban intensification between 2005 (*left*) and 2021 (*right*) (©Google Earth 2023).

scavengers or degraded by road traffic). On the other hand, roadkill tends to be more conspicuous compared with other causes of death and so may be more likely to be recorded.

Although it has been suggested that inexperienced dispersing juveniles are more likely to be killed by road traffic (Slater 2002), studies have reported that the majority of recorded road deaths are adult squirrels (Shuttleworth 2001, LaRose et al. 2010, Shuttleworth et al. 2015b, Fey et al. 2016, Blackett et al. 2018). This may be due to adults ranging more widely during the breeding season or to exploit known food resources (Shuttleworth 2001), resulting in them encountering and crossing roads more frequently than juveniles.

Shuttleworth (2001) found that there was a clear seasonal pattern to road traffic mortality, with a peak in the autumn months. Due to increased natural food resources in the autumn, squirrels spend more of their active time on the ground foraging and scatter-hoarding, which could result in their crossing roads more frequently as they search for and cache food items (Shuttleworth 2000, 2001). The provision of supplemental food may also contribute to the autumnal peak in road traffic mortality by encouraging increased terrestrial activity, as red squirrels spend on average 67% of their active time foraging in the canopy (Kenward & Tonkin 1986) whereas this falls to around 50% when supplementary food is available (Shuttleworth 2000, 2001). To mitigate this, aerial rope bridges are increasingly being installed at road traffic mortality 'hotspots' to provide an alternative route and discourage squirrels from crossing the roads at ground level (Magris & Gurnell 2002).

3.1.3.3.2. Diseases

Studies have shown that diseases accounted for 25.6% (*n* = 245; LaRose et al. 2010), 35% (*n* = 163; Simpson et al. 2013), and 34.4% (*n* = 337; Blackett et al. 2018) of recorded red squirrel deaths. As previously discussed in Chapters One and Two, SQPV is a significant concern for red squirrel populations and ongoing conservation efforts across the UK, with the potential to spread rapidly and lead to high population mortality (Rushton et al. 2006, Chantrey et al. 2014). However, SQPV can be difficult to diagnose based on appearance alone, as it is visually similar to other diseases such as

Staphylococcus aureus-associated FED and leprosy, and so requires confirmation through laboratory analysis. This could lead to some suspected SQPV-infected individuals being euthanised but later testing negative for the disease, which could put additional pressure on already vulnerable populations (Collins et al. 2014).

In addition to SQPV, red squirrels are susceptible to a wide range of other diseases. One such emerging threat is adenovirus (Everest et al. 2014), which has now been identified in several red squirrel populations across the UK (e.g. Everest et al. 2008, 2010, 2012) and mainland Europe (e.g. Peters et al. 2011, Romeo et al. 2014, Côrte-Real et al. 2020). Although grey squirrels have been identified as carriers of this enteric disease (Everest et al. 2009b), evidence suggests that interspecific transmission also occurs from other small rodents such as wood mice (*Apodemus sylvaticus* Linnaeus 1758), as cases have been identified in red squirrels in sites where grey squirrels are absent (Everest et al. 2013, 2014). Adenovirus may present as a clinically significant infection resulting in the death of the infected individual, but it may also present as an asymptomatic infection with no apparent signs of the disease (Everest et al. 2014).

Another recently identified disease is leprosy, which similarly has been detected in red squirrel populations across the UK (e.g Meredith et al. 2014, Simpson et al. 2015, Avanzi et al. 2016, Schilling et al. 2019), although evidence suggests that the disease is unlikely to be a significant cause of mortality (Schilling 2020). On the other hand, FED has been highlighted as a cause for concern in the closed, isolation populations on the islands of Jersey and the Isle of Wight (Simpson et al. 2010, Blackett et al. 2018, Fountain et al. 2021), although only one possible case has been detected in mainland UK, on Anglesey in North Wales (Shuttleworth et al. 2015b). It has been suggested that the disease is acquired from a currently unidentified reservoir host, possibly rats or bank voles (*Myodes glareolus* Schreber 1780), rather than from squirrel to squirrel transmission (Fountain et al. 2021).

Disease outbreaks may be exacerbated in urban red squirrel strongholds due to higher population densities and supplemental feeders acting as sources for the spread of disease. Studies continue to identify other emerging and novel diseases in red squirrel populations, such as rotavirus (Everest et al. 2009a, 2011) and dicistrovirus (Dastjerdi et al. 2021).

3.1.3.3.3. Predation and Other Sources of Mortality

Predation seems to only account for a small proportion of overall mortality in urban red squirrel populations but often appears to be attributable to free-ranging domestic or feral cats and dogs (Magris & Gurnell 2002, Simpson et al. 2013c, Shuttleworth et al. 2015b, Fey et al. 2016, Blackett et al. 2018). This is most likely as a result of companion animals being associated with human presence and so they can occur at high densities in urban environments, whereas other predators, such as pine martens, are sensitive to human disturbance and tend to avoid inhabiting urban areas (Twining et al. 2020b). Apart from Magris & Gurnell (2002) who reported cat predation as being responsible for 36% of recorded red squirrel mortality on Jersey, the small number of predation events generally reported in the published literature suggests that it has not significantly contributed to the red squirrels' decline.

Other reported causes of death include poisoning, most likely accidentally by anticoagulant rodenticides (n = 1/245; LaRose et al. 2010, n = 1/60; Simpson et al. 2013, n = 4/337; Blackett et al. 2018), electrocution (LaRose et al. 2010, Simpson et al. 2013c), and drowning (n = 1/163; Simpson et al. 2013, n = 1/119; Shuttleworth et al. 2015). The few reported casualties suggests that these situations are not commonplace in red squirrel populations.

3.1.4. Chapter Aim and Objectives

The aim of this chapter is to evaluate the resources and risks present for red squirrels in the urban environment using Formby as a study site, specifically supplemental feeding, the availability of natural food sources (i.e. habitat quality), and mortality threats, which were identified as key topics in Chapter One. The data that are analysed in this chapter will also be incorporated into the home range analysis in Chapter Four.

The objectives of are:

1. To assess the availability of supplemental food in the study site, including the potential risk of calcium deficiency from peanuts.
2. To determine the availability of natural food sources (i.e. habitat quality) across the study site.
3. To evaluate mortality in the red squirrel population in the study site, including the predominant cause(s) of death, potential ‘hotspots’ of mortality, and the prevalence of adenovirus and SQPV in the population.

3.2. METHODS

All the necessary permissions (e.g. for transportation and storage of the red squirrel carcasses; Appendix III) and ethical approval were obtained prior to commencing data collection and renewed annually as required. Ethical approval was granted by NTU’s ARES Research Ethics Committee and AWERB (ARE595/ARE666).

Descriptive statistics and qualitative analyses were conducted in Microsoft Excel, mapping and spatial analyses were conducted in ArcGIS (v10.5.1, ESRI 2017), and statistical tests were conducted in R Statistical Software (RStudio Team 2022). All results graphs were produced in Microsoft Excel.

3.2.1. Supplemental Feeding Survey

A public survey was conducted from March 2018 to April 2020 to identify the locations of feeders across the study site and to assess the supplemental feeding practices of the residents in Formby, including what food they are providing, how much, and when they are providing it. The supplemental feeding survey, research consent information sheet, and participant consent form (Appendix V) could either be provided as hard copies or emailed as electronic forms. The research consent information sheet was produced to ensure that the participants were fully informed regarding the purpose of the research, how the information that they provided was going to be utilised, and their rights as participants (e.g. to withdraw their responses and to remain anonymous). Participants were requested to sign and submit the consent form with the completed survey. An online version of the survey was also created using the Jisc Online Surveys tool to increase the reach of the survey and the number of responses. Consent was taken by their participation and completion of the online survey, which was stated in the introduction. Due to the survey topic, it was highly unlikely that participation would cause any emotional or physical distress to any of the participants, but nevertheless the survey was designed so that questions could be skipped if required.

Several different channels were used to advertise the survey as widely as possible: the live-capture trapping volunteers (see section 2.2.2, p. 42) were contacted directly, a short article was published in the National Trust Formby volunteer e-newsletter, the LWT posted the online link on their social media sites, and hard copies were distributed via a mail drop. As Formby has a population of approximately 23,000 (measured at the 2011 census), it was unfeasible to distribute the survey to all the residents via the mail drop. Instead, approximately 1500 surveys were distributed by volunteers between December 2019 and February 2020, targeting the areas where the radio-collared squirrels were located. Hard copies of the mail drop survey could either be returned via post, scanned and emailed to the provided email address, or submitted to the local library to be collected by the principal researcher. The accompanying information sheet also provided the Jisc Online Surveys web address, so that respondents had the option to complete the survey online.

3.2.1.1. Data Analysis

Duplicates were checked for via the address provided and only the first response was included in the analyses. In any instances where a respondent selected multiple answers, the more conservative answer was included in the analyses. Peanuts and monkey nuts were considered as separate food items within the survey, as squirrels appeared to prefer eating shelled food items (*pers. obvs*.). The question regarding provision of water was incorporated into the survey at a later date, so the first 27 respondents were unable to answer this question. For the question regarding cleaning of feeders, three respondents did not provide answers and so were excluded from the analysis of this question. This question was also not applicable for one respondent, as they provided supplemental food in nets that were disposed of once emptied.

3.2.2. Natural Food Sources

The availability of natural food sources across the study site, as an indicator of habitat quality, was assessed via cone and seed crop abundance transect surveys using methods adapted from Lurz et al. (1997). A grid was generated over a map of the study site with a point marked in the centre of each grid-square, using the Grids and Graticules Wizard in ArcGIS (Fig. 3.2). An 8x8 grid was selected as the optimum distribution of transects across the study site, with the most even spread in both woodland (columns one and two in Fig. 3.2) and urban locations (columns three to eight in Fig. 3.2), resulting in a total of 64 transects at a density of approximately one transect per 0.04 ha.

The co-ordinates of the central points were downloaded from ArcGIS, so that the locations could be found in the study site via GPS. If the central point was within an area of woodland, one of the nearest trees was selected as the start point for the transect and, using a random number generator, a bearing was randomly selected for the direction of the transect. If the central point was within an urban or open (e.g. sand dune, grassland) area, then the nearest patch of woodland or publicly accessible trees (i.e. not within a private garden) within the grid-square were selected (Fig. 3.2). As with the woodland grid-squares, one of the nearest trees and a random bearing were then used. A red indicator was marked as the start point at the base of the tree using forestry marker paint, so that the same start point could be located each year. If a whole grid-square was not accessible due to landowner access permissions (as occurred with A1 – A4, B3, and B4), despite likely containing suitable red squirrel habitat, the transect was not conducted. C4 also could not be accessed in 2019 due to a new housing development and so was excluded, which resulted in 57 transects being included in the data analysis.

The transects were marked out along the randomly selected bearing at 10 m long by 1 m wide, after which all the tree cones (e.g. pine species, European larch *Larix decidua* Miller 1768) and seeds (specifically beech, oak, hazel *Corylus avellana* Linnaeus 1758, sweet chestnut *Castanea sativa* Miller 1768, and horse chestnut *Aesculus hippocastanum* Linnaeus 1758) were counted and cleared from the transect area. The transects were initially cleared to remove the old seed crop in June to August 2017, before repeating the surveys in June to July 2018 and June to early August 2019 to assess the annual seed crop.

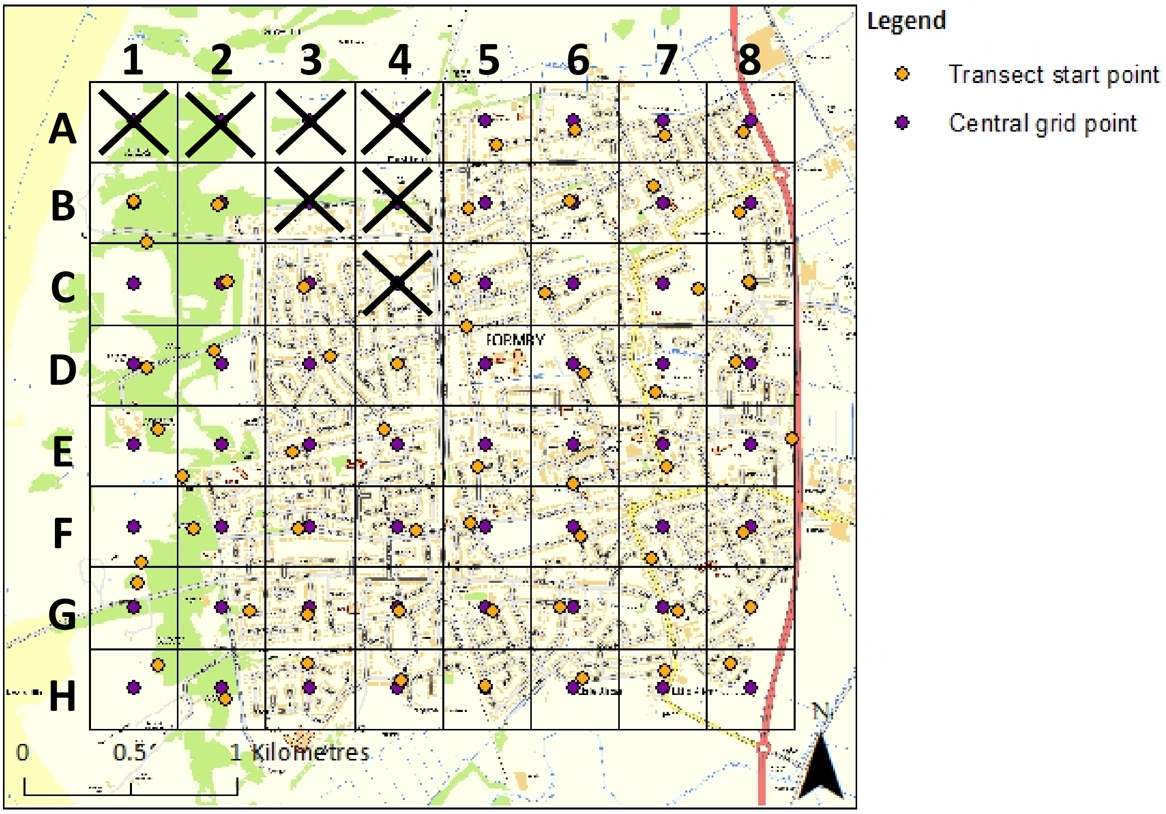


Figure 3.2. Locations of the cone/seed crop surveys, with the original central grid point and the adjusted start point marked as points. Transects that were not conducted, due to access permissions, are marked with an 'X' (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

3.2.2.1. Data Analysis

The annual cone and seed crops, for both the woodlands and urban area in 2018 and 2019, were estimated by calculating the number of cones/seeds per m². In addition, the relative energy content (kJ/g) of various natural food sources has previously been calculated and published in the literature (Table 3.1; Reher et al. 2016), so this was used to calculate the overall energy content (kJ) of the cones and seeds in each transect. The overall energy content per transect was then determined as being 'high' (> 10,000 kJ), 'medium' (1000 – 10,000 kJ), 'low' (< 1000 kJ), or 'none' (no cone or seed crop counted during the surveys), as an approximate indicator of habitat quality across the study site. This was visualised in ArcGIS by creating colour-coded polygon shapefiles using the 'Editor' toolbar.

Fisher's Exact tests (from the 'stats' package that is included in RStudio) were used, due to small expected cell counts (< 5), to compare the frequency of transects with high, medium, low, and no cone/seed crop between the woodlands and urban area in 2018 and 2019. Using the mean energy content for each transect for both years and excluding those with no mean cone/seed crop, a Mann Whitney U test (again from the 'stats' package) was used, due to the small sample sizes (n woodland = 14, n urban = 14) and right-skewed distribution of the data, to compare the energy content in the woodlands and the urban area.

Table 3.1. Relative energy content (kJ/g) of natural food sources, adapted from Reher et al. (2016).

Natural Food Source	kJ/g	Reference
Scots pine (*Pinus sylvestris*)	25.64	Grodziński & Sawicka-Kapusta (1970)
Douglas fir (*Pseudotsuga menziesii*)	29.84	Smith (1968)
European larch (*Larix decidua*)	23.07	Grodziński & Sawicka-Kapusta (1970)
Beech (*Fagus sylvatica*)	25.08	Grodziński & Sawicka-Kapusta (1970)
Oak (*Quercus robur*)	18.50	Grodziński & Sawicka-Kapusta (1970)
Hazel (*Corylus avellana*)	26.36	USDA (2015)
Horse chestnut (*Aesculus hippocastanum*)	20.33	Papageorgiou (1978)
Sweet chestnut (*Castanea sativa*)	17.90	Cicek & Tilki (2007)

3.2.3. Population Mortality

Since 2015, residents within the Merseyside red squirrel stronghold have been reporting sick or dead red squirrels to the LWT. Sick squirrels were caught and taken to a local veterinary practice, either for treatment and release or to be euthanised. Dead squirrels were collected and frozen in a standard freezer (-4 ^{o}C) by the LWT for future analysis. Ideally the date, location, age, sex, and any observations regarding possible causes of death were recorded when the carcass was collected. In cases where the carcass could not be collected, typically only the date, location, and any observations by the reporter were recorded.

3.2.3.1. Necropsies

Necropsies were conducted with permission from the LWT (Appendix III), to determine the potential cause of death and to collect samples for further analyses. From 2015 to 2019, there were 439 recorded instances of red squirrel mortality across the Merseyside stronghold. From 2015 and 2016, 28 and 30 carcasses respectively were collected and necropsied. From 2017 and 2018, 11 carcasses were unlabelled and so had no recorded details for identification: nine were necropsied and one was sent to the Wellcome Sanger Institute for genome sequencing (Appendix VI; Mead et al. 2020). From 2017, 45 carcasses were collected and 42 were necropsied. From 2018, 98 carcasses were collected and 89 were necropsied, including one suspected leprosy case that was sent to the Royal (Dick) School of Veterinary Studies at the University of Edinburgh for testing. From 2019, 97 carcasses were collected although only two were necropsied from January, as it was decided to halt the data collection once the carcasses up to the end of 2018 had been necropsied due to the extensive number collected. Overall, 200 red squirrels were necropsied between July 2017 to November 2019.

3.2.3.2. External and Internal Examinations

The carcasses were thoroughly defrosted for between 12 to 24 hours prior to conducting the necropsies. Each individual was assigned a unique identification number. Prior to commencing the necropsy, their age stage (adult, sub-adult, or juvenile), sex, and breeding condition were recorded if not already determined when the carcass was collected. These were determined using the same methods as for live-capture trapped individuals (see section 2.2.2.3, p. 50).

A thorough external examination was conducted to identify any signs of trauma or disease. All the limbs, joints, and digits were manipulated to feel for broken bones, including the whole length of the spine down to the tip of the tail. The skull and mandibles were also gently pressed to determine the presence of any fractures, and the inside of the mouth was checked for any damaged or missing teeth. A visual check was conducted for any lesions, cuts, wounds, or the presence of blood that may be a sign of injury or disease. Any ulcerated or scab-like lesions around the eyes, nose, lips, paws, ears, and genitals were noted as a potential sign of SQPV infection. In addition, the base of the tail and hind legs were checked for faecal staining as an indication of a potential gastro-intestinal infection. Finally, the ectoparasite burden was assessed by brushing the fur vigorously with a flea comb for 30 seconds over a white sheet of paper (see Fig. 2.7 in section 2.2.2.3, p. 52; categories 'very low' and 'low' were combined, so parasite burden was determined as either 'low', 'moderate' or 'high'). However, ecto-parasites will tend to abandon a deceased host, so this was likely to provide an underestimate of parasite burdens in comparison with the live population.

Following the external examination, a ventral mid-line incision was made in order to conduct a thorough internal examination. The internal cavities and all organs were checked for any signs of trauma, disease, or abnormalities (e.g. bleeding, bruises, cuts, enlarged or discoloured organs). Any breeding females were checked for the presence of embryos. Finally, the consistency and colour of the gut contents were also recorded, as a liquid consistency that was yellow-green in colour may indicate a potential gastro-intestinal infection.

3.2.3.3. Sample Collection

Once the external examination was completed and prior to commencing the internal examination, external samples were collected for further analyses. These included the lower lip, any tissues with signs of potential SQPV lesions (Fig. 3.3), whiskers, tail hairs, and tibia. Approximately 12 whiskers and 30 tail hairs were collected by holding the bottom of the shafts and plucking them out of the skin to ensure that the bulbs remained intact. The tibia was removed from the left leg and dissected free of any remaining soft tissues, although if the tibia in the left leg was broken then it was collected from the right leg instead.

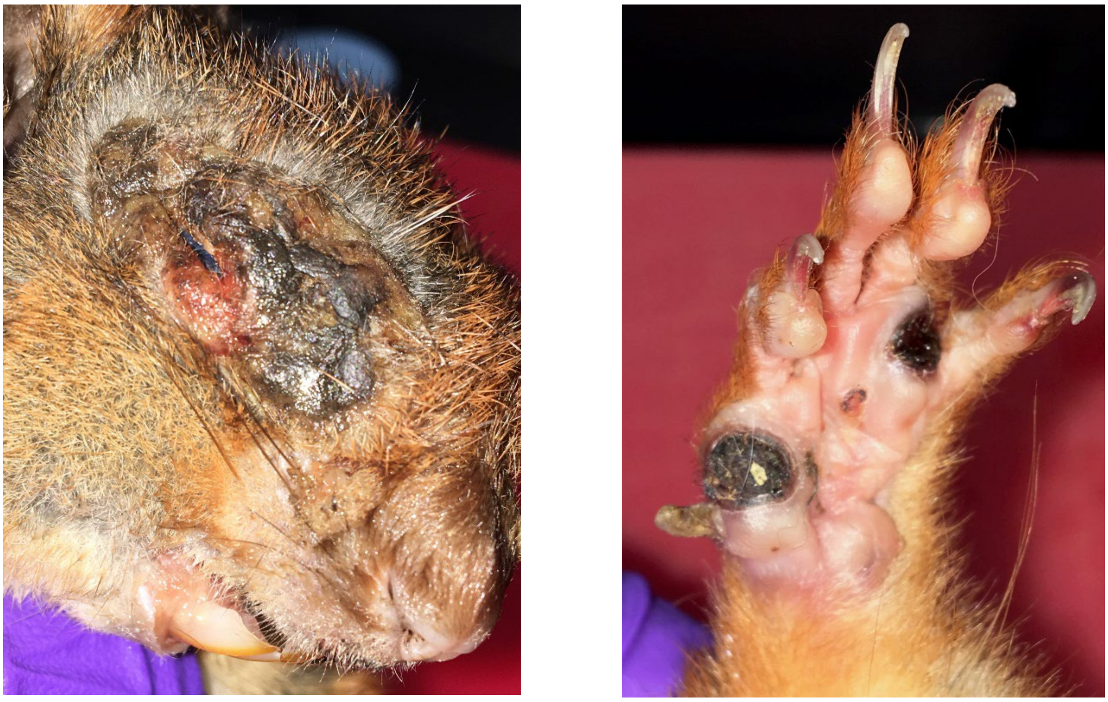


Figure 3.3. Signs of potential SQPV infection, visible as ulcerated or scab-like lesions most likely present on the eyes (*left*), nose, lips, ears, paws (*right*) and genitals (photos: J Smith).

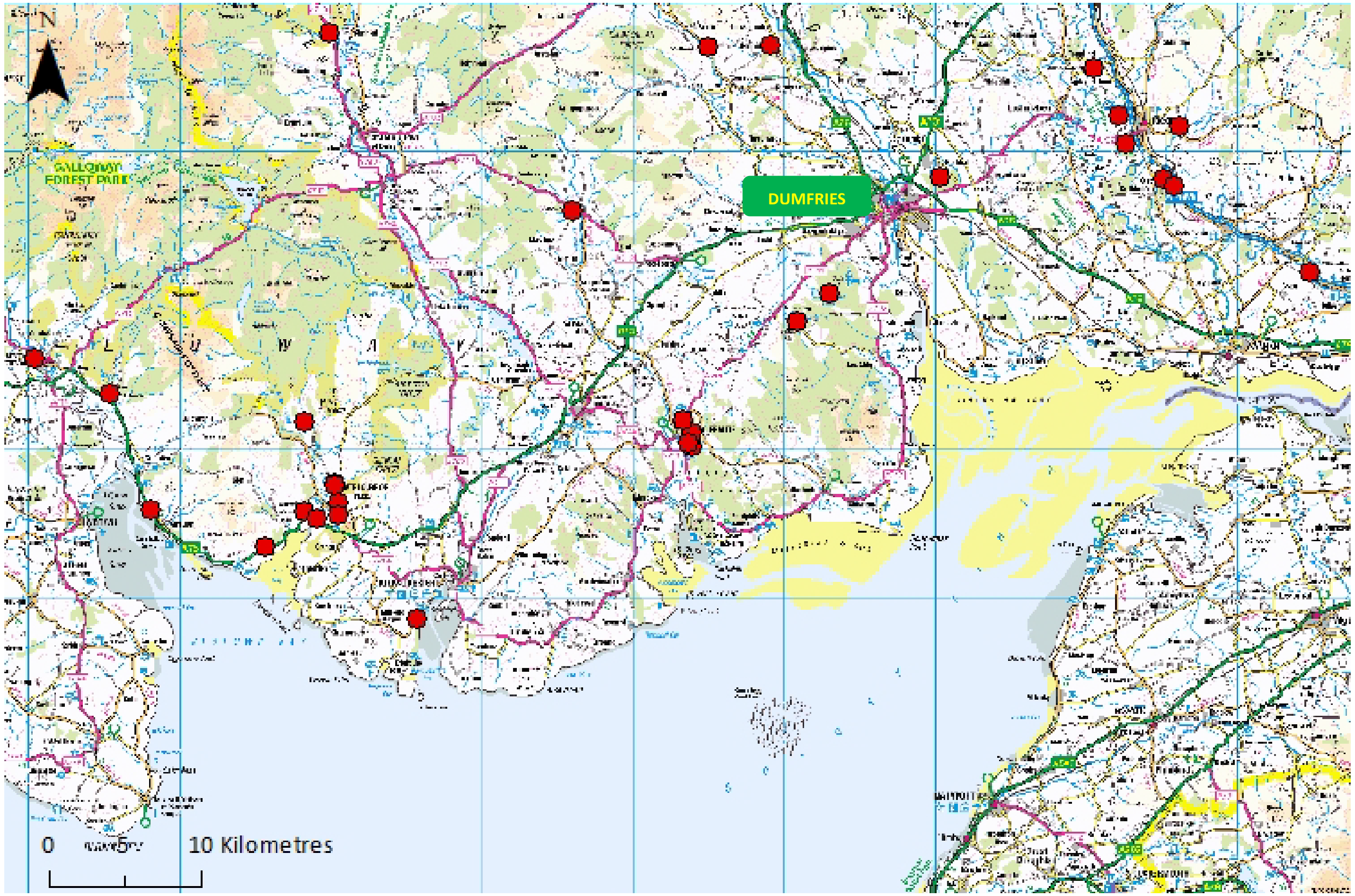
Following the internal examination, additional samples were taken including kidney, spleen, liver, large intestine, and gut contents. Approximately half of one kidney was collected, and similar sample sizes were collected of liver, spleen, and large intestine. The sample of large intestine was obtained from the vermiform appendix, after which a sample of the gut contents could be gathered by squeezing the large intestine. However, it is worth noting that the external/internal examinations and sample collections were dependent on the level of decomposition of the carcasses, so not all aspects of the necropsy could be conducted on every individual.

All the dissection equipment was thoroughly disinfected in Virkon^{TM} between collecting samples to prevent any cross-contamination. All samples were dry-stored in either an Eppendorf (2 ml), Bijou tube (7 ml), or small sample bag depending on the size of the sample and frozen in a standard freezer (-4 ^{o}C). All sample containers were labelled twice with the designated identification number for each squirrel and a code identifying the type of sample (LIP: lower lip, LES: skin lesion samples where multiple samples would be LESa, LESb, etc., WHK: whiskers, TAIL: tail hairs, KID: kidney, SPLN: spleen, LIV: liver, INT: large intestine, GUT: gut contents, TIBIA: tibia from left leg).

Dumfries & Galloway in Scotland was recommended as a suitable site to collect samples for comparative analyses (R Ogden, University of Edinburgh, *pers. comm.*), as the squirrels were less likely to have access to supplemental feeding due to the rural nature of the area. As such, tibias were similarly collected from red squirrels from Dumfries & Galloway (Fig. 3.4), where the carcasses were also collected between 2015 to 2018, with permission from the Royal (Dick) School of Veterinary Studies, University of Edinburgh.

3.2.3.4. Sample Analyses

The tibias were analysed at Brackenhurst Campus, Nottingham Trent University (see section 3.2.3.4.1). The remaining samples (whiskers, tail hair, lower lip, suspected SQPV lesions, kidney, spleen, liver, large intestine, and gut contents) were couriered to the Animal & Plant Health Agency (APHA), Weybridge, to undergo testing for SQPV and adenovirus (see section 3.2.3.4.2).



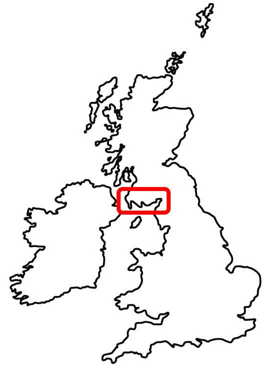


Figure 3.4. Approximate locations of dead red squirrels in Dumfries & Galloway (marked as points), from which tibias were collected for comparison with the Merseyside red squirrels. Note that locations were only recorded for 30 of the 37 individuals and in many cases only as 6-figure grid references (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2023)).

3.2.3.4.1. Bone Strength Analysis

Of the 200 Merseyside squirrels that were necropsied, tibias were collected from 197 individuals whilst the carcasses of the remaining three were too decomposed. Tibias were also collected from 43 Dumfries & Galloway squirrels. Once any remaining soft tissues were removed from the tibias, the tibias were weighed (g) using a digital analytical balance with an accuracy of ± 0.0001 g. Their length (mm) was also measured using digital callipers (Fig. 3.5), as this can be used as an indicator of body size (e.g. Finnegan et al. 2008).

Figure 3.5. Set up of measuring the lengths of the tibias using digital callipers (photo: K Hamill).

Bone strength was determined in Newtons (N) using a texture analyser (Stable Micro Systems, Surrey, UK) with recommended pre-determined settings (A Kemp, NTU, *pers. comm.*; Table 3.2). For the longer bones from adult squirrels, the gap in the jig was set to 2 cm (Fig. 3.6) and reduced to 1.2 cm for the smaller bones from juveniles.

Once all the bones were snapped, they were ashed overnight in a furnace (Nabertherm, Germany) at 650 ^{o}C for 12 hours, reaching the required temperature over an initial two-hour heating-up period (Kemp 2021). The crucibles, which were individually coded (Fig. 3.7), were initially weighed using a digital analytical balance and the snapped bones were then placed inside. The associated crucible and bone identification codes were recorded to later enable the ash weight to be assigned to the correct individual, before the crucibles were placed in the furnace. Following combustion as described above and once cooled to room temperature, the crucibles containing the ash were weighed again and the ash weight was calculated by subtracting the initial crucible weight. Mineral content (% of calcium and phosphorous) was then calculated from the ash weight as a proportion of the initial bone weight, using the equations:

$$\textit{Bone ash weight (g)} = \textit{Full crucible weight (with ash) (g)} - \textit{Empty crucible weight (g)}$$

$$\textit{Bone mineral content (\%)} = \left(\frac{\textit{Bone ash weight (g)}}{\textit{Bone weight (g)}} \right) \times 100$$

Table 3.2. Texture analyser settings used to measure bone strength (N) of red squirrel tibias.

Test mode	Compression
Pre-test speed (mm/sec)	5.00
Test speed (mm/sec)	3.00
Post-test speed (mm/sec)	40.00
Target mode	Distance
Distance (mm)	10.000
Trigger type	Auto (Force)
Trigger force (N)	0.050

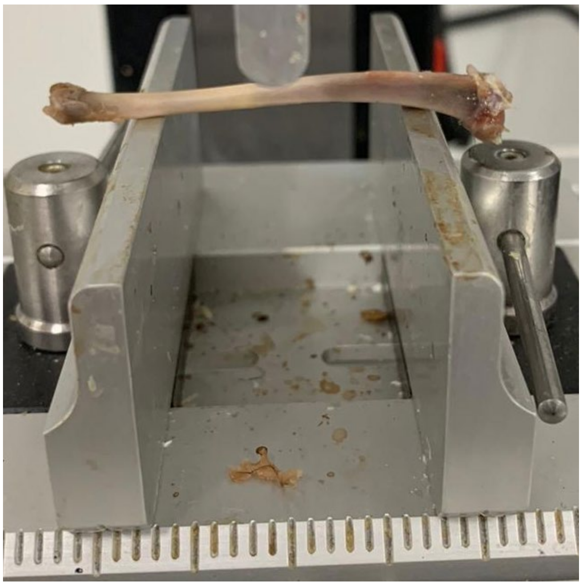


Figure 3.6. Set up of the texture analyser for measuring the strength of an adult squirrel tibia (photo: A Kemp).

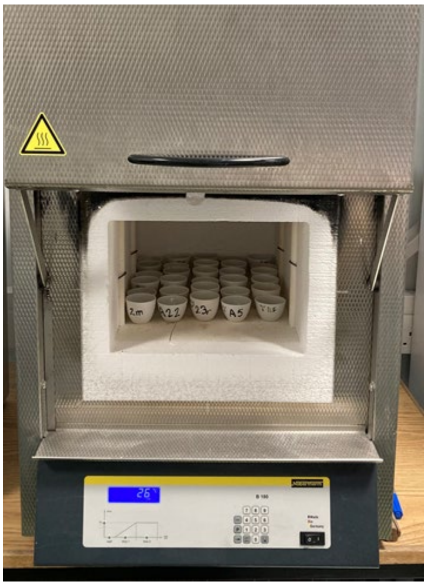


Figure 3.7. Set up of the furnace, highlighting the individual crucible codes (photo: K Hamill).

3.2.3.4.2. Disease Analysis

Samples collected during the necropsies were tested for SQPV and adenovirus by APHA, using previously described methodologies for negative contrast stain transmission electron microscopy (TEM; Everest et al. 2010) and polymerase chain reaction (PCR) assays (Everest et al. 2012b). In brief, negative stain TEMs were conducted using 3 mm copper/rhodium (100 µm mesh) support grids, which were pre-treated through immersion into 0.4% formvar in chloroform followed by carbon coating and then subjected to plasma glow discharge to provide a stable, hydrophilic sample platform. Approximately 0.25 cm^3 of each sample, using skin lesions to test for SQPV and gut contents to test for adenovirus in this study, was ground in 2 cm^3 of 0.1 M Sorenson's phosphate buffer (pH 6.6) to form a suspension, of which a 50 µl aliquot of each sample was pipetted on to dental wax and then a support grid placed copper-side upwards on to the aliquot for 30 seconds. Finally, each grid was placed on to a drop of 2% phosphotungstic acid (pH 6.6) for 10 seconds to counterstain the grid and each sample was analysed using an electron microscope to confirm the presence of SQPV (Fig. 3.8) or adenovirus (Fig. 3.9) particles (see Everest et al. 2010 for the full methodology). Only those individuals with suspected SQPV lesions were tested to confirm the disease, as asymptomatic cases (i.e. no visible lesions) are highly unlikely.

If no adenovirus particles were detected using negative stain TEMs, then PCR assays were conducted using either gut contents or spleen tissue. In brief, DNA was extracted from the samples using the DNeasy® Blood & Tissue Kit and associated protocols (QIAGEN 2006) and a nested PCR was used for increased sensitivity of detection. The reactions were carried out in 25 µl volumes containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 25 pmol of each primer (AdVPol_1F and AdVPol_1R), 1.25 U Taq DNA polymerase (Promega), and 2.5 µl of extracted DNA. PCR reactions were carried out using an ABI Veriti Thermal Cycler under the following conditions: (1) two minutes at 94 ^{o}C, (2) 40 cycles of 94 ^{o}C for 30 seconds, 55 ^{o}C for one minute, and 72 ^{o}C for one minute, and then (3) seven minutes at 72 ^{o}C. For the second-round PCR, 2.5 µl of the first-round product was amplified using internal primers AdVPol_3F and AdVPol_3R, with the same cycling conditions as the first-round PCR. The PCR products were then visualised by ethidium bromide gel electrophoresis using 2 % agarose gels and UV light, and sequenced using direct dye-termination sequence reactions (ABI Big Dye Terminator Cycle Sequencing Ready Reaction; see Everest et al. 2012b for the full methodology). Any PCR-positive results for adenovirus were considered to be an asymptomatic infection, whereas a pathogenic infection would be detected by TEM (Everest et al. 2014).

Unfortunately, not all suspected samples were tested due to delays as a result of the COVID-19 pandemic, as laboratories were redirected to COVID-related work. Only samples from 24 individuals were tested, of which nine were tested for adenovirus only, eight were tested for SQPV only, and seven were tested for both diseases. In addition, one suspected leprosy case was sent to the Royal (Dick) School of Veterinary Studies at the University of Edinburgh for testing (Avanzi et al. 2016).

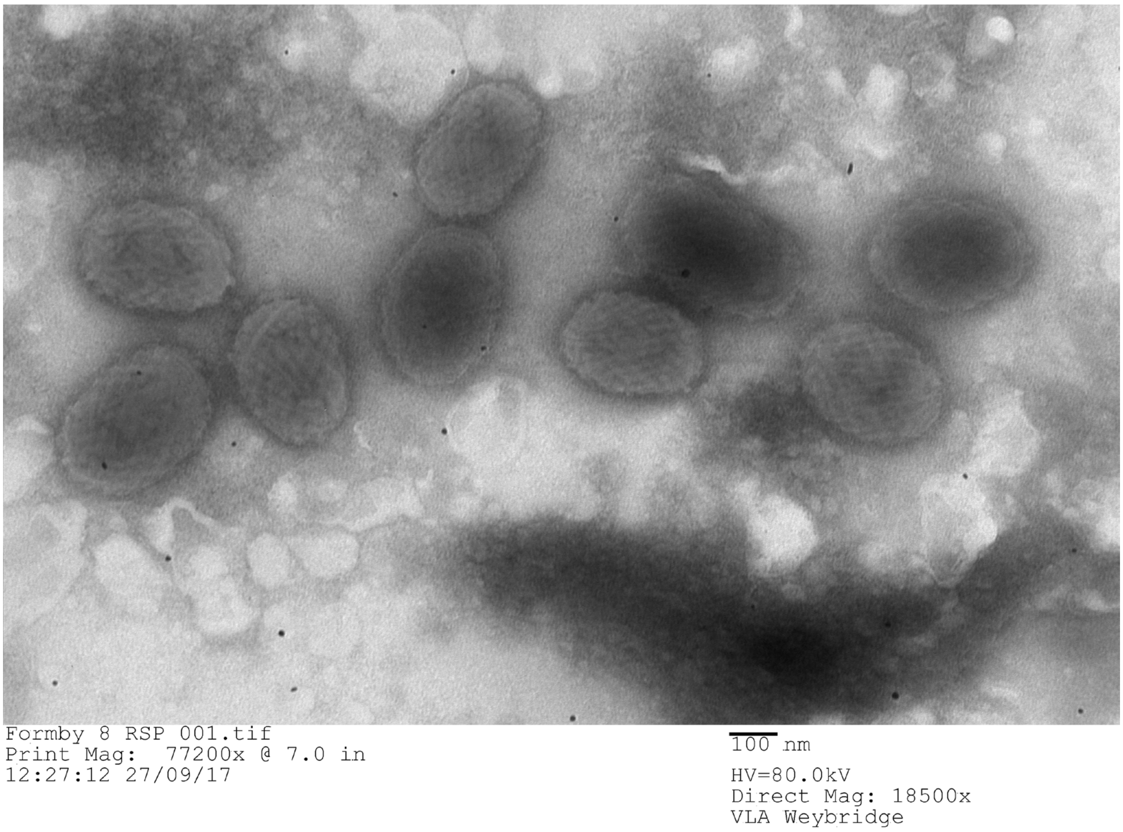


Figure 3.8. Negative stain TEM image of SQPV particles from a sample collected during the necropsies, indicating a SQPV-positive red squirrel (photo: D Everest).

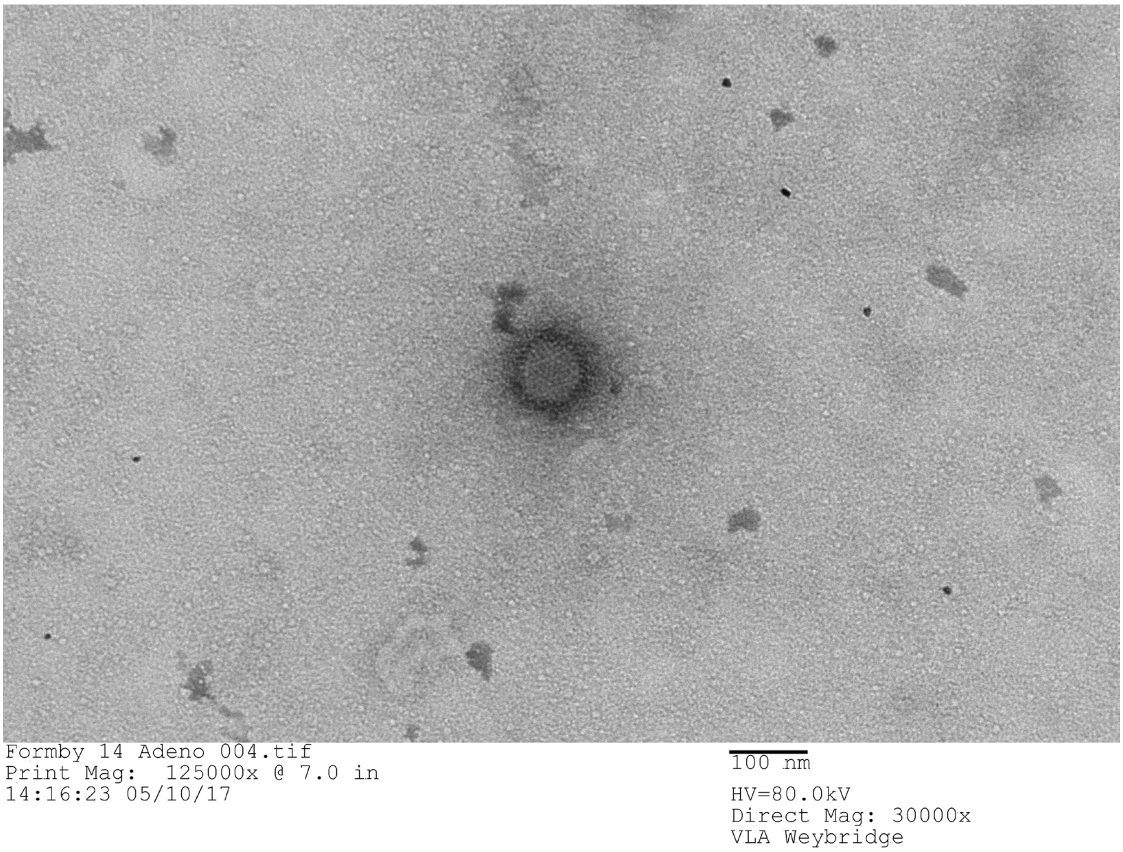


Figure 3.9. Negative stain TEM image of an adenovirus particle from a sample collected during the necropsies, indicating a pathogenic adenovirus infection in a red squirrel (photo: D Everest).

3.2.3.5. Cause of Death

The most likely cause of death (Table 3.3) for each necropsied individual was determined through a combination of the notes recorded when the sick or dead individual was originally collected (e.g. the habitat, such as they were found on a road or in a garden, and any additional information, such as the individual was observed being hit by a car or behaving lethargically), the notes recorded during the necropsy (e.g. signs of injury or disease), and, if tested, the results of the disease tests (see section 3.2.3.4.2). If sick individuals were taken to the vets and had to be euthanised due to the extent of their injures or disease, the primary cause of death was recorded as 'Euthanised' but a secondary cause of death was also identified. In some cases, there appeared to be multiple contributing factors, for example facial injuries (i.e. trauma) leading to starvation or dehydration due to an inability to eat or drink properly.

Table 3.3. Criteria used to determine the potential cause of death for necropsied individuals.

Cause of Death	Criteria
Inconclusive	– EITHER no obvious signs of injury or disease, so unable to determine the cause of death. – OR too decomposed to identify any signs of injury or disease. – OR signs of both injuries AND disease, but no obvious link or additional information (e.g. location) to help determine the primary cause of death.
Euthanised (with secondary cause of death)	– Individual found alive and taken to the vets, but euthanised due to the extent of its injuries or disease. – Secondary cause of death determined, if possible, as per criteria below.
Trauma	
Road traffic	– Found on a road. – May have been observed being run over by the reporter. – Major injuries, typically numerous broken bones (including fractured skull and jaw), damaged organs, AND obvious internal and external bleeding.
Trauma (likely road traffic)	– Cause of trauma likely to be due to road traffic due to extent of injuries, but was not found on a road (instead was in a garden or woodlands) – Major injuries, typically numerous broken bones (including fractured skull and jaw), damaged organs, AND obvious internal and external bleeding.
Trauma (likely fall)	– Cause of trauma likely to be due to a fall (e.g. from a tree or drey, or house guttering or roof). – Major injuries, typically broken limbs, broken or ruptured spine, AND internal bleeding. – Often found at the base of a tree, below a drey, or next to a house, or the fall was observed by the reporter.
Trauma	– Unknown cause of trauma. – Signs of one or more injuries, typically lacerations, broken bones, damaged organs, OR internal and/or external bleeding. – Injuries typically less severe/numerous than a suspected road traffic accident.
Disease	
SQPV	– Signs of SQPV lesions (e.g. scabby or ulcerated lesions on either the lips, ears, nose, eyes, paws, and/or genitals). – In some cases, confirmed via disease testing.
Other disease	– EITHER identified by the vets or during necropsy. – OR signs of infection (e.g. enlarged spleen) but unable to identify the disease. – In some cases, adenovirus confirmed via disease testing.
Other	
Accidental poisoning	– Typically suspected by the reporter. – Underweight, observed acting in a lethargic or disorientated manner, or found alongside other dead animals.
Anaemia	– Very high flea burden, but no obvious signs of injury or disease.
Malnutrition/dehydration	– Very underweight (e.g. visible ribs and pelvis, minimal visceral fat). – Minimal stomach contents. – Sometimes a high flea burden.
Predation	– Signs of bite and/or puncture wounds, sometimes with limbs missing. – Often observed by the owner if predated by a pet cat or dog.

3.2.3.6. Data Analysis

3.2.3.6.1. Population Mortality

Population demographics and causes of death were investigated and compared using descriptive statistics in Microsoft Excel. Due to the small sample sizes, data for sub-adults and juveniles were collated for comparison with adults.

Annual and seasonal (spring: March – May, summer: June – August, autumn: September – November, winter: December – February) frequencies of population mortality were analysed using Chi-Square Goodness of Fit tests from the 'stats' package that is included in RStudio. These included: (1) overall mortality between years, (2) overall mortality between seasons, (3) road traffic mortality between seasons, (4) disease mortality between years, and (5) disease mortality between seasons. Chi-Square Tests of Independence, or Fishers Exact tests where the expected cell counts were small (< 5), from the 'stats' package were used to investigate associations between: (1) frequencies of male and female casualties between seasons, (2) frequencies of adult and sub-adult/juvenile casualties between seasons, (3) frequencies of breeding and non-breeding casualties between seasons, (4) frequencies of male and female road traffic casualties between seasons, (5) frequencies of adult and sub-adult/juvenile road traffic casualties between seasons, and (6) parasite burdens of adults and sub-adults/juveniles.

The kernel density analyses, for identifying 'hotspots' of road traffic and SQPV mortality, were conducted by importing the co-ordinates of the locations where the dead squirrels were found to create a shapefile, from which a raster layer was generated using the 'Kernel Density' tool available in the ArcToolbox in ArcGIS.

3.2.3.6.2. Bone Strength and Mineral Content

Four Merseyside individuals were excluded from the data analysis, due to a mislabelling error and subsequent inability to distinguish between the samples. One Dumfries & Galloway individual was also excluded from the data analysis, as both tibias were too damaged due to the squirrel perishing in a road traffic incident. Only tibias collected from adult squirrels (Merseyside: n = 133, Dumfries & Galloway: n = 37) were included in the data analysis due to the range in ages and development of the juveniles, from newly weaned through to sub-adult.

A Spearman's rank-order correlation (from the 'stats' package that is included in RStudio) was used to determine whether there was collinearity between bone strength (N) and ash weight (g) (i.e. mineral content) for adult squirrels from Merseyside and Dumfries & Galloway. It was found that there was a statistically significant positive correlation between bone strength and ash weight (r_s = 0.886, df = 168, $p < 0.0001$), in other words higher ash weight and therefore mineral content equates to stronger bones, so only bone strength was used in the statistical analyses because this variable is easier to measure and so has more practical application.

In addition, an unpaired t-test (from the 'stats' package) was used to determine if there was a significant difference between the bone lengths (mm) of adult red squirrels from Merseyside and Dumfries & Galloway, as it is known that an increased body size is associated with increased bone strength (Mielke et al. 2018, Rickman et al. 2023). It was found that the squirrels from Merseyside had significantly longer bones (60.93 mm ± 1.66 SD) compared to those from Dumfries & Galloway (59.30 mm ± 1.92 SD; $t_{(168)}$ = -5.073, $p < 0.0001$). Therefore, bone strength was standardised by regression using the equation:

$$\textit{Bone strength} = -321.12 + (7.11 \times \textit{Bone length})$$
$$(F_{1,168} = 87.17,\ p < 0.0001)$$

The residual bone strengths were then compared between Merseyside (n = 133) and Dumfries & Galloway (n = 37) individuals.

Comparative analyses of bone strength and length were conducted within the Merseyside population, between: (1) males (n = 58) and females (n = 74; total n = 132, as one individual was excluded as their sex could not be determined due to decomposition); (2) breeding (n = 52) and non-breeding (n = 80) individuals (total n = 132, as one individual was excluded as their breeding condition could not be determined due to decomposition); and (3) individuals located in the urban area (n = 89) and woodlands (n = 18; total n = 107, as 26 individuals were excluded as the carcass locations were not recorded). Comparative analyses of bone strength and length were also conducted within the Dumfries & Galloway population between males (n = 20) and females (n = 17; total n = 37). Comparative analyses could not be conducted between breeding and non-breeding individuals, as these data were not recorded when the carcasses were submitted to the Royal (Dick) School of Veterinary Studies, University of Edinburgh, or between individuals located in more urbanised areas and woodlands, as many of the recorded locations of the carcasses were only approximate with six-figure grid references and so the habitat could not be accurately determined. These analyses were conducted using unpaired t-tests (again from the 'stats' package) as, although the parametric assumption of homogeneity of variances was met, the parametric assumption of normality was not met but t-tests are relatively robust to violations of the normality assumption (Skovlund & Fenstad 2001, as cited in Fagerland 2012).

3.3. RESULTS

3.3.1. Supplemental Feeding Survey

There were 170 respondents, although one was the National Trust and so was evaluated separately to the households (see section 3.3.1.5). Six respondents did not fully complete the survey and were excluded from the analyses, so 163 responses were analysed.

3.3.1.1. Sightings of Red Squirrels?

75.5% of the respondents see a red squirrel in their garden at least once a week, with 41.7% of respondents seeing a red squirrel every day (Fig. 3.10). It was most common to see one or two squirrels at the same time ($\bar{x} \pm SD = 1.48 \pm 0.74$), reported by 57% and 31.9% of respondents respectively (Fig. 3.11). Only 8.6% of respondents reported seeing three or more squirrels at the same time in their gardens.

3.3.1.2. Provision of Supplemental Food?

75.5% of the respondents provide supplemental food to the red squirrels in their gardens, even if indirectly through feeding the birds. These respondents were not necessarily the same 75.5% who reported seeing a red squirrel in their garden at least once a week: 45% of the 40 respondents who do not provide supplemental food reported seeing a red squirrel at least once a week. Only those respondents who provide supplemental food (*n* = 123) were then requested to complete the remainder of the survey.

Hanging bird feeders are the most common type of supplemental feeder used in the urban gardens, followed by squirrel boxes (Fig. 3.12). 51.2% of the respondents have multiple types of feeders, with 199 hanging bird feeders, 74 squirrel boxes, and 60 bird tables recorded in total. Of the 16 respondents who selected ‘other’, they reported simply scattering the supplemental food on the floor or placing the food in dishes on the ground.

Peanuts were the most commonly provided supplemental food item and, when taken into consideration with monkey nuts, were provided by 81.3% of respondents (Fig. 3.13). Typically, ‘other’ types of food items reported were store-purchased wild bird seed mix or fat balls. 66.7% of the respondents provide more than one type of food item. Of the 41 respondents that provide one type of food item, 75.6% provide only peanuts or monkey nuts. In addition, only 3.3% of respondents provide additional sources of calcium, such as deer antlers or cuttlefish bones.

78% of the respondents estimate that they provide 3 kg or less of supplemental food per month, with 36.6% of respondents supplying less than 1 kg (Fig. 3.14). Only a small proportion of respondents (8.1%) estimate that they provide 8 kg or more of supplemental food per month. 81.3% of the respondents provide supplemental food throughout the year. Of the remaining 23 respondents, 95.7% do not provide supplemental food during the summer (June to August), instead only supplying food during the autumn (September to November), winter (December to February), and/or spring (March to May) months.

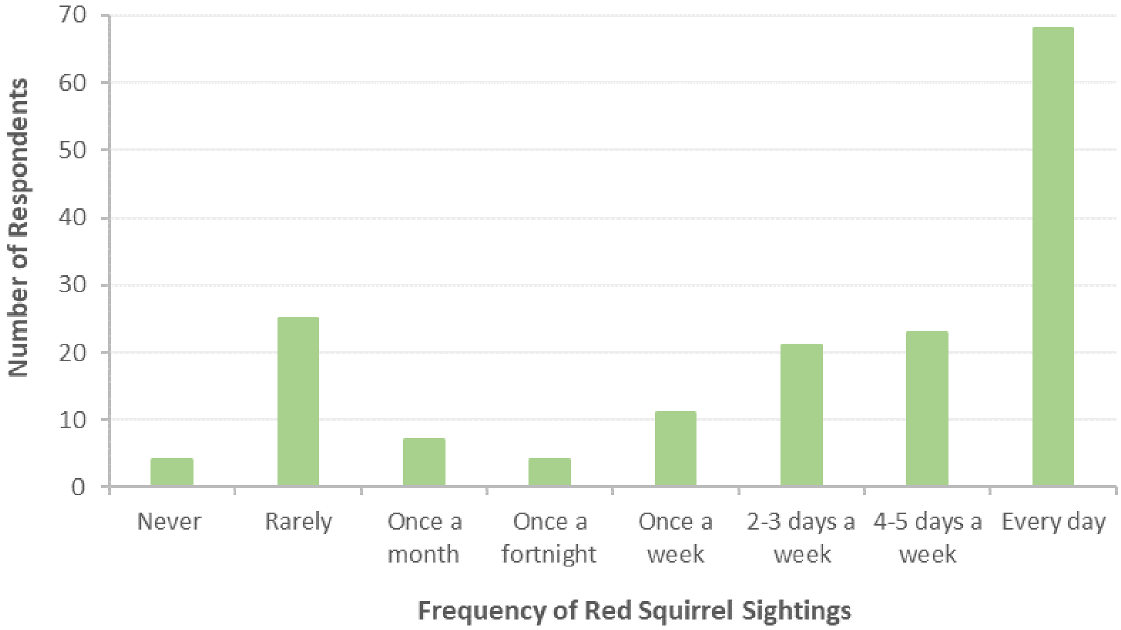


Figure 3.10. Summary of responses (n = 163) to the question 'on average, how regularly do red squirrels visit or pass through your garden?'.

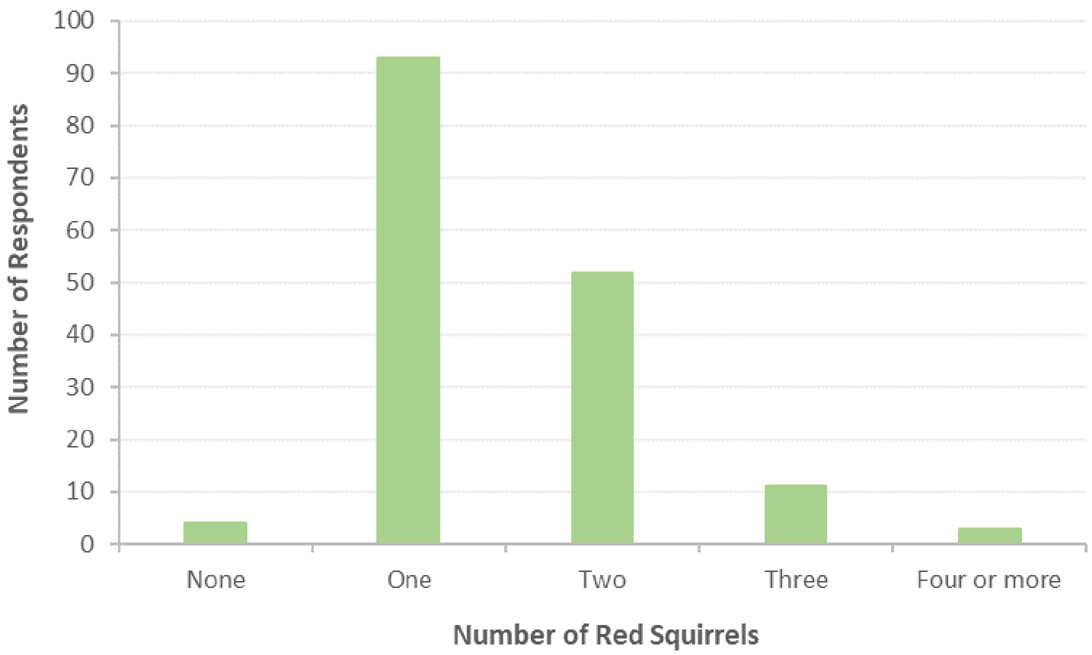


Figure 3.11. Summary of responses (n = 163) to the question 'on average, how many red squirrels do you see in your garden at the same time?'.

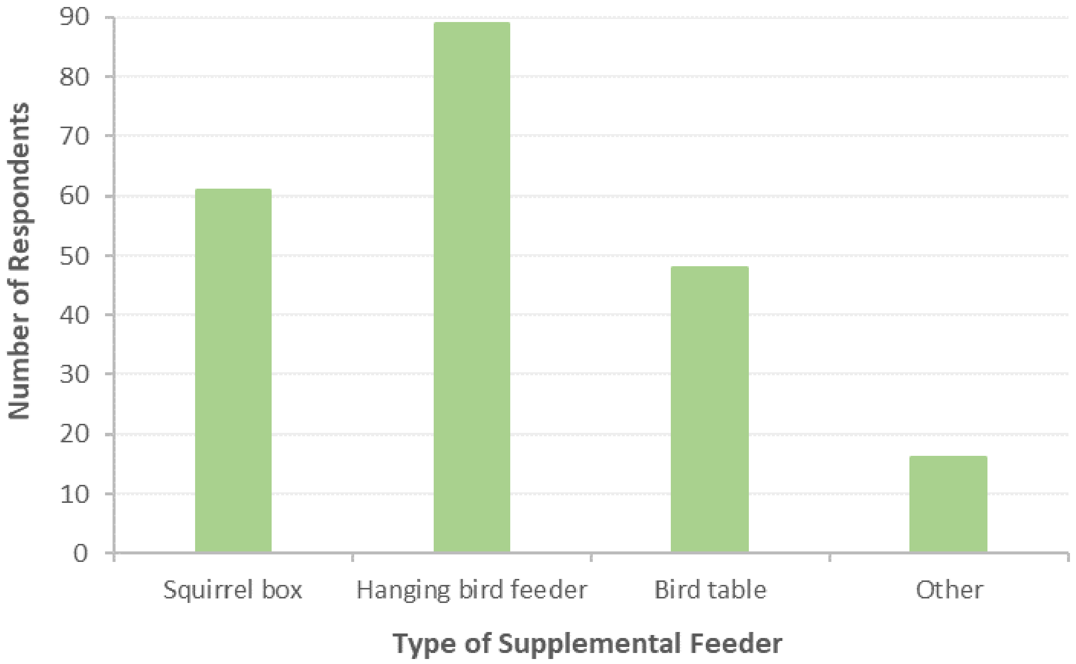


Figure 3.12. Numbers of different supplemental feeders available in the urban gardens (*n* = 123), with approximately half of the respondents providing multiple types of feeders.

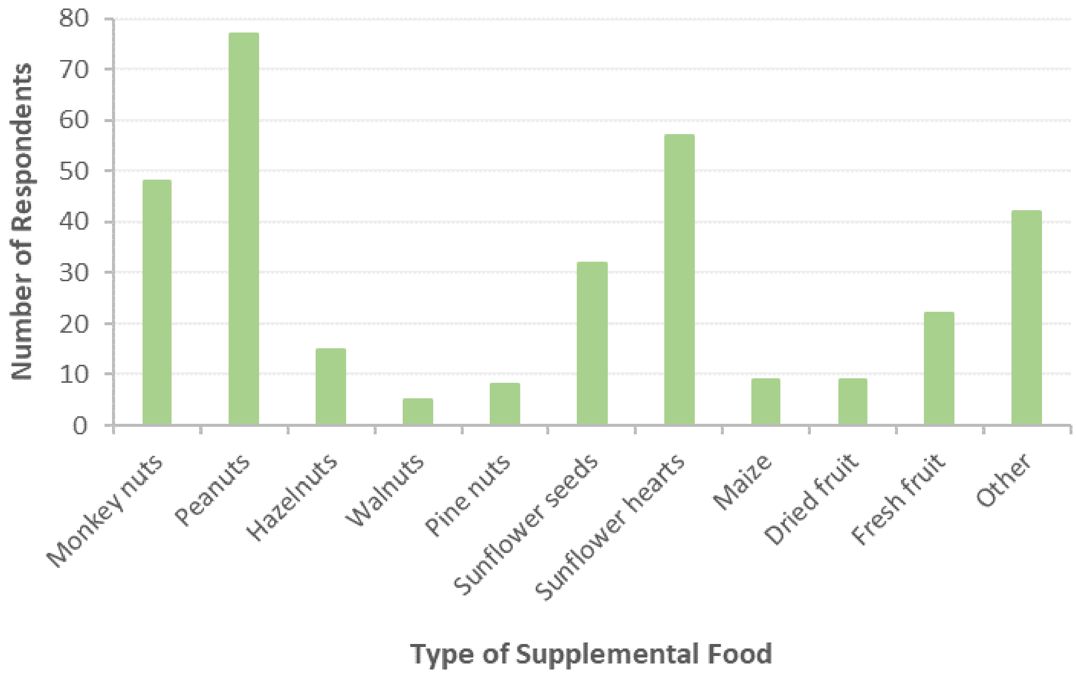


Figure 3.13. Number of respondents providing different types of supplemental food (*n* = 123), with approximately two-thirds of the respondents providing multiple types of food items.

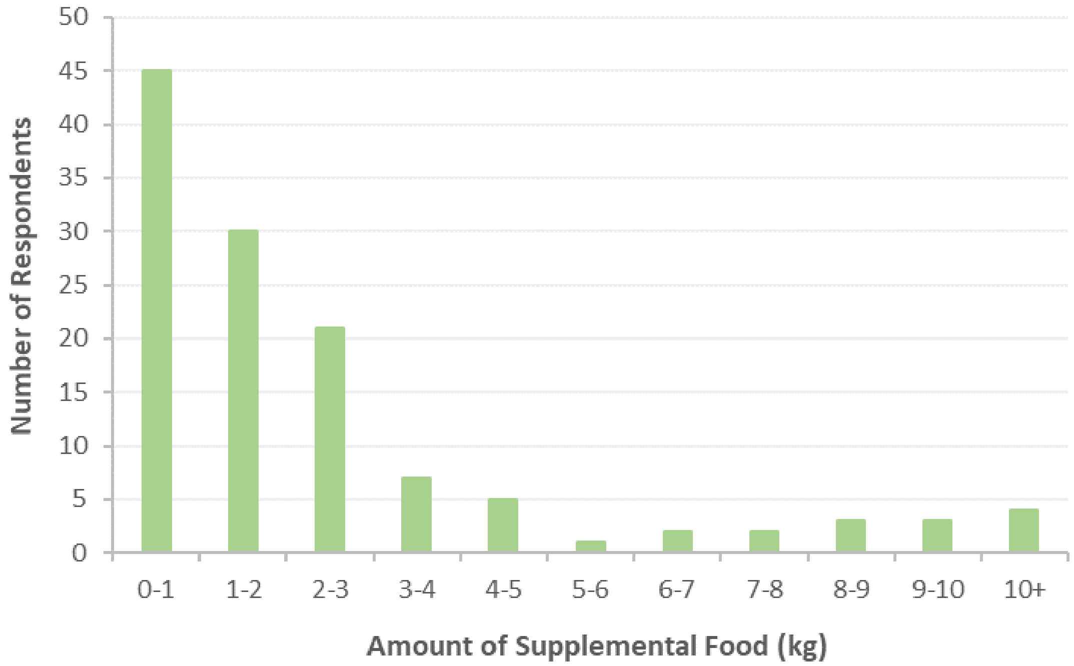


Figure 3.14. Estimated amount of supplemental food (kg) provided by the respondents (*n* = 123) per month.

3.3.1.3. Provision of Water?

Of the 96 respondents who answered this question, 86.5% reported that they provide water in their gardens and 36.5% have more than one type of water source available, with bird baths being the most common type of water source available (Fig. 3.15).

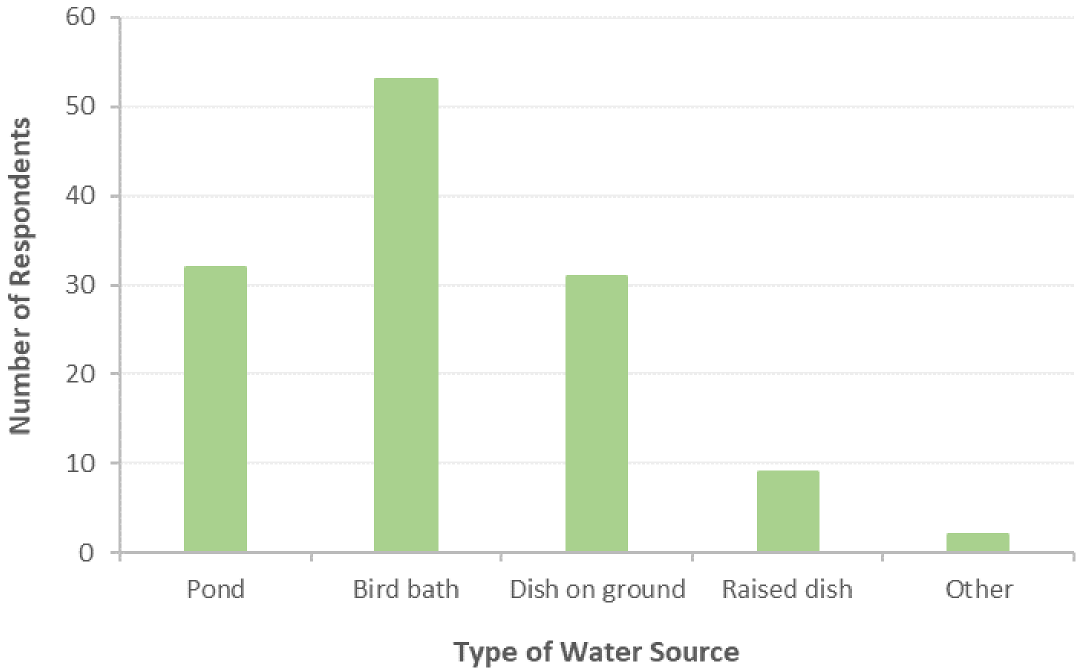


Figure 3.15. Numbers of different water sources available in the urban gardens (*n* = 93), with approximately one-third of the respondents providing multiple types of water sources.

3.3.1.4. Hygiene of Supplemental Feeders?

Of the 119 responses that were included, 39.5% either never clean their feeders, or only do so once or twice per year (Fig. 3.16). Generally, respondents were most likely to clean their feeders either monthly (21.9%) or every two to three months (24.4%). Excluding the 18 respondents who never cleaned their feeders, 43.4% of respondents (*n* = 101) incorporate soap or disinfectant into their cleaning practices, whilst 56.6% only use water. Only 18.5% of the 119 respondents cleaned their feeders at least monthly and using soap and/or disinfectant.

Figure 3.16. Summary of responses (n = 119) to the question 'how often do you generally clean your feeder?'.

3.3.1.5. National Trust Red Squirrel Reserve

At the time of the survey, the National Trust were supplying a mixture of peanuts, monkey nuts, and sunflower seeds in the Red Squirrel Reserve and reported seeing four or more squirrels every day. The supplemental food was provided in six custom-made feeders distributed around 'Squirrel Walk' (Fig. 3.17), designed to exclude larger birds such as carrion crows (*Corvus corone* Linnaeus 1758) and jays (*Garrulus glandarius* Linnaeus 1758), as well as one squirrel box adjacent to the Victoria Road car park, which were all cleaned monthly using disinfectant. For years, over 10 kg of supplemental food was provided each month throughout the year, although the National Trust permanently stopped supplemental feeding in mid-2018 due to a SQPV outbreak and the potential risk of future outbreaks. Water was available year-round via several dog water fountains at the entrance to the Victoria Road car park (Fig. 3.18). There were also seasonal ponds that form in the dune slacks, although these sometimes dry up completely during drought years.

Figure 3.17. A National Trust volunteer placing supplemental food in one of the custom-made feeders on 'Squirrel Walk' (photo: K Hamill).

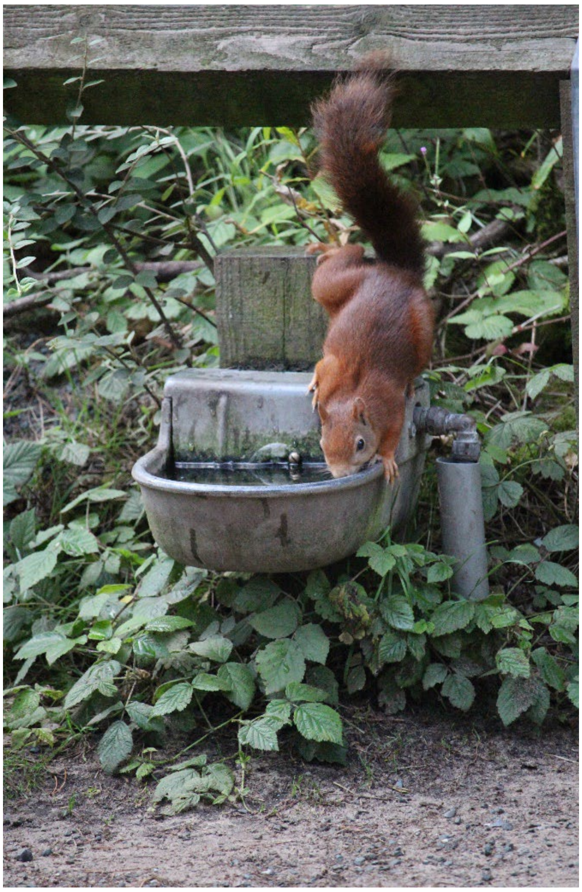


Figure 3.18. A red squirrel drinking from a dog water fountain near the entrance to the National Trust Red Squirrel Reserve (photo: K Hamill).

3.3.2. Habitat Quality

The mean cone crop (cones/m²) was 24.5 (2018) and 11.8 (2019) in the woodlands, compared with 3.3 (2018) and 2.6 (2019) in the urban area. The mean proportion of cones eaten by squirrels was 21.4% (2019) – 23.1% (2018). The mean seed crop (seeds/m²) was 12.6 (2018) and 7.4 (2019) in the woodlands, compared with 1.2 (2018) and 0.03 (2019) in the urban area.

In 2018, 52.6% (n = 30) of the 57 transects had no cone/seed crop, of which 29 transects were located in the urban area (Fig. 3.19a). Of the 13 woodland transects with a cone/seed crop, two were classified as 'low' quality, seven as 'medium', and four as 'high'. Of the 14 urban transects with a cone/seed crop, eight were classified as 'low', five as 'medium', and one as 'high'. There was a statistically significant association between the habitat quality and the location of the transect

(Fisher's Exact test: $p < 0.0001$). A *post hoc* pairwise comparison with FDR correction found that there was a higher number of transects with no cone/seed crop found in the urban area.

In 2019, 64.9% (n = 37) of the 57 transects had no cone/seed crop, of which 36 transects were located in the urban area (Fig. 3.19b). Of the 13 woodland transects with a cone/seed crop, two were classified as 'low', ten as 'medium', and one as 'high'. Of the seven urban transects with a cone/seed crop, four were classified as 'low', two as 'medium', and one as 'high'. Again, there was a statistically significant association between the habitat quality and the location of the transect (Fisher's Exact test: $p < 0.0001$). A *post hoc* pairwise comparison with FDR correction again found that there was a higher number of transects with no cone/seed crop found in the urban area.

There was a significant difference in the energy content of the transects in the woodlands compared to the urban area (Mann Whitney U test: $W = 52$, $p = 0.036$), with the woodland transects having a higher median energy content ($\tilde{x} = 2583.23$ kJ) compared to the urban transects ($\tilde{x} = 440.34$ kJ).

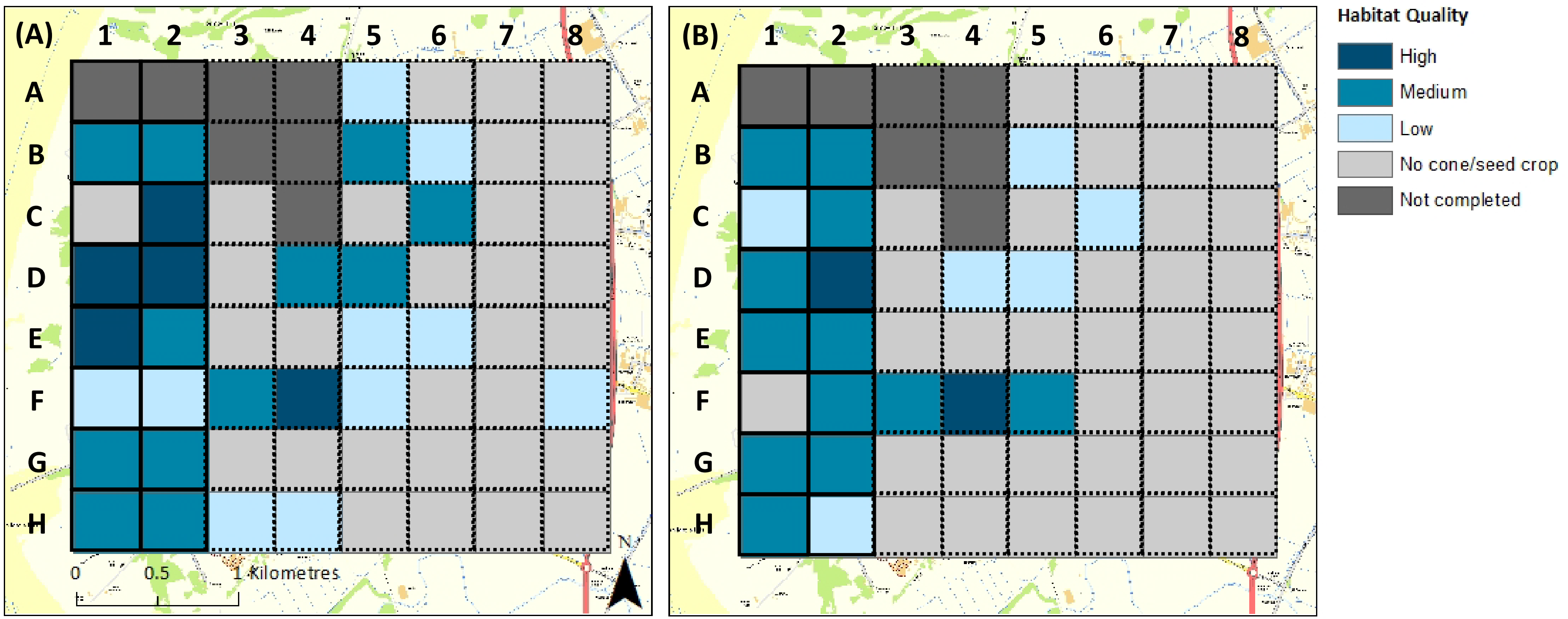


Figure 3.19. Habitat quality, based on the total energy content calculated per cone/seed crop transect survey, across the study site in (a) 2018 and (b) 2019. The grid squares of the woodland transects (columns one and two) are highlighted by solid outlines and the grid squares of the urban transects (columns three to eight) are highlighted by dotted outlines (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

3.3.3. Bone Strength and Mineral Content

There was a significant difference between the residuals of bone strengths of adult red squirrels from Merseyside and Dumfries & Galloway (t_{168} = -4.436, p < 0.0001). The squirrels from Merseyside had significantly stronger bones (115.15 N ± 19.09 SD) and higher mineral content (36.27% ± 2.82 SD), compared to bone strengths (89.40 N ± 22.08 SD) and mineral content (33.92% ± 3.04 SD) of the squirrels from Dumfries & Galloway (Fig. 3.20).

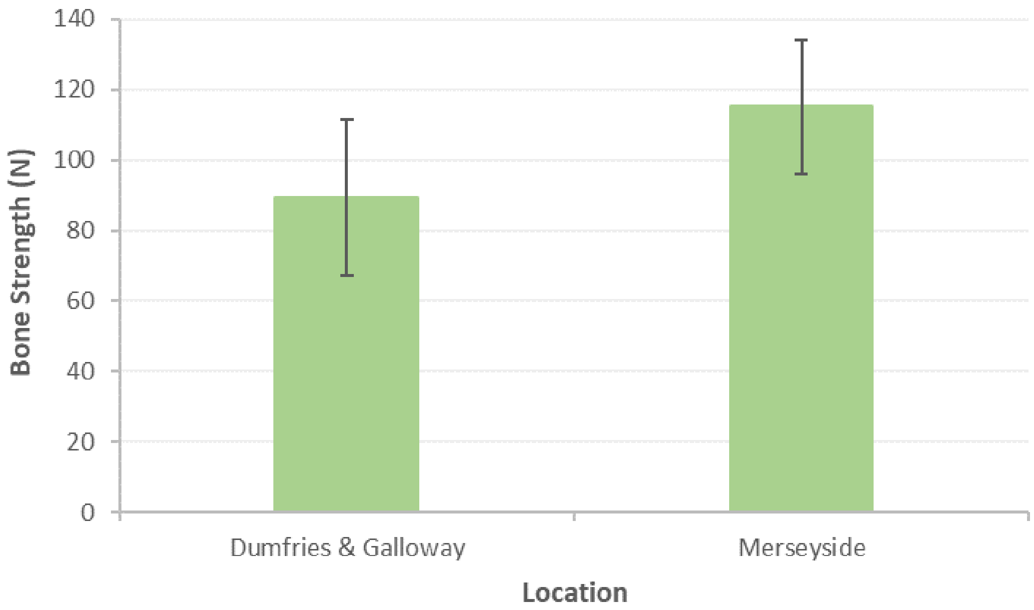


Figure 3.20. Mean bone strength (N ± SD) of tibias collected from necropsied red squirrels from Merseyside (n = 133) and Dumfries & Galloway (n = 37).

Within Dumfries & Galloway, there was no significant difference in bone strength between males (93.20 N ± 18.88 SD) and females (84.92 N ± 25.18 SD; t_{35} = -1.143, p = 0.26), or bone length between males (59.36 mm ± 1.87 SD) and females (59.24 mm ± 2.03 SD; t_{35} = -0.184, p = 0.86). Within Merseyside, there was no significant difference in bone strength between males (114.08 N ± 19.26 SD) and females (115.65 N ± 18.96 SD; t_{130} = 0.470, p = 0.64), or bone length between males (60.75 mm ± 1.76 SD) and females (61.05 mm ± 1.59 SD; t_{130} = 1.028, p = 0.31). There was also no significant difference in bone strength between breeding (117.03 N ± 19.58 SD) and non-breeding (113.62 N ± 18.68 SD) individuals (t_{130} = 1.006, p = 0.31). In addition, there was no significant difference in bone strength between individuals located in the woodlands (115.01 N ± 14.33 SD) and those from the urban area (114.89 N ± 20.51 SD; t_{105} = -0.023, p = 0.98), or bone length between individuals located in the woodlands (61.09 mm ± 1.26 SD) and those from the urban area (60.80 mm ± 1.76 SD; t_{105} = -0.654, p = 0.51).

3.3.4. Population Mortality

3.3.4.1. Annual and Seasonal Patterns in Population Mortality

From all 439 recorded instances of red squirrel mortality, there was a significant difference in the frequency of mortality between years (Chi-Square Goodness of Fit test: X^2 = 83.20, df = 4, p < 0.0001; Fig. 3.21). A *post hoc* pairwise comparison with FDR correction found that population mortality in both 2018 (n = 118) and 2019 (n = 147) was significantly higher than in 2015 (n = 54), 2016 (n = 50), and 2017 (n = 70).

There were two clear peaks of population mortality, one in July and another in September/October (Fig. 3.21). There was a significant difference in the frequency of mortality between seasons (Chi-Square Goodness of Fit test: X^2 = 134.84, df = 3, p < 0.0001). A *post hoc* pairwise comparison with FDR correction found that population mortality in the summer (n = 163) and autumn (n = 177) was significantly higher compared to spring (n = 59) and winter (n = 40).

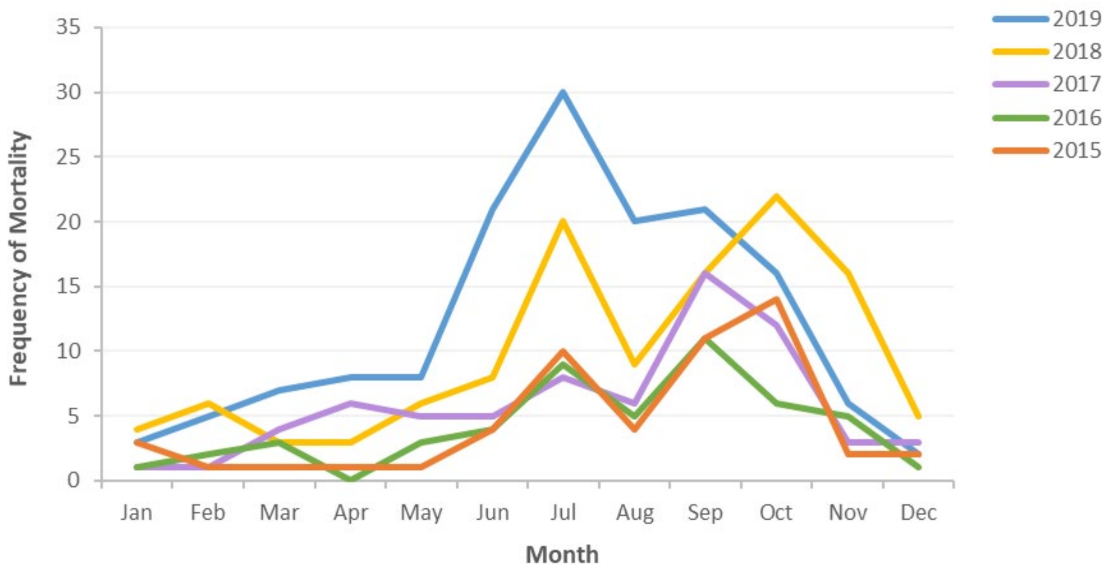


Figure 3.21. Monthly frequencies of red squirrel mortality in the Merseyside stronghold, from 2015 to 2019 (n = 439).

From only the necropsied individuals (n = 186, excluding the nine unlabelled individuals and any individuals that could not be sexed due to decomposition), there were no significant associations in male and female mortality between seasons (Chi-Square Test of Independence: $X^2 = 3.26$, df = 3, p = 0.35; Fig. 3.22), or in sub-adult/juvenile and adult mortality between seasons (Chi-Square Test of Independence: $X^2 = 4.96$, df = 3, $p = 0.17$; Fig. 3.23). However, there was a significant association between breeding condition and seasonal mortality (Fishers Exact test: $p < 0.0001$). A *post hoc* pairwise comparison with FDR correction found that there were more deaths of non-breeding individuals in autumn (Fig. 3.24).

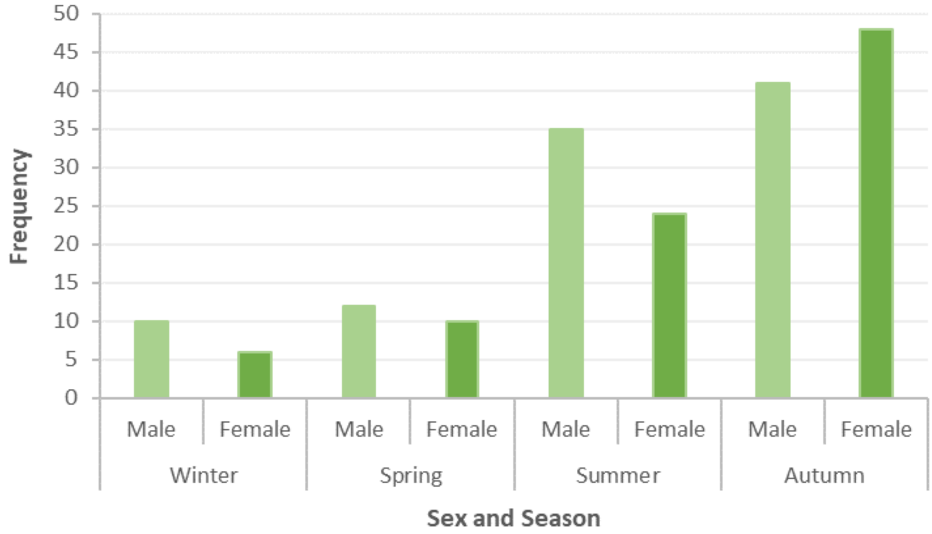


Figure 3.22. Seasonal frequencies of male and female casualties, identified from necropsies (n = 186).

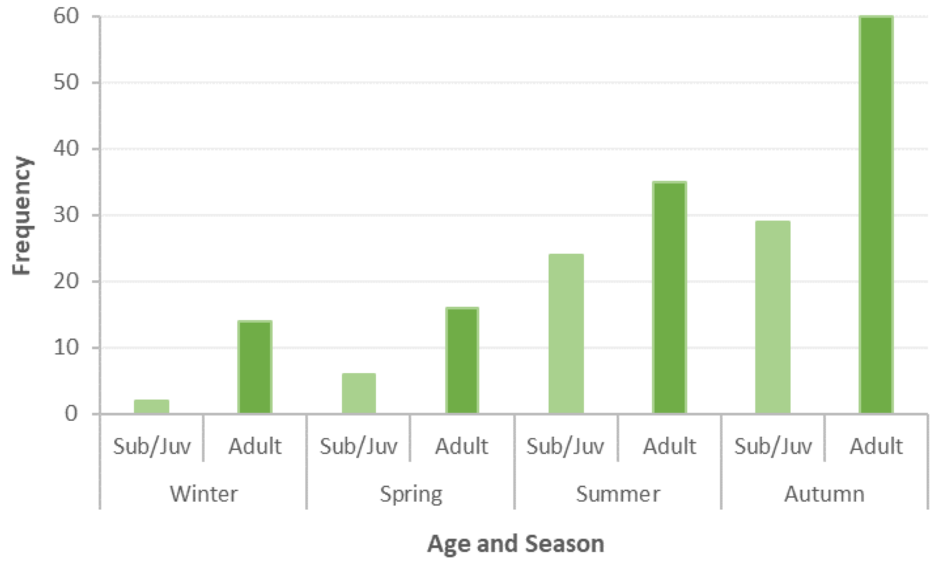


Figure 3.23. Seasonal frequencies of sub-adult/juvenile and adult casualties, identified from necropsies (n = 186).

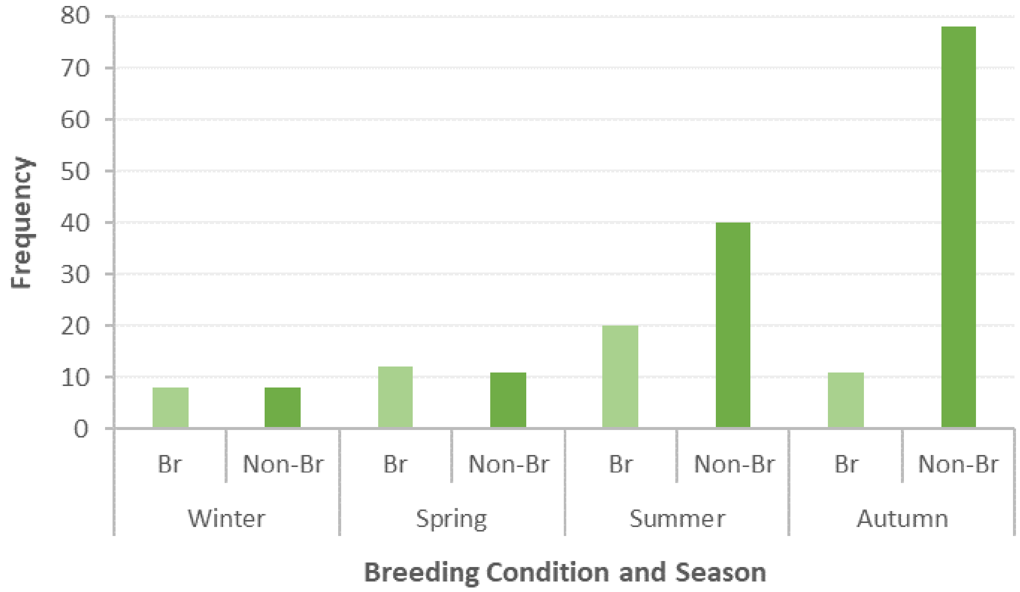


Figure 3.24. Seasonal frequencies of breeding (Br) and non-breeding (Non-Br) casualties, identified from necropsies (n = 186).

3.3.4.2. Population Demographics

Of the 200 individuals that were necropsied, 67.5% were adults, 11.5% were sub-adults, and 21% were juveniles (Fig. 3.25). Furthermore 51% (n = 102) were males, of which 28.4% were in breeding condition, and 47.5% (n = 95) were females, of which 25.3% were in breeding condition. It was not possible to sex three individuals (two juveniles and one adult) due to the level of decomposition. Two of the breeding females were found to be pregnant, each with three embryos.

Parasite burdens were determined to be high for 7.5% of individuals, moderate for 8.5%, and low for 30.5%, but there was no parasite burden for 53.5% of the carcasses (Fig. 3.26). Excluding individuals with no parasite burden, there was a statistically significant association of parasite burden with age (Chi-Square Test of Independence: X^2 = 13.04, df = 2, p = 0.0015). A *post hoc* pairwise comparison with FDR correction found that adults were more likely to have low parasite burdens. In comparison with the live population (as determined during the live-capture trapping, as discussed in Chapter Two), excluding the dead individuals with no parasite burden, there was a higher proportion of the necropsied individuals with a moderate/heavy parasite burden (34.4%) compared with the live population (16.4%).

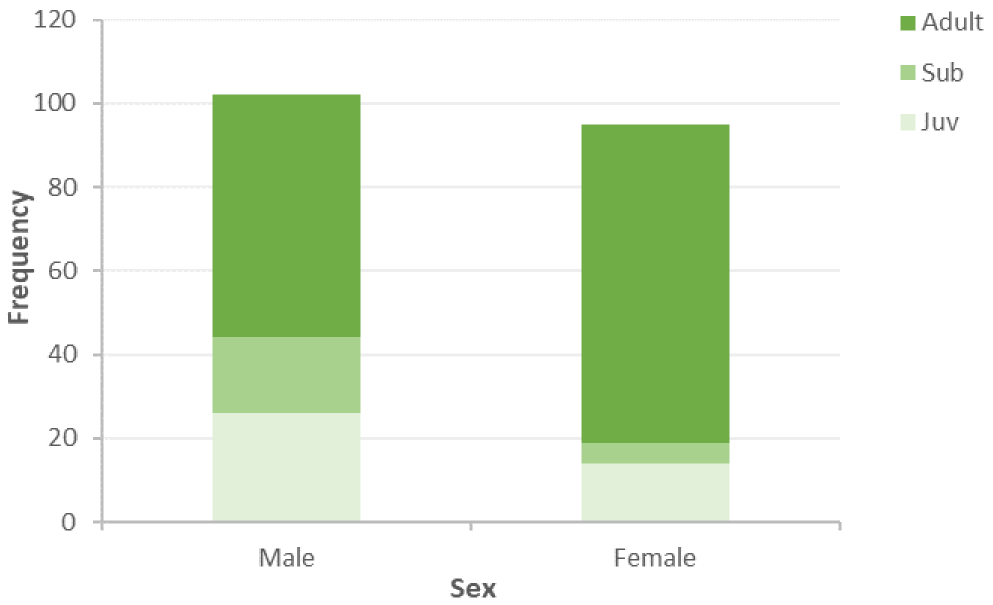


Figure 3.25. Frequencies of adult, sub-adult and juvenile males and females identified during necropsies (n = 197).

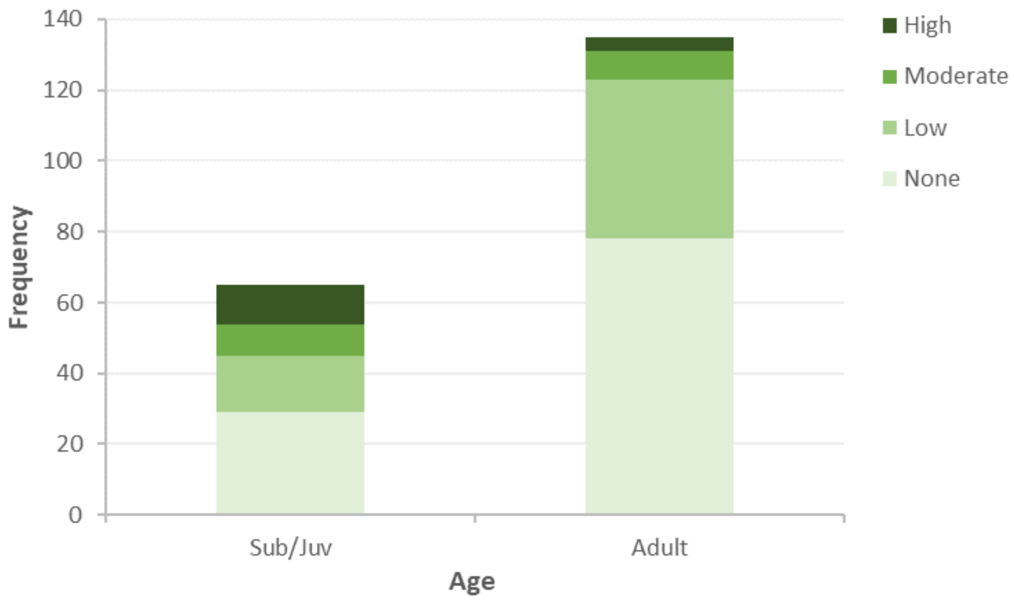


Figure 3.26. Parasite burdens of sub-adult/juvenile and adult squirrels identified during necropsies (n = 200).

3.3.4.3. Causes of Mortality

Of the 200 individuals that were necropsied, 15.5% died or were euthanised whilst in veterinary care due to the extent of their injuries or disease and 20% were determined as 'inconclusive'.

3.3.4.3.1. Road Traffic

Road traffic (both confirmed and suspected) accounted for 32.5% of mortality. It is also likely that at least some of the other casualties with traumatic injuries, where the cause of injury could not be determined, may have been due to road traffic, in which case road traffic could account for up to 47.5% of mortality. Of the 65 road traffic casualties across the Merseyside stronghold, 47.7% (n = 31) were males, of which 32.3% (n = 10/31) were in breeding condition, and 49.2% (n = 32) were females, of which 40.6% (n = 13/32) were in breeding condition, whilst two individuals could not be sexed due to the level of decomposition. In addition, 80% of the road traffic casualties were adults.

There was a significant difference in the seasonal frequencies of road traffic casualties (Chi-Square Goodness of Fit test: $X^2 = 18.27$, df = 3, $p < 0.001$). A *post hoc* pairwise comparison with FDR correction found that the greatest proportion of road traffic casualties occurred in the autumn (40%) and summer (30.8%) compared to the winter and spring (10.8% in both seasons; Fig. 3.27). However, there were no significant associations in the frequencies of male and female road traffic casualties (Fisher's Exact test: $p = 0.85$) or of sub-adult/juvenile and adult (Fisher's Exact test: $p = 0.68$) road traffic casualties between seasons.

Figure 3.27. Monthly frequencies, totalled across 2015 to 2018, of road traffic casualties (*n* = 60, excluding the unlabelled individuals for which the date of death was unknown).

Of the 65 road traffic casualties, 83.08% (*n* = 54) were located within the study site, with the highest numbers of casualties reported near the entrance to the National Trust woodlands on Victoria Road and Larkhill Lane (box A in Fig. 3.28) and the northern end of Gores Lane (box B). There were additional 'hotspots' of road traffic mortality on Freshfield Road (box C), near Freshfield Caravan Park (box D), on Kirklake Road and Bushbys Lane (box E), and around Duke Street Park (box F).

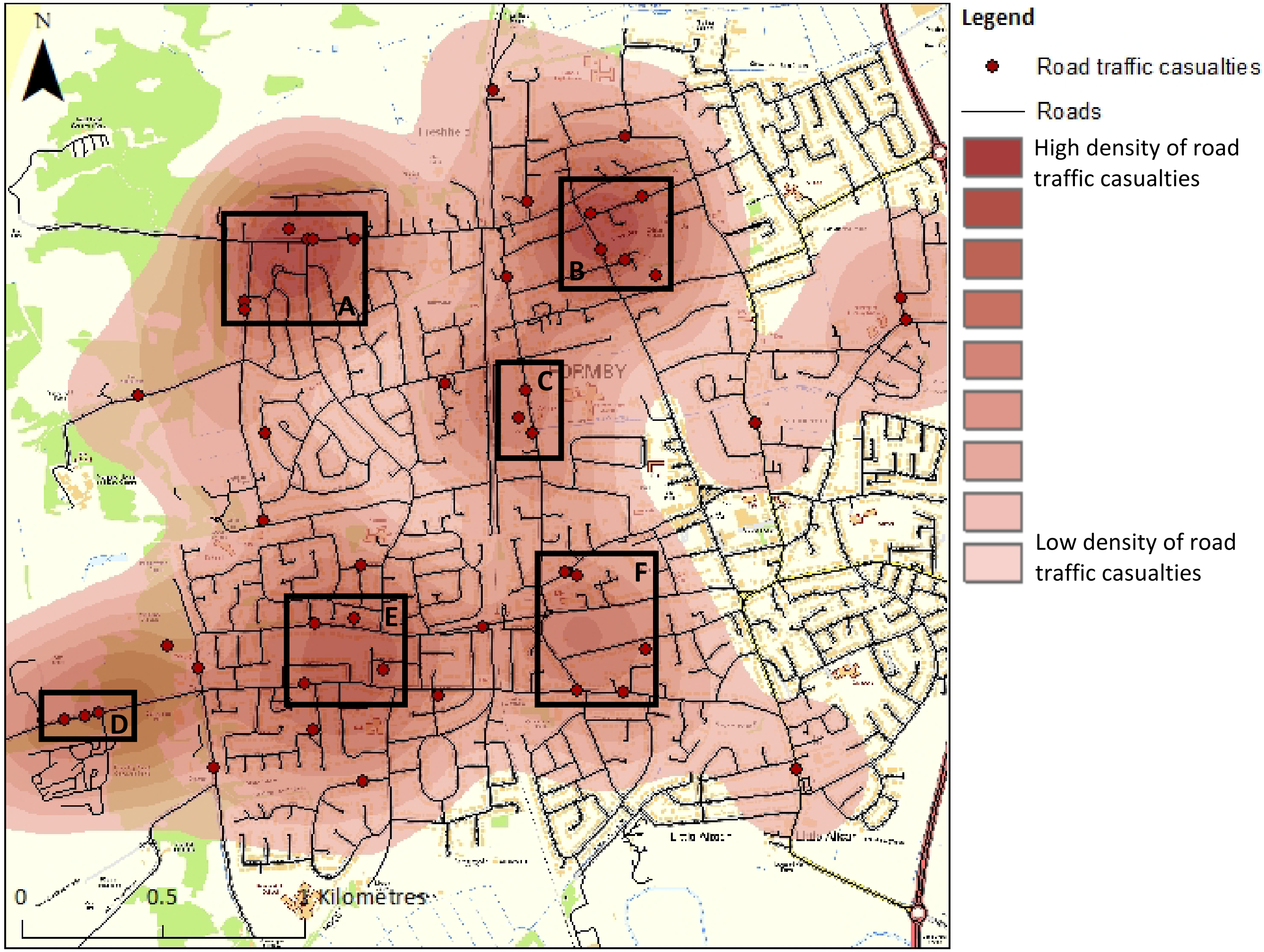


Figure 3.28. 'Hotspots' of road traffic mortality, with locations of individual road traffic mortality incidents marked as points (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

3.3.4.3.2. Other Causes of Trauma

A suspected fall (e.g. from a tree, drey, or building) accounted for 5.5% of mortality, of which 72.7% of the casualties were juveniles or sub-adults (Fig. 3.29). Of the three adults, there was only one breeding male whilst the other male and female were non-breeding. Four of the juveniles were likely to have been littermates, having all been found in the same location within a day of each other.

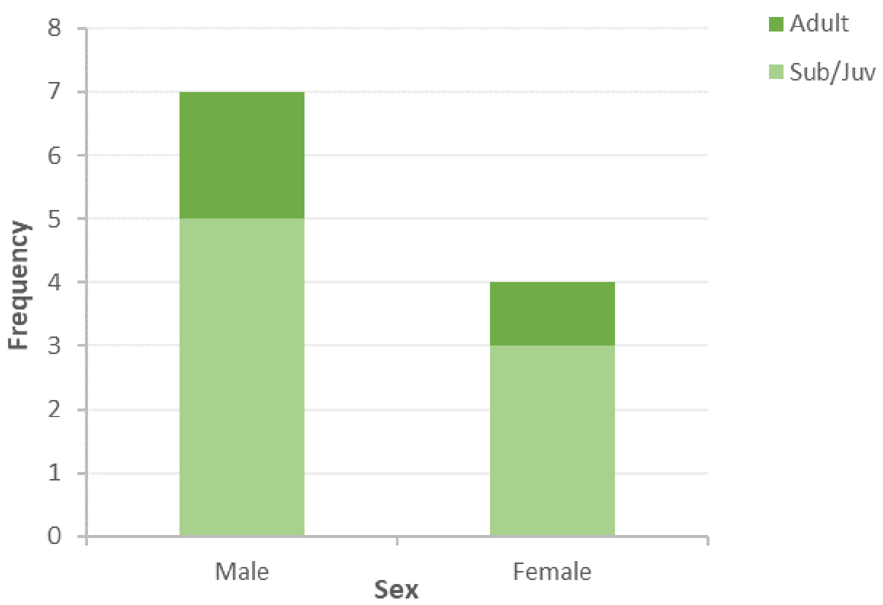


Figure 3.29. Frequencies of sub-adult/juvenile and adult male and female casualties of suspected falls (*n* = 11).

Other trauma, where the cause of injury could not be determined, accounted for 15% of mortality. Of these 30 individuals, two also showed signs of suspected SQPV, two showed signs of a subsequent infection from the trauma, and three showed signs of malnutrition. Overall, there were relatively equal numbers of males (53.3%) and females (46.7%), adults (53.3%) and sub-adults/juveniles (46.7%; Fig. 3.30). However, a greater proportion of the sub-adults/juveniles were male (78.6%), whilst a greater proportion of the adults were female (68.8%).

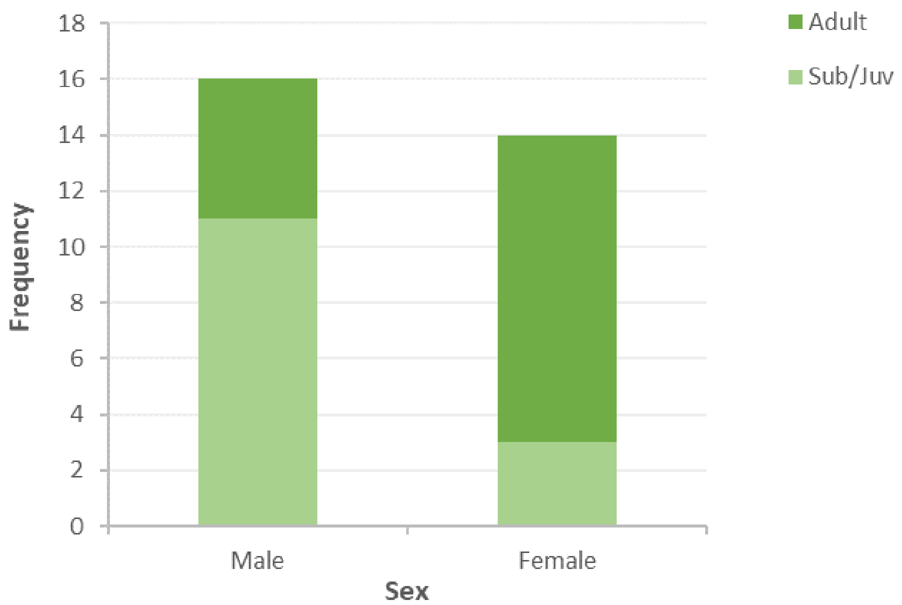


Figure 3.30. Frequencies of sub-adult/juvenile and adult male and female casualties of other traumatic injuries (n = 30).

3.3.4.3.3. Disease

Diseases (both confirmed and suspected) accounted for 20.5% of mortality. There was a significant difference in the annual frequencies of disease-related casualties (Chi-Square Goodness of Fit test: X^2 = 15.29, df = 3, p = 0.0016). A *post hoc* pairwise comparison with FDR correction found that disease-related mortality was higher in 2018, of which a higher proportion of casualties were confirmed/suspected SQPV deaths compared with the preceding three years (Fig. 3.31). There was, again, a similar seasonal pattern of mortality (Chi-Square Goodness of Fit test: X^2 = 21.93, df = 3, p < 0.0001), with a *post hoc* pairwise comparison with FDR correction indicating that a higher number of disease-related casualties were recorded in autumn (53.7%) compared to summer (26.8%), spring (14.6%), and winter (4.9%).

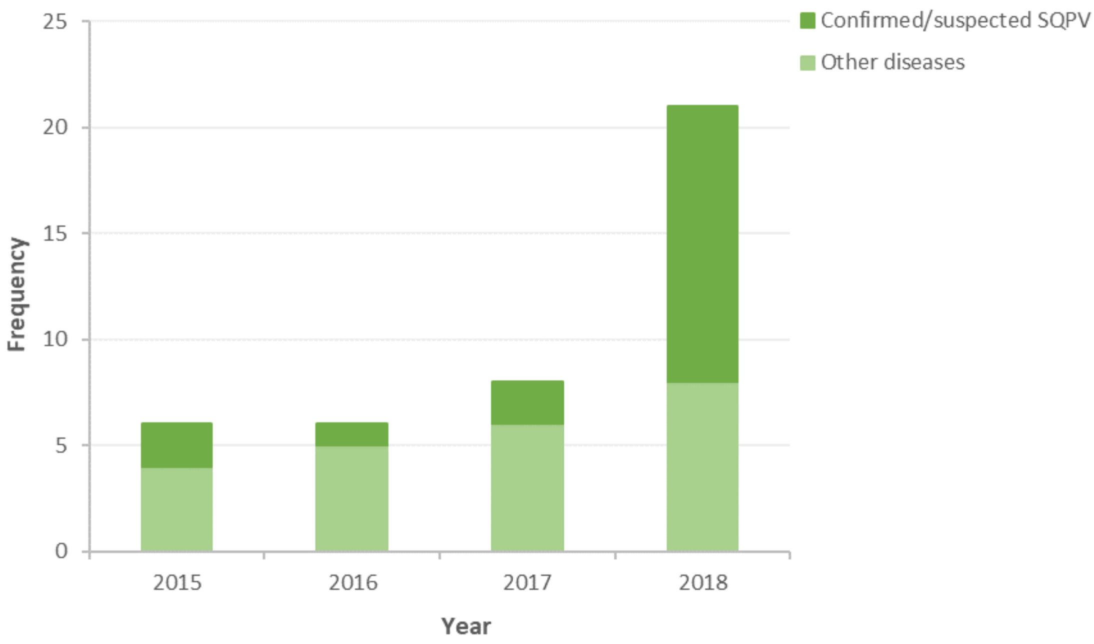


Figure 3.31. Annual disease-related mortality from 2015 to 2018 (*n* = 41).

Of those individuals that were tested for SQPV (*n* = 15), 46.7% tested positive. There were also 15 suspected cases in addition to the seven confirmed cases of SQPV, for which the disease was considered to be the primary cause of death for 11 individuals. For the remaining four individuals, the primary cause of death was attributed to road traffic or other trauma. There were an additional two suspected SQPV cases in 2015 and 2018, for which the carcasses were not collected and so could not be necropsied, as well as 26 suspected cases in 2019, for which the carcasses were not necropsied.

Prior to 2018, all of the confirmed/suspected SQPV cases were located outside of the study site in the wider Merseyside stronghold (e.g. in Churchtown, Scarisbrick, Blundellsands), with one or two cases each year from 2015 to 2017. From late summer/autumn of 2018 onwards, there are a number of SQPV casualties within Formby, predominantly in the southern woodland and the immediate adjacent urban area (Fig. 3.32).

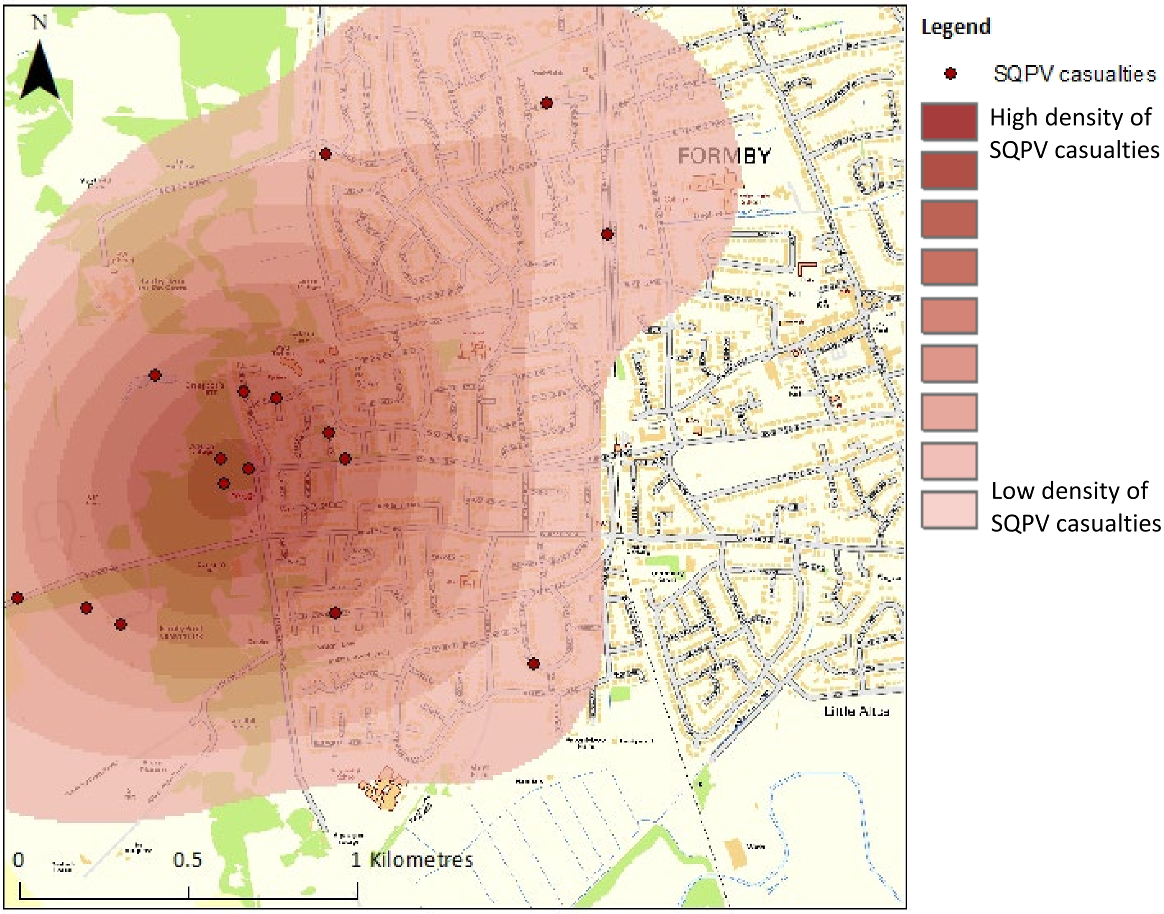


Figure 3.32. SQPV mortality in 2018, with locations of confirmed and suspected SQPV casualties marked as points (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Digimap Ordnance Survey Service (2016)).

Of those individuals that were tested for adenovirus (n = 16), 81.3% tested positive. Only one individual tested positive by TEM, which suggests a pathogenic case of adenovirus, whereas the remaining 12 individuals tested positive by PCR that suggests these were asymptomatic cases. Of the seven individuals that were tested for both SQPV and adenovirus, one tested positive for both diseases whilst the others only tested positive for adenovirus. The one suspected leprosy case that was sent to the University of Edinburgh tested negative.

There were also 22 individuals showing signs of other diseases, some of which were identified by the veterinary surgeon (e.g. one individual with a suspected abdominal hernia) or during necropsy (e.g. one individual with spinal tumours). The others showed signs of infection (e.g. enlarged spleen, faecal staining around the anus) but the disease could not be confirmed.

3.3.4.3.4. Predation

Predation only accounted for 2% of deaths, where one individual was confirmed as predated by a domestic cat, one by a domestic dog, and two by an unknown species. Three of the casualties were males (two adults, one of which was in breeding condition and the other was non-breeding, and one juvenile) and the fourth casualty was a juvenile female. The two juveniles were predated in the summer of 2017 and the spring of 2018, the latter of which was attacked by a cat and then euthanised at the vets. The breeding male was predated in early 2018, whilst the non-breeding male was killed by a dog in the autumn of 2015.

There were an additional four predation events recorded, three of which were due to domestic cats and one suspected by a fox. However, two of the carcasses were not collected and so could not be necropsied, whilst the other two were collected in 2019 and so were not necropsied. The three cat predation events occurred in the autumn of 2016 (unknown age and sex), the spring of 2017 (juvenile of unknown sex), and the autumn of 2019 (non-breeding adult male). The suspected fox predation event occurred in the summer of 2019, of an adult male squirrel suspected of suffering with SQPV.

3.3.4.3.5. Other Causes of Death

There were two cases (1%) of suspected accidental poisoning, mostly likely with rodenticide, of one sub-adult male in 2015 and one adult breeding female in 2018. There were also two cases (1%) of suspected anaemia due to particularly high flea burdens, which were two juvenile females from the same litter in 2017. In addition, there were 11 individuals (5.5%) with suspected malnutrition or dehydration, six of which also exhibited signs of injury or disease and seven were sub-adults/juveniles. Excluding one unlabelled individual, as the date collected was unknown, 70% of these cases were recorded in the summer and the remaining cases were recorded in the autumn.

3.4. DISCUSSION

Key findings from each topic of the results section (supplemental feeding including the impact on bone strength and mineral content, natural food sources, and population mortality) have been summarised below (Table 3.4).

Table 3.4. Summary of key findings from Chapter Three.

Topic	Key Findings
Supplemental feeding survey	• 75.5% of respondents saw a red squirrel at least once a week, with 41.7% seeing one every day • 75.5% of respondents provide supplemental food, with 81.3% providing peanuts and monkey nuts • 78% of respondents provide < 3 kg of supplemental food per month • 81.3% of respondents provide supplemental food throughout the year • 86.5% of respondents provide water sources • 18.5% of respondents clean their feeders at least monthly using soap and/or disinfectant
Habitat quality	• Mean cone and seed crops were higher in the woodlands compared to the urban area • Mean cone and seed crops were higher in 2018 compared to 2019 • 52.6% (2018) and 64.9% (2019) of transects had no cone/seed crop, of which 96.7% (2018) and 97.3% (2019) were located in the urban area ($p < 0.0001$) • Energy content was higher in the woodlands compared to the urban area ($p < 0.05$)
Bone strength and mineral content	• Merseyside squirrels had stronger bones and higher mineral content than Dumfries & Galloway squirrels ($p < 0.0001$) • No significant differences in bone strength or length between males and females within the Dumfries & Galloway population • No significant differences in bone strength or length between males and females, breeding and non-breeding individuals, or urban and woodland individuals within the Merseyside population
Population mortality	• Population mortality was higher in 2018 – 19 compared to 2015 – 17 ($p < 0.0001$), due to higher SQPV mortality • Population mortality was higher in summer and autumn compared to spring and winter ($p < 0.0001$)

	• No significant seasonal differences in the frequency of casualties between males and females or adults and sub-adults/juveniles, but there were higher frequencies of non-breeding individuals in autumn ($p < 0.0001$) • 67.5% of casualties were adults, 11.5% were sub-adults, and 21% were juveniles • 51% of casualties were males (28.4% in breeding condition) and 47.5% were females (25.3% in breeding condition) • Adults were more likely to have low parasite burdens than sub-adults/juveniles ($p < 0.01$) • 53.5% of casualties had no parasite burden • 34.4% of casualties had a moderate/heavy parasite burden compared to 16.4% of live population • Up to 32.5% of casualties were considered to be road traffic, 20.5% due to diseases, 5.5% due to a suspected fall, 15% due to other trauma, 2% due to predation, 1% due to suspected accidental poisoning, 1% due to suspected anaemia, 2.5% due to suspected malnutrition or dehydration, and 20% were inconclusive • Disease-related mortality was higher in autumn compared to summer, spring, and winter ($p < 0.0001$) • 81.3% of 16 individuals tested positive for adenovirus, although all but one were asymptomatic • 80% of road traffic casualties were adults • Road traffic mortality was higher in summer and autumn compared to spring and winter ($p < 0.001$) • No significant seasonal differences in the frequency of road traffic casualties between males and females or adults and sub-adults/juveniles • 'Hotspots' of road traffic casualties were identified on Victoria Road and Gores Lane, as well as Freshfield Road, Freshfield Caravan Park, Kirklake Road and Bushbys Lane, and around Duke Street Park

3.4.1. Human-Provided and Natural Food Resources

3.4.1.1. Supplemental Feeding

The squirrels' preference for shelled food items (e.g. monkey nuts, hazelnuts) may be due to a phenomenon known as 'contra-freeloading', which has been observed in captive species, where animals prefer to work for a resource (e.g. via the action of having to open the shell) rather than easily accessing it (Osborne, 1977). It may also be due to these items being high in energy and less perishable, and therefore ideal for caching (Kostrzewa & Krauze-Gryz 2020).

The high density of red squirrels in the vicinity of 'Squirrel Walk' (see section 2.4.4, p. 78) was likely due to the daily provision of large quantities of supplemental food by the National Trust that occurred until mid-2018. Following the cessation of supplemental feeding due to the 2018/19 SQPV outbreak, it is suspected that some of the squirrels may have subsequently dispersed into other parts of the woodland with more ample natural food resources (R Cripps, *pers. comm.*). This may impact the number of visitors to the site if they are less likely to see squirrels at 'Squirrel Walk', although it is likely (albeit discouraged) that some visitors will continue to bring their own supplemental food to feed the squirrels.

It is to be expected that most respondents only see one squirrel at a time, as arboreal squirrels are generally solitary (Bosch & Lurz 2012). The number of respondents reportedly seeing squirrels every day (41.7%) or at least once per week (75.5%) was lower in comparison with another previous study conducted in Formby, in which approximately 66.5% of respondents reported seeing squirrels daily and 87.5% at least once per week (n = 64; Hughes 1991, *unpublished data*, as cited in Shuttleworth 1996). In addition, the mean number of squirrels reported (1.48 ± 0.74 SD) was lower (3.06 ± 0.2 SD; Hughes 1991, *unpublished data*, as cited in Shuttleworth 1996). This may be because the public survey unintentionally coincided with the 2018/19 SQPV outbreak and so may reflect the reduced population abundance as a result of the increased disease mortality. One respondent also noted that they had several squirrels regularly visiting a decade prior, but this had not been the case since the previous 2007/08 SQPV outbreak. This suggests that, although the population had recovered (see section 2.3.3, p. 66), the red squirrels may not have dispersed and reoccupied their previous distribution within the study site, possibly due to changes in food availability or habitat corridors. Another respondent noted that they had noticed a reduction in the number of squirrels visiting their garden since a nearby corridor of trees had been removed, so urban intensification may be another reason for the difference between this study's survey and the previous survey (Shuttleworth 1996), impacting how the squirrels move around the urban landscape.

The public survey suggests that the provision of supplemental food and water for wildlife is widespread throughout Formby, including approximately half of the respondents specifically providing food for the squirrels via feeder boxes. In addition, residents with hanging bird feeders and tables appear to refrain from using baffles, which are often installed to prevent grey squirrels from accessing bird feeders, which suggests they are content to allow the red squirrels access to the bird food. This reflects the residents' enthusiasm and connection with their local red squirrel population. It is also positive that the majority of the respondents provide water in their gardens, as this may become more vital with more frequent heatwaves (as occurred in 2018; McCarthy et al. 2019) associated with climate change.

The public survey suggests that most people only supply relatively small quantities (< 3 kg) of food each month, but with so many people providing supplemental food throughout the year, including some large quantities (> 8 kg per month), this accumulates to relatively large amounts of human-provided food resources across the study site. In addition to supporting higher numbers of squirrels, such extensive supplemental feeding may be encouraging the squirrels to remain within the local area rather than dispersing into the wider stronghold (Starkey & delBarco-Trillo 2019). By encouraging the residents to feed less, both in terms of quantity and frequency, alongside potentially installing supplemental feeders in nearby woodlands, this may attract the squirrels to disperse out of Formby into the wider stronghold (Starkey & delBarco-Trillo 2019). This would have the benefits of both reducing the population density within Formby, thereby reducing the risk of disease outbreaks, as well as contributing to the population recovery across North Merseyside and West Lancashire, which is one of the aims of the LWT Red Squirrel Project. However, it is important to highlight that ongoing grey squirrel control would be essential to allow the dispersing red squirrels to survive and establish in the wider stronghold.

Although supplemental feeding can clearly be beneficial for urban wildlife, in terms of providing abundant and reliable food sources and encouraging positive human-wildlife interactions, there are also risks associated with higher population densities and sharing of feeders that could lead to local disease outbreaks. General guidance from wildlife conservation organisations (e.g. RSPB, the Wildlife Trusts) states that feeders should be cleaned at least once a month using soap or disinfectant, but this public survey highlighted that less than a fifth of respondents maintain this standard of hygiene. Furthermore, the LWT Red Squirrel Project team do encourage residents to temporarily remove feeders following reports of SQPV cases, but this is not always successful due to the public perception that the squirrels will starve without access to the supplemental food (*pers. obvs.*), which can then exacerbate SQPV outbreaks. These findings raise concerns for potential future disease outbreaks in the study site, particularly in such a high-density red squirrel population and one that has already suffered from two previous SQPV outbreaks.

3.4.1.1.1 Provision of Peanuts

The public survey highlights that peanuts (including monkey nuts) are the most widespread and abundant supplemental food item. As previously discussed in Chapters One and section 3.1.3.1, peanuts are high in fat but very low in calcium, so over-consumption may lead to malnourishment and calcium deficiencies (Bosch & Lurz 2012). However, the findings from the bone strength and mineral content analyses suggest that this is not the case in the Merseyside red squirrel population, which may be due to the availability of natural food sources (which will be further discussed in section 3.4.2). As reported by Shuttleworth (2000), peanut consumption was not determined by its availability but instead was negatively correlated with pine cone abundance, as was the consumption of alternative low-energy but calcium-rich natural food sources such as buds and flowers. This suggests that the chemical composition of peanuts may limit the amount that squirrels can consume and that they can compensate for any nutritional deficiencies as long as there are a variety of natural food sources available (Shuttleworth 2000, Thomas et al. 2018). This is further supported by the fact that supplemental feeding regimes have been ineffective in preventing red squirrel population declines when natural food sources are scarce (Shuttleworth 1997). This, again, highlights the importance of maintaining high-quality and diverse urban greenspaces to provide a wide variety of natural food sources for the red squirrel population.

However, it is likely that the Dumfries & Galloway squirrels may have had at least some access to supplemental food, as supplemental feeding of wildlife is so prevalent across the UK and some of the dead squirrels were found in residential gardens or on the outskirts of villages. Further comparative analyses with other red squirrel populations, both across the UK and from mainland Europe, would be beneficial.

3.4.1.2. Natural Food Resources

It is well-known that seed-consumers, such as red squirrels, are impacted by the availability of pulsed resources and mast seeding (e.g. Salmaso et al. 2009, Selonen et al. 2015). It is likely that the differences in the mean cone and seed crops between 2018 and 2019 were as a result of these natural fluctuations in resources. It is also unsurprising that the mean cone and seed crops, and consequently the overall energy content, were higher in the woodlands compared to the urban area, particularly considering the number of transects across the latter that had no cone or seed crop. In addition, the availability of natural food resources closely corresponds with the population distribution (see section 2.3.2, p. 64), with limited or no seed crop in the eastern half of the study site and correspondingly fewer red squirrel sightings. However, there are high-quality greenspaces (e.g. parks, woodland patches) embedded within the urban landscape, providing natural food resources to support the urban population of red squirrels, which is also reflected in the population distribution. It is also likely that many private residential gardens, which were inaccessible for this survey, contain natural food sources. These urban greenspaces are particularly important, considering the widespread supplemental feeding especially of peanuts, to allow the squirrels to forage for more nutritionally balanced items and compensate for any potential malnourishment from the supplemental food (Thomas et al. 2018). Therefore the loss of these greenspaces through infill densification (*pers. obvs.,* Pauleit et al. 2005) is concerning with regard to the long-term availability of habitat corridors for dispersal, nesting sites, and natural food resources for the urban population of red squirrels.

3.4.2. Population Mortality

The increase in mortality in 2018 and 2019 compared with the preceding three years is unsurprising considering the SQPV outbreak that occurred in the latter half of 2018 through to 2019, which will be further discussed in section 3.4.2.2.

There is a clear seasonal pattern in overall population mortality, with a peak in July followed by another higher peak in September/October. The same seasonal pattern was separately identified for both disease and road traffic mortality, which suggests these causes of death were contributing to the overall seasonal pattern. This supports Shuttleworth's (2001) study, who also found a seasonal pattern in road traffic mortality with a peak in the autumn. There were no sex- or age-related seasonal differences in mortality, which suggests that the seasonal pattern was not due to higher mortality of males/females or adults/juveniles, although there was a higher mortality of non-breeding individuals. However, Shuttleworth's (2001) study found that adult males, particularly those that were reproductively active, were more likely to be killed by road traffic during the winter. In contrast, the findings from this research suggest the seasonal pattern in mortality is not due to breeding individuals of either sex looking for mates nor dispersing juveniles establishing home ranges (as previously discussed in Chapter One and section 3.1.3.3.1). Instead, increased natural food availability in the autumn and the provision of supplemental food both lead to increased terrestrial foraging and scatter-hoarding behaviours (Shuttleworth 2000, 2001). This could result in squirrels traversing roads more frequently as they search for and cache food items, as well as increased disease spread through sharing feeders (Shuttleworth 2001, Chantrey et al. 2014). The latter may also be exacerbated when both natural and cached food sources are depleted during the summer months, resulting in squirrels relying more heavily on supplemental food sources during the summer and early autumn until natural food sources become available again. Furthermore, dominant breeding individuals may hold more stable, higher quality territories with more reliable food sources, whilst non-breeding individuals may have to shift their home ranges in response to food availability or may hold larger, poorer quality territories (Wauters & Dhondt 1992, Wauters et al. 2001b). This may result in non-breeding individuals having to move further to locate food sources, therefore encountering roads more frequently or having to share feeders with other individuals.

Of the individuals that were necropsied, there was an approximate 1:1 sex ratio, which is comparable to the live population (see section 2.3.1.2, p. 61) and suggests there is no sex-biased mortality in this study. However, there was a higher proportion of juveniles compared to the number that was live-capture trapped. As discussed earlier in this section and will be further discussed in section 3.4.2.1, this appears not to be due to dispersing juveniles encountering road traffic. Instead, it may be that juveniles are more prone to predation, starvation, or disease.

In comparison with the live population, there were twice as many necropsied individuals with a heavy/moderate parasite burden, which is to be expected considering the association between poor body condition and a higher ecto-parasite burden (LaRose et al. 2010). Additionally, as with the live population, necropsied sub-adults/juveniles tended to have higher parasite burdens in comparison with the adults. There were two cases, both juveniles from the same litter, where the heavy flea burden and resultant anaemia was considered to be the primary cause of death, suggesting that the higher parasite burdens in sub-adults/juveniles may be due to the parasite loads that build up in the dreys.

Whilst all the live individuals had at least some ecto-parasites, parasites were absent from the majority of the necropsied individuals due to the fact that ecto-parasites will tend to abandon a deceased host. As many of the necropsied individuals were collected several hours after being reported, or even days if found by chance, this would have allowed time for the ecto-parasites to abandon the carcasses. These findings are consistent with the published literature, which also found that ecto-parasites were only present on approximately 29 – 34% of the carcasses that were examined (LaRose et al. 2010, Simpson et al. 2013c).

3.4.2.1. Road Traffic

Although slightly lower compared with other published research (e.g. Shuttleworth 2001, LaRose et al. 2010, Simpson et al. 2013, Shuttleworth et al. 2015, Blackett et al. 2018), road traffic mortality was still the greatest cause of death in this study compared with other causes of mortality such as disease, predation, falls, etc. However, as previously discussed in both Chapter One and section 3.1.3.3.1, it is unclear whether roadkill records are being over- or under-estimated. The approximate 1:1 sex ratio of the road traffic casualties corresponds with the sex ratio of the live population, which suggests that there is not a sex bias in road traffic mortality. The autumnal peak in mortality and the fact that the majority of the casualties were adults is supported by other published studies (Shuttleworth 2001, LaRose et al. 2010, Shuttleworth et al. 2015, Fey et al. 2016, Blackett et al. 2018).

In addition to mortality, roads can indirectly impact on wildlife populations through habitat fragmentation and acting as barriers to movement (Rondinini & Doncaster 2002). As discussed in Chapter One, it appears that red squirrels' movement ability is not restricted within the urban landscape (Fey et al. 2016, Selonen et al. 2018, Hämäläinen et al. 2019), which suggests that this indirect impact may not be as much of a concern to urban red squirrel populations as the direct impact of mortality. The potential impact of roads acting as a barrier to movement will be further investigated in Chapter Four.

All the road traffic mortality 'hotspots' were located on the busier thoroughfares that are lined with corridors of trees, rather than the quieter residential streets. This suggests that the squirrels make use of these corridors for dispersing and moving throughout the town, which is supported by the fact that the locations of road traffic casualties closely correspond with the population distribution (see section 2.3.2, p. 64). In many locations the canopy cover does not fully meet from one side of the road to the other (*pers. obvs*.), which would force any squirrels to cross these busy roads at ground level and therefore increase the chances of encountering road traffic. In addition, the main locations of road traffic mortality were identified on Victoria Road, immediately at the entrance to the National Trust woodlands (see box A in Fig. 3.28 in section 3.3.4.3.1) and further eastwards at the intersection with Gores Lane (see box B in Fig. 3.28). This provides further support to the suggestion that the woodlands, particularly the northern part where the population density is higher, may be acting as a source population from where individuals disperse out into the urban area.

3.4.2.2. Diseases, including SQPV and Adenovirus

As previously mentioned, it is unsurprising that disease-related mortality increased in 2018, considering the confirmed SQPV outbreak in 2018/19 that reduced the population by approximately 50% (see section 2.3.3, p. 66). There appears to be occasional SQPV cases each year within the wider Merseyside stronghold, specifically in areas where the distributions of both red and grey squirrels overlap, thus increasing the likelihood of interactions between individual red squirrels and SQPV-carrying grey squirrels. However, due to the high population density within Formby, a lone SQPV-carrying grey squirrel can trigger an outbreak by infecting one or a few red squirrels followed by intraspecific transmission, which can be exacerbated by the presence of supplemental feeders acting as sources of disease spread (Chantrey et al. 2014). The 2018/19 outbreak appears to have been successfully contained in the south of the study site, potentially through positive actions by the LWT and local residents (e.g. rapid removal of infected individuals, removal and cleaning of supplemental feeders), preventing its spread to the Red Squirrel Reserve in the northern woodland.

SQPV testing was targeted to individuals with suspected symptoms (e.g. visible lesions), so those that tested negative were potentially suffering from a visually similar disease such as *Staphylococcus aureus*-associated FED. FED has already been identified as a cause for concern in two island populations on Jersey and the Isle of Wight (Simpson et al. 2010, Blackett et al. 2018, Fountain et al.

2021), so it may be beneficial to test for this disease in the isolated population in Formby, particularly as there were several casualties with visible lesions but negative SQPV tests. Although only a small number of random samples were tested for adenovirus, a high proportion tested positive for the disease. This suggests that the prevalence of adenovirus may be high in the population and further testing may continue to identify cases, although the majority of cases appear to be asymptomatic. It has been suggested that rats may act as a reservoir host for FED (Fountain et al. 2021) and wood mice for adenovirus (Everest et al. 2013, 2014). Therefore, red squirrels may be at risk of contracting these diseases even within red squirrel-only strongholds, particularly in urban environments where higher population densities of squirrels, rats, and mice will all be exploiting the available supplemental feeders.

Despite the SQPV outbreak, overall disease mortality appears to be lower (20.5%) compared with several other published studies (approximately 30% in LaRose et al. 2010, 35% in Simpson et al. 2013b, 34% in Blackett et al. 2018). This may be due to the fact that histological examinations were not conducted in this study, so some diseases (e.g. pneumonia, *T. gondii* infection) that were detected in the published research were not identified in this study.

3.4.2.3. Other Causes of Mortality

Predation, typically by free-ranging domestic cats, only accounted for a very small proportion of the overall mortality, which supports the findings in the existing published literature (Simpson et al. 2013c, Shuttleworth et al. 2015b, Fey et al. 2016, Blackett et al. 2018). However, predation events may be under-estimated if the carcasses are mostly consumed or carried away by predators, which may explain why most predation events can be attributed to domestic cats that often bring their prey home. Mitigation measures may help to reduce instances of predation by pets, for instance by asking owners to keep their cats inside during the early morning when squirrels are most active (Tonkin 1983). In reality, this may be difficult to achieve due to public perceptions of welfare implications associated with keeping cats indoors (Foreman-Worsley et al. 2021). Therefore, as there is little evidence to suggest that predation has significantly contributed to the red squirrels' decline, it would be more effective to focus any limited resources on other conservation management strategies.

Similarly to the published literature (LaRose et al. 2010, Simpson et al. 2013, Blackett et al. 2018), there was a small number of suspected accidental poisonings, most likely with anticoagulant rodenticides, although this is unlikely to significantly impact the red squirrel population.

Although only a small proportion of the overall population mortality, the majority of casualties from suspected falls were juveniles and sub-adults, which suggests that the exploring young may be most at risk from an accidental fall rather than, for example, breeding adults during a mating chase. In addition, it is likely that four of the juveniles were littermates, which may have resulted from their drey falling out of the tree (e.g. during a storm), although the drey itself was not found, or the litter may have abandoned the drey due to a parasite infestation, as all four individuals were found to have high flea burdens.

For those individuals with suspected malnutrition or dehydration, more than half also exhibited signs of injury or disease so it was likely that their symptoms were preventing foraging or eating/drinking adequately. For example, there was one individual with a fractured orbital socket that was severely underweight, which was suspected to have received a glancing blow from a car and the subsequent injuries were preventing it from eating adequately. Another individual, who was suspected to have died due to dehydration in the 2018 heatwave, also had a fractured front arm that may have hindered its ability to move and access water sources. In addition, the majority of suspected malnourished or dehydrated individuals were recorded in the summer months. This may be due to natural food sources not yet being available (as nuts and seeds typically mature in the autumn) alongside their over-winter food caches becoming depleted, which may disproportionately affect sub-adults and juveniles who may not yet have food caches to rely upon and may be prevented from accessing the remaining limited natural food sources or supplemental feeders by dominant adults.

This may also be exacerbated due to the seasonal ponds in the woodlands drying up during the hotter summer months.

3.5. CONCLUSION

These findings, in conjunction with the findings from Chapter Two, indicate that supplemental food sources are widespread and abundant, thereby supporting the high-density red squirrel population. There also appears to be an association between the habitat availability and quality, and therefore natural food sources, with the population distribution across the study site, particularly regarding greenspaces (e.g. parks, woodland patches) embedded within the urban landscape. Despite the extensive provision of peanuts, the squirrels do not appear to be suffering from calcium deficiencies, potentially due to the availability of natural food sources allowing them to switch their diet based on their nutritional requirements. However, it would still be beneficial to encourage residents to feed a wider variety of food items and potentially provide additional sources of calcium (e.g. deer antlers, cuttlefish bones). These findings also emphasise the importance of maintaining the urban greenspaces, including residential gardens, in terms of providing reliable and nutritionally balanced natural food sources, nesting sites, and habitat corridors for dispersal. In addition, it would be beneficial to improve the habitat within the woodland reserve, for example through management to provide more variety of natural food resources and additional water sources, to support the red squirrel population.

However, these findings also highlight the various risks to the red squirrel population. Such extensive supplemental feeding supporting a high population density and discouraging dispersal into the wider stronghold, combined with the lack of suitable feeder hygiene practices, threatens the population with further SQPV outbreaks. Therefore, it would be beneficial to encourage residents to provide less supplemental food, feed less frequently, clean their feeders regularly with disinfectant, and remove feeders promptly in response to any positive SQPV cases. It is also essential that grey squirrel control continues to prevent any incursion into the stronghold and to create space in the wider stronghold into which the red squirrels can disperse. In addition, urban greenspaces are being lost through infill densification, which is likely to impact the red squirrel population considering the importance of these resources in term of food availability and dispersal corridors.

Although grey squirrels and SQPV are the main threats to red squirrel populations, road traffic may put the population under additional pressure. Therefore, it may be beneficial to install aerial bridges at one or more of the identified mortality 'hotspots' to reduce this pressure. It would also be beneficial to further investigate adenovirus and its potential impact on the population, as the disease appears to be prevalent within the population but it is not yet fully understood.

The impacts of these resources and risks will be further investigated in relation to the red squirrels' spatial ecology in Chapter Four, incorporating both the findings from this chapter and Chapter Two.

4.0. Chapter Four: Home Ranges of Red Squirrels in an Urban Environment

Chapter Three examined the resources and risks for the red squirrel population in the study site of Formby, Merseyside, specifically supplemental feeding, the availability and quality of greenspaces and natural food sources, and mortality threats (e.g. road traffic, disease). This chapter analyses the home ranges of red squirrels in relation to these resources and risks, incorporating the findings from Chapter Three, to investigate how red squirrels use the urban environment in the study site.

4.1. INTRODUCTION

Understanding the spatial movements and associated habitat use of wildlife species is crucial for effective conservation management. Management actions can include defining protected areas (e.g. Kramer & Chapman 1999), controlling pest or invasive species (e.g. Lambert et al. 2008, Smith et al. 2015), decision-making for endangered species recovery (e.g. Plotz et al. 2016), and assessing the suitability of reintroductions (e.g. Schadt et al. 2002). These can be informed through quantitative analyses of home ranges, which Burt (1943) defined as the "area traversed by the individual in its normal activities of food gathering, mating, and caring for young". Burt (1943) also suggested that occasional exploratory forays outside the area should not be considered part of the home range and that animals will often move to other areas during their lifetime. Many animals may also have one or more core areas within their home range, which are used more intensively to exploit higher density patches of resources that are unequally distributed across the landscape (Powell 2000). The shape and size of home ranges are known to be influenced by factors such as body size, sex, diet (i.e. carnivorous or herbivorous), food availability, population density, and landscape structure (Massei et al. 1997, Anderson et al. 2005, Dahle et al. 2006, Naidoo et al. 2012, Tucker et al. 2014, Schoepf et al. 2015).

With the rapid global increase in urban expansion and intensification altering landscape structure and composition, there is an increasing need to understand the subsequent impact on the spatial ecology of wildlife species, particularly considering current global biodiversity loss. Many studies have indicated that home range sizes across taxa (mammals, birds, and reptiles) are smaller in urban environments compared with rural environments (O'Donnell & delBarco-Trillo 2020). This may be due to habitat loss and fragmentation limiting the extent of home ranges to the remaining suitable habitat, with barriers (e.g. roads, buildings) preventing movement between fragments, or the increased availability of anthropogenic food (e.g. supplemental feeding, bins, allotments) reducing the need to travel as far for resources (Rondinini & Doncaster 2002, Tigas et al. 2002, Mitsuhashi et al. 2018, Thomas et al. 2018, Bauder et al. 2020, Bista et al. 2022). Correspondingly, there is a relationship between home range size and population density, with urban areas typically supporting higher population densities of urban-adaptable species than conspecifics in rural habitats (Ditchkoff et al. 2006, Bateman & Fleming 2012, Šálek et al. 2015).

For red squirrels, home range size has been shown to vary in response to food availability (Lurz et al. 2000, Wauters et al. 2001b, 2005, Pierro et al. 2007, Reher et al. 2016), sex (Wauters & Dhondt 1992, Andrén & Delin 1994, Lurz et al. 2000, Wauters et al. 2001b, Verbeylen et al. 2009), population density (Haigh et al. 2017b), habitat type (Wauters & Dhondt 1992, Thomas et al. 2018, Krauze-Gryz et al. 2021), habitat fragmentation (Wauters et al. 1994, Verbeylen et al. 2009), and different personality types (Wauters et al. 2021). For example, the polygynous-promiscuous mating system of red squirrels means that males and females have different behavioural strategies to improve their reproductive success. Typically, male home ranges show a level of dependence on female availability to increase mating opportunities, whilst female home ranges are more dependent on resource availability to successfully raise their offspring (Wauters & Dhondt 1992, Lurz et al. 2000). Therefore patterns of space use differ between the sexes, with males having larger home ranges than females (Table 4.1) and overlapping extensively with multiple females as well as neighbouring males, whereas females tend to have a high degree of intrasexual territoriality where they defend exclusive core areas against other females (Wauters & Dhondt 1992, Lurz et al. 2000, Pierro et al. 2007).

Table 4.1. Overview of mean home range and core area sizes (in hectares) of male (M) and female (F) red squirrels in different habitat types, adapted from Bosch & Lurz (2012).

Home Range (Core Area) ($\bar{x}$ ± SD)	Habitat Type		Reference (Country)
3.5 ± 2.7 (0.3 ± 0.2)	Urban	City centre	Thomas et al. 2018 (Germany)
6.7 ± 1.7 (1.6 ± 0.4)		Semi-natural cemetery	
M: 2.36 ± 0.14 (0.94 ± 0.08) F: 1.73 ± 0.09 (0.55 ± 0.04)	Urban	City centre park	Krauze-Gryz et al. 2021 (Poland)
M: 11.24 ± 1.53 (3.57 ± 0.46) F: 5.66 ± 0.07 (0.95 ± 0.26)		Forest	
M: 4.45 ± 0.15 (2.30 ± 0.15) F: 2.84 ± 0.15 (1.20 ± 0.07)	Woodland	Coniferous	Wauters & Dhondt 1992 (Belgium)
M: 6.39 ± 0.39 (4.45 ± 0.45) F: 2.62 ± 0.30 (1.86 ± 0.16)		Deciduous	
M: 17.75 ± 6.20 (2.17 ± 0.68) F: 6.29 ± 0.64 (1.39 ± 0.25)	Woodland	Coniferous	Lurz et al. 2000 (England)
M: 7.57 ± 3.42 (3.32 ± 1.75) F: 2.70 ± 0.80 (1.06 ± 0.38)	Woodland	Mixed	Wauters et al. 2001 (Italy)
M: 5.57 ± 3.20 (2.16 ± 1.60) F: 3.29 ± 2.51 (1.21 ± 0.92)	Woodland	Mixed	Verbeylen et al. 2009 (Belgium)

As male red squirrel home ranges also overlap with other males, which increases encounters with potential competitors, there is a dominance hierarchy between neighbouring individuals, with larger and heavier (i.e. dominant) males holding larger and higher quality home ranges than smaller subordinate males such as dispersing sub-adults (Wauters & Dhondt 1992, Wauters et al. 2001b, Reher et al. 2016). Similarly, dominant females defend their high-quality core areas against other females and so are strongly spaced apart with no overlap, whilst smaller subordinate females occupy less preferred habitat patches, either settling on the edges of the home ranges of multiple dominant females (avoiding their core areas) or behaving as 'floaters' moving between habitat patches without settling (Wauters & Dhondt 1992, Wauters et al. 2001b, Reher et al. 2016). These subordinate females will then claim the high-quality core areas if they become vacant, should the dominant female either move or perish (Wauters & Dhondt 1992).

Furthermore, spatial and temporal variations in food availability have been shown to influence home ranges for both sexes, although typically more strongly for females due to their dependence on resource availability, with home range and core area size being inversely correlated with food abundance (Lurz et al. 2000, Wauters et al. 2001b, 2005, Pierro et al. 2007, Reher et al. 2016). Therefore, home ranges of red squirrels inhabiting more urbanised areas are typically smaller compared to conspecifics in more rural locations (Table 4.1), most likely due to increased anthropogenic food availability. However, there may also be an impact of roads acting as a barrier to movement; Fey et al. (2016) found that urban red squirrels appear to avoid crossing roads during daily routine activities (e.g. foraging) within their home ranges.

It is necessary to understand the spatial ecology of red squirrels in urban environments to contribute to conservation efforts across their distribution range, particularly in the UK where they are endangered and in parts of mainland Europe where populations are declining. More broadly, a greater understanding of urban wildlife ecology and implementation of effective urban management would benefit other wildlife species, as well as the human inhabitants.

4.1.3. Chapter Aim and Objectives

The aim of this chapter is to examine the home ranges of the red squirrels in the study site in relation to the resources and risks (as evaluated in Chapter Three) present in the urban environment.

The objectives are:

1. To compare home range sizes within the study site and with the published literature
2. To investigate the effect of the following variables on core area size:
    a. Sex
    b. Natural food availability (i.e. habitat quality)
    c. Supplemental food availability
    d. Canopy cover (i.e. habitat availability)
    e. Presence of roads

4.2. METHODS

4.2.1. Live-Capture Trapping and Processing

Squirrels were live-capture trapped and processed as detailed in Chapter Two (see section 2.2.2, p. 42). In summary, two phases of trapping were conducted between mid-May and mid-August in 2018 and 2019. During the initial four-week trapping period (Phase 1, from mid-May until mid-June), population demographics were recorded and radio collars were attached to a sub-sample (see below). During the re-trapping period (Phase 2, from the end of July to mid-August), targeted trapping was undertaken for up to three weeks to re-trap the radio-collared individuals to remove their radio collars (note that Phase 2 in 2019 was postponed to May 2020 due to the SQPV outbreak).

During Phase 1, a sub-sample of trapped individuals had a radio collar attached (BioTrack Ltd., UK; PIP-3 brass collar). A minimum sample size required for radio-tracking was calculated based on a power analysis (Appendix VIII; Minitab 2023) and a literature review of previous research into red squirrel home ranges. This was determined to be approximately 20 individuals (10 males and 10 females) per research season. BioTrack Ltd. were consulted to determine which collar model was the lightest and smallest design to minimise any potential impact on the squirrels, but with an appropriate range and battery life for the location and length of this study. The recommended collars weighed approximately 8.5 g, which is less that the required limit of 5% of the squirrels' body weight (Home Office 2016).

All trapped squirrels underwent a health-check to determine whether they would be suitable to radio collar. Only adults were considered, to avoid any risk associated with juveniles or sub-adults still growing whilst wearing the collars, although breeding females were also not collared to avoid any risk to dependant young. Individuals needed to be free of any signs of disease or injury, have a low or medium parasite burden, and weigh more than 250 g to be suitable for a radio collar. If any radio-collared squirrels were recaptured during Phase 1, they were checked for signs of abrasion and, if this was the case, the collar would be removed as per the fieldwork protocols (Appendix IV). The radio collars were also colour-coded using nail varnish (Fig. 4.1), which lasted for the duration of the fieldwork, for ease of individual identification from a distance or if the squirrel was sighted opportunistically.

Figure 4.1. A radio-collared red squirrel in a handling cone showing the colour code, which in this case was grey and yellow (photo: K Hamill).

At the end of each fieldwork season, Phase 2 of trapping was undertaken to remove the radio collars. This consisted of up to three weeks of trapping effort targeting the areas where the collared squirrels were located, to maximise the chances of re-trapping the target individuals whilst reducing the risk of trapping other squirrels. However, should individuals not be re-trapped, the collar design was such that they could be worn long-term without inflicting harm or distress. In addition, other studies have monitored red squirrels via radio-tracking for extended time periods (e.g. Wauters et al. 2010, Fey et al. 2016) with no adverse effects of the collars on the squirrels.

4.2.2. Radio-Tracking

During both fieldwork seasons in 2018 and 2019, the collared squirrels were radio-tracked ideally once per day, but at least once every two to three days. Radio-tracking started from when they were first trapped during Phase 1 in late May/early June until either they were recaptured in Phase 2 in late July/early August, or the fieldwork season ended in mid-August. In 2019, as Phase 2 was delayed to May 2020 due to the SQPV outbreak, trained LWT volunteers continued to radio-track the squirrels from mid-August until mid-November when the collar batteries expired.

In more open and accessible habitats, typically in the woodlands, the collared squirrels were either recorded as 'sighted' (i.e. the squirrel was physically seen by the researcher) or 'located' (i.e. the squirrel was not physically seen due to e.g. foliage, but the researcher was confident of its location in a specific tree or group of trees based on the radio signal). Once a squirrel was sighted or located, the date, time, and GPS co-ordinates were recorded.

In more inaccessible habitats, typically in the urban area where the squirrels were often in residential gardens, the squirrels were triangulated by taking three GPS co-ordinates and three bearings from different angles around the location of the strongest signal. The triangulations were later calculated in ArcGIS using the 'RemoteLocationXY' toolbox (Moore & Sarichev 2014). Within the parameters of the software, if the signal location could not be triangulated, the bearings could be adjusted by up to 10^{o} (Bartolommei et al. 2013) and the GPS co-ordinates adjusted by up to 5 m in any direction (Wing et al. 2005), but only a maximum of two adjustments could be made per triangulation. If any triangulations continued to be unsuccessful following the adjustments, they were excluded from the dataset. These adjustments were considered to be reasonable, as there can be errors when taking GPS co-ordinates under canopy cover (up to 10 m under closed canopy; Wing et al. 2005) and through 'bounce-back' of the radio signal from obstacles such as buildings or hilly terrain (up to 12^{o}; Bartolommei et al. 2013).

4.2.3. Home Range Analysis

Descriptive statistics were conducted in Microsoft Excel, mapping and spatial analyses were conducted in ArcGIS (v10.5.1, ESRI 2017), and all statistical tests were conducted in R Statistical Software (RStudio Team 2022). All results graphs were produced in Microsoft Excel.

Home ranges were calculated using the 'adehabitatHR' package in RStudio (Calenge and contributions from Fortmann-Roe S 2023) and exported as shapefiles for spatial analysis in ArcGIS (see section 4.2.3.1). Total home range sizes were determined by 100% minimum convex polygons (MCPs). Although MCPs have known limitations (including assuming that animals use all parts of their home ranges equally and having a high sensitivity to extreme data points), they are a widely used method of estimating animals' home ranges (Powell 2000, Nilsen et al. 2008) and so allow for comparison with previous studies (e.g. Reher et al. 2016, Thomas et al. 2018), as well as highlighting exploratory forays (Sievert et al. 2022). Core areas were determined by kernel density estimation (KDE) using the least-squares cross-validation (LSCV) smoothing factor, with 70% of fixes. KDEs are a recommended and widely used method for estimating home ranges, although they are sensitive to the choice of smoothing factor (i.e. bandwidth (h); Worton 1989, Seaman et al. 1999, Powell 2000, Gitzen & Millspaugh 2003). For example, the reference bandwidth (h_{ref}) tends to over-smooth, producing larger home range estimates (Kie 2013, as cited in Reher et al. 2016). As recommended by Gitzen et al. (2006), h_{LSCV} was selected as squirrels use small patches of resources and this smoothing factor is better at identifying these areas of intensive use. The most common problem associated with using h_{LSCV} for home range estimation is failure of the models (Gitzen et al. 2006, Bauder et al. 2015) but, as discussed below, this only occurred for one individual and so was not an issue in this study. Core areas were estimated using 70% of fixes because the utilisation distribution curves showed an inflection point at approximately this point (Fig. 4.2; Powell 2000), therefore representing the area of most intensive use by excluding exploratory forays, and also to allow for comparison with previous studies (e.g. Wauters & Dhondt 1992, Wauters et al. 1994, Verbeylen et al. 2009).

Any public sightings and the GPS co-ordinates of when the individuals were initially trapped and collared were not included in the dataset. A minimum of 30 – 40 fixes adequately represents the

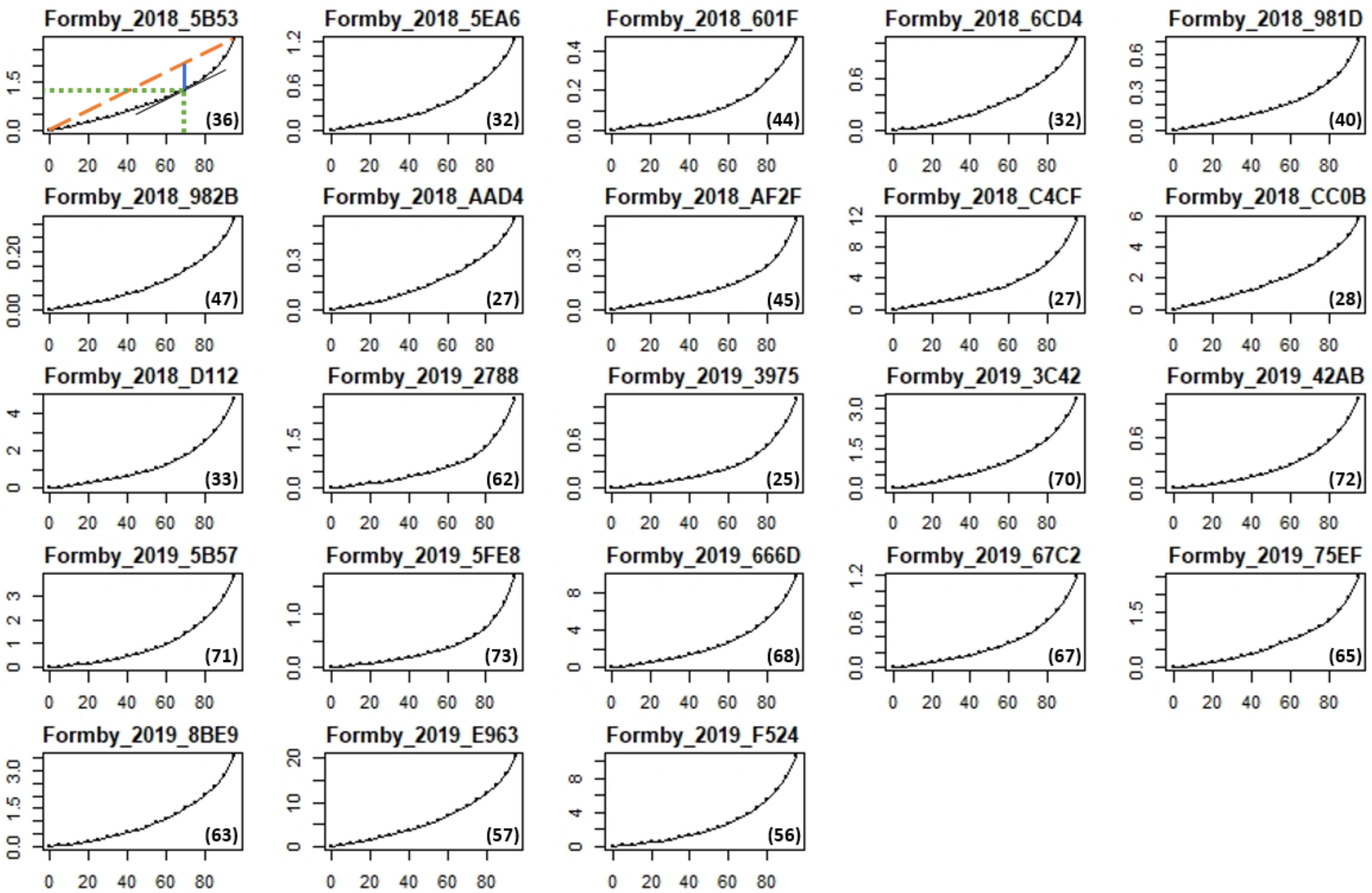


Figure 4.2. Utilisation distribution curves for KDE_{LSCV} home range estimations for each individual squirrel, to determine the percentage of fixes that best represents the core area (see Fig. 3.6B in Powell 2000). The x-axis is the cumulative percentage of the number of fixes (the total number of fixes per individual is noted in brackets on each graph) and the y-axis is the home range area (ha). Using individual 5B53 as an example, the orange dashed line represents if all areas of the home range were randomly used, while the solid blue line shows the point of maximum difference between this and the utilisation distribution curve. Below this inflection point represents the most intensively used area (i.e. the core area, shown by the dotted green lines), which indicates the approximate percentage of fixes required to represent the core area and the approximate size of the core area (ha).

home range of an individual squirrel (Wauters & Dhondt 1992, Lurz et al. 2000, Wauters et al. 2001b, Reher et al. 2016). Therefore, two individuals with fewer than 10 fixes were excluded, one of which died of suspected dehydration in the 2018 heatwave and the other was predated on by a cat in 2019. Another individual with 20 fixes, who died in the SQPV outbreak in 2019, was also excluded later as the home range analysis showed that the KDE_{LSCV} model failed to converge. However, four individuals with between 25 – 28 fixes were included as the home range analysis showed that the KDE_{LSCV} models successfully converged, giving a final sample size of 23 individuals.

4.2.3.1. Spatial Analysis of Resources and Risks

Spatial analyses were conducted only using the 70% KDE_{LSCV} core areas, to investigate how the resources and risks affect this area of intensive use rather than exploratory forays within the wider 100% MCP home range. The area (ha) of the core areas, which was calculated using the 'adehabitatHR' package in RStudio, was also cross-checked in the attribute table of the 70% KDE_{LSCV} polygon shapefile using the 'Calculate Geometry' tool.

Four variables were spatially analysed in relation to the core areas, for inclusion in the data analysis: (1) canopy cover (i.e. habitat availability), (2) the presence of roads, (3) the presence of supplemental food sources, and (4) the availability of natural food sources (i.e. habitat quality). The canopy cover was obtained from the canopy polygon data from the National Tree Map™ (©Bluesky International Limited 2018). The 'Intersect' tool in ArcGIS was used to determine the extent of overlap between the canopy cover polygons and the 70% core area polygons for each individual, from which the proportion of available habitat within each core area was calculated by dividing the area of canopy cover overlap (ha) by the core area size (ha).

The locations of roads were manually mapped over a basemap of the study site (EDINA Aerial Digimap Service 2019), using the 'Line' tool available in the 'Create Features' toolbox in ArcGIS. The locations of supplemental feeders were obtained via the public survey (see section 3.2.1, p. 91) and mapped as points in ArcGIS using the GPS co-ordinates. The 'Near' tool in the 'Proximity' toolset in

ArcGIS was used to determine both the distance to the nearest supplemental feeder (m) and the distance to the nearest road (m) from the edge of each individuals' 70% core area.

Finally, the habitat quality (categorised as high, medium, low, or none from the seed crop abundance surveys in Chapter Three) across the study site was mapped by creating polygon shapefiles using the 'Editor' toolbar in ArcGIS. The 'Intersect' tool in ArcGIS was then used to determine the height of the trees (obtained from the points data from the National Tree Map^{TM}) that overlapped with each of the habitat quality polygons. This highlighted that there was a general positive trend between the habitat quality and the mean tree height (Fig. 4.3), as larger, mature trees typically produce more seeds (Krannitz & Duralia 2004, Ordóñez et al. 2005). Therefore, rather than using the habitat quality polygons from the seed crop abundance surveys, the mean tree height from the points data from the National Tree Map^{TM} was used to determine the habitat quality within the 70% core areas, as these data were more robust. As such, the mean tree height (m) within each individuals' 70% core area was calculated using the 'Intersect' tool in ArcGIS to determine the overlap between the points data and the core area polygons for each individual.

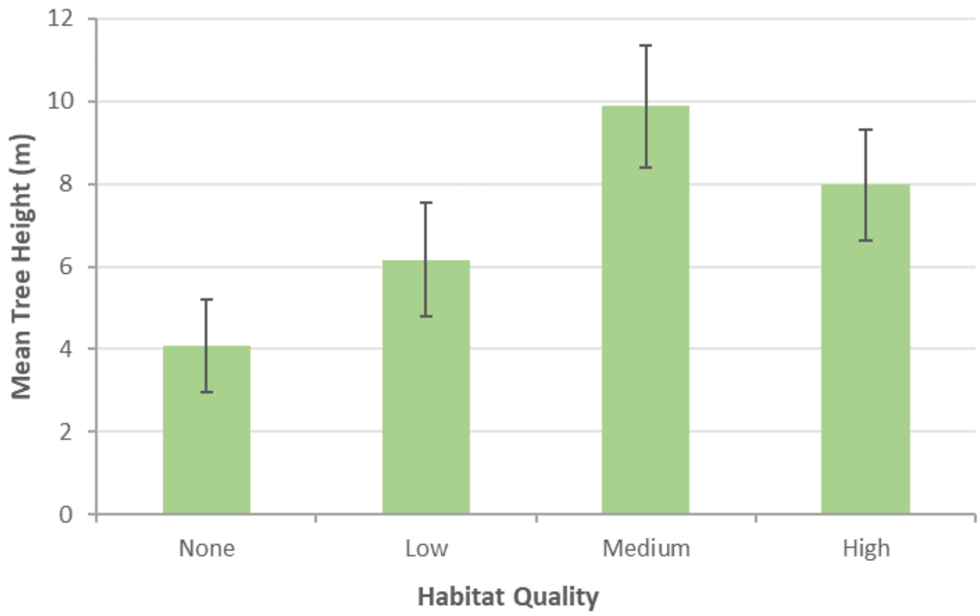


Figure 4.3. Mean tree height (m ± SD) for each category of habitat quality, with habitat quality determined from the seed crop abundance surveys (from Chapter Three) and mean tree height calculated from the National Tree Map^{TM} data (©Bluesky International Limited 2018) using ArcGIS (v10.5.1, ESRI 2017).

4.2.3.2. Data Analysis

Comparative analyses of the 100% MCP home range sizes were conducted to explore trends within the study site and for comparison with the published literature. As there were no females radio-collared in the urban environment (as the only urban female died in the SQPV outbreak and so was excluded from the home range analysis when the KDE_{LSCV} model failed to converge), only males (*n* = 16) were used to compare home range size between habitats (*n* woodland = 9, *n* urban = 7) and only the woodlands (*n* = 16) were used to compare home range size between sexes (*n* males = 9, *n* females = 7). Mann Whitney U tests, from the 'stats' package that is included in RStudio, were used due to the small sample sizes and right-skewed distributions of the data.

Using the spatial analysis data (see section 4.2.3.1), a gamma generalised linear model (GLM) was fitted to the data to predict 70% KDE_{LSCV} core area sizes (ha) in relation to the independent variables (canopy cover, mean tree height, distance to nearest supplemental feeder, distance to nearest road, and sex), as the gamma distribution is typically used for continuous response variables that are positive (i.e. no zeros or negative values) and right-skewed (Smith et al. 2020). The function 'glm' from the 'stats' package that is included in RStudio was used to fit the model. The canonical link function, which for the gamma distribution is the inverse link function, and the logarithmic (log) link function are both often used for gamma GLMs to link the response variable and the covariates (Dunn & Smyth 2018, Smith et al. 2020). In addition, canopy cover and mean tree height were highly correlated (R^2 = 0.9) but are both potentially important predictors of squirrels' core area sizes. Therefore, several models were built either excluding one or including both of these variables and using either the inverse or log-link function, from which the AICs were then used to select the most suitable model (Table 4.2; Ng & Cribbie 2017, Gregorich et al. 2021, Eustace et al. 2022). As Model 3 had the lowest AIC, this model was selected and validated (i.e. verification of homogeneity of residual variance, model misfit, normality of residuals, and the absence of influential observations; Smith et al. 2020).

Due to the different radio-tracking periods (May to August in 2018 and May to November in 2019) and as home range size is known to vary seasonally (Bosch & Lurz 2012), differences in home range size were not compared between years and the variable 'year' was not included in the GLM.

Table 4.2. Summary of gamma GLM models, from which the most suitable model (Model 3) was selected based on the lowest AIC.

Model	Link Function	Variables	AIC
1	Inverse	Mean tree height + Distance to nearest road + Distance to nearest feeder + Sex	43.24
2	Inverse	Canopy cover + Distance to nearest road + Distance to nearest feeder + Sex	40.88
3	Inverse	Canopy cover + Mean tree height + Distance to nearest road + Distance to nearest feeder + Sex	**36.69**
4	Log	Mean tree height + Distance to nearest road + Distance to nearest feeder + Sex	45.53
5	Log	Canopy cover + Distance to nearest road + Distance to nearest feeder + Sex	40.18
6	Log	Canopy cover + Mean tree height + Distance to nearest road + Distance to nearest feeder + Sex	40.02

4.3. RESULTS

Overall, 26 individuals were radio-collared (Table 4.3): 12 in 2018 and 14 in 2019. In 2018, there were nine individuals (five males and four females) in the woodlands and three (all males) in the urban area (Fig. 4.4 – 4.6). In 2019, there were eight individuals (five males and three females) in the woodlands and five (four males and one female) in the urban area (Fig. 4.7 – 4.9). The three individuals that were excluded from the data analyses (due to having ≤ 20 fixes and so the KDE_{LSCV} models failed to converge) were one woodland male in 2018 and two urban individuals, one male and the female, in 2019 (as indicated in Table 4.3), resulting in a final sample size of 23 individuals.

Of the total number of fixes (n = 1295), 21.85% (n = 283) were triangulations, of which 19.43% (n = 55) were adjusted (see section 4.2.2) and 19.79% (n = 56) were excluded (i.e. the triangulation was unsuccessful). The mean (± SD) estimated error of the triangulations was 7.69 m ± 7.14. The average number of fixes per individual was 49.6 ± 17.3 (min. = 25, max. = 73).

Table 4.3. Summary of the radio-collared red squirrels, including the three individuals that were excluded from the data analysis (4B5A, 785D, and A9C5).

Individual ID	Year	Habitat	Sex	100% MCP Home Range Size (ha)	70% KDE_{LSCV} Core Area Size (ha)
5B53	2018	Woodlands	M	1.75	1.27
601F	2018	Woodlands	M	0.38	0.17
6CD4	2018	Woodlands	M	1.77	0.45
981D	2018	Woodlands	M	0.33	0.28
4B5A	2018	Woodlands	M	N/A	N/A
5EA6	2018	Woodlands	F	1.05	0.51
982B	2018	Woodlands	F	0.37	0.13
AAD4	2018	Woodlands	F	0.42	0.26
AF2F	2018	Woodlands	F	0.45	0.19
C4CF	2018	Urban	M	13.38	4.20
CC0B	2018	Urban	M	4.36	2.83
D112	2018	Urban	M	3.36	1.74
2788	2019	Woodlands	M	3.86	0.86
3975	2019	Woodlands	M	1.86	0.35
3C42	2019	Woodlands	M	5.55	1.36
5B57	2019	Woodlands	M	15.77	1.39
75EF	2019	Woodlands	M	2.50	0.99
42AB	2019	Woodlands	F	2.03	0.38
5FE8	2019	Woodlands	F	4.99	0.51
67C2	2019	Woodlands	F	1.74	0.43
666D	2019	Urban	M	9.59	3.63
8BE9	2019	Urban	M	9.75	1.42
E963	2019	Urban	M	22.97	9.14
F524	2019	Urban	M	22.88	3.67
785D	2019	Urban	M	N/A	N/A
A9C5	2019	Urban	F	N/A	N/A

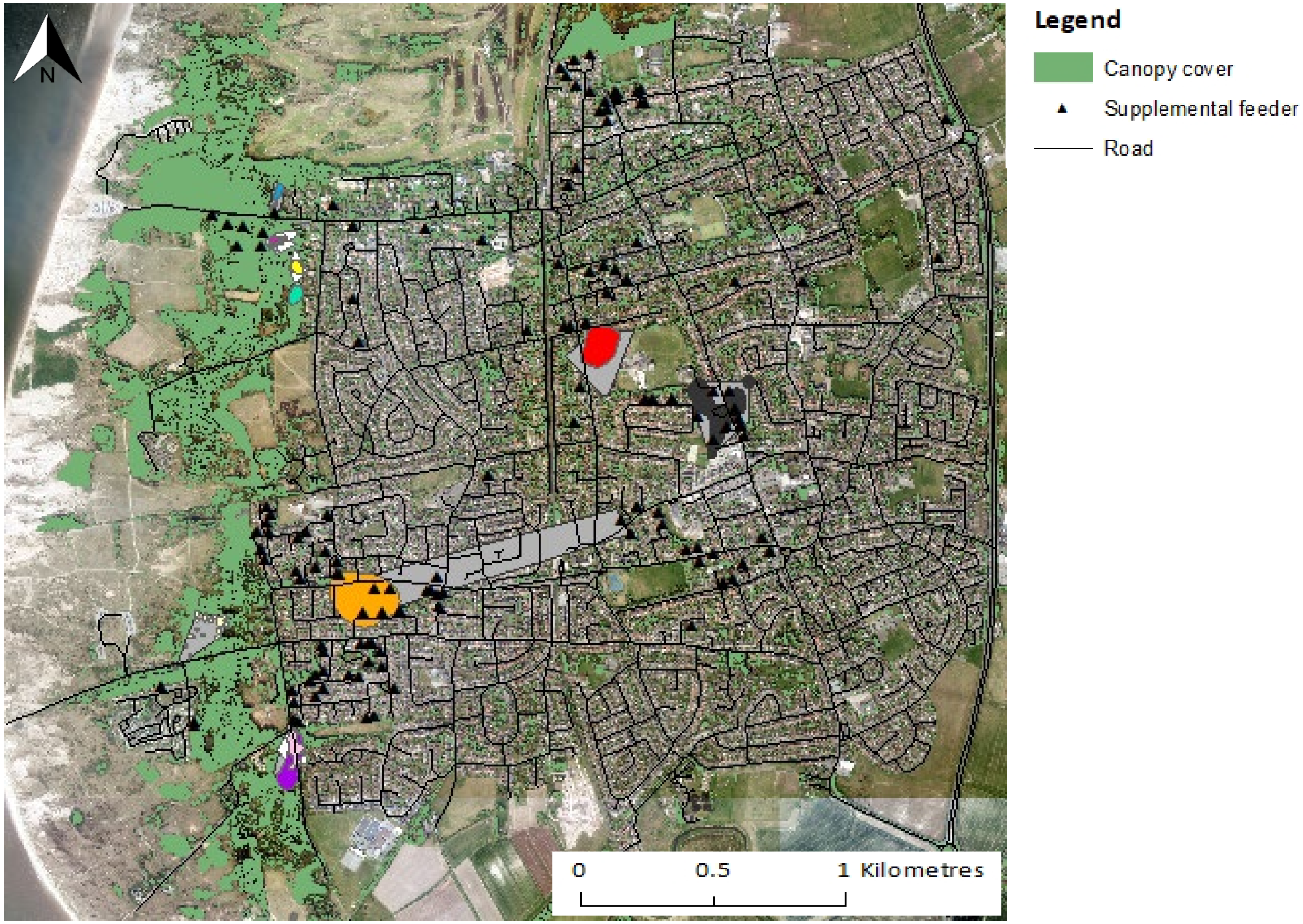


Figure 4.4. Overview of the 100% MCP home ranges (grey polygons for males and white polygons for females) and 70% core areas (coloured polygons, with different colours representing different individuals) in 2018. Canopy cover, locations of supplemental feeders, and roads are also mapped (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Aerial Digimap Service (2019)).

Figure 4.5. 100% MCP home ranges (grey polygons for males and white polygons for females) and 70% core areas (coloured polygons, with different colours representing different individuals) in the northern (*left*) and southern woodlands (*right*) in 2018. Canopy cover, locations of supplemental feeders, and roads are also mapped (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Aerial Digimap Service (2019)).

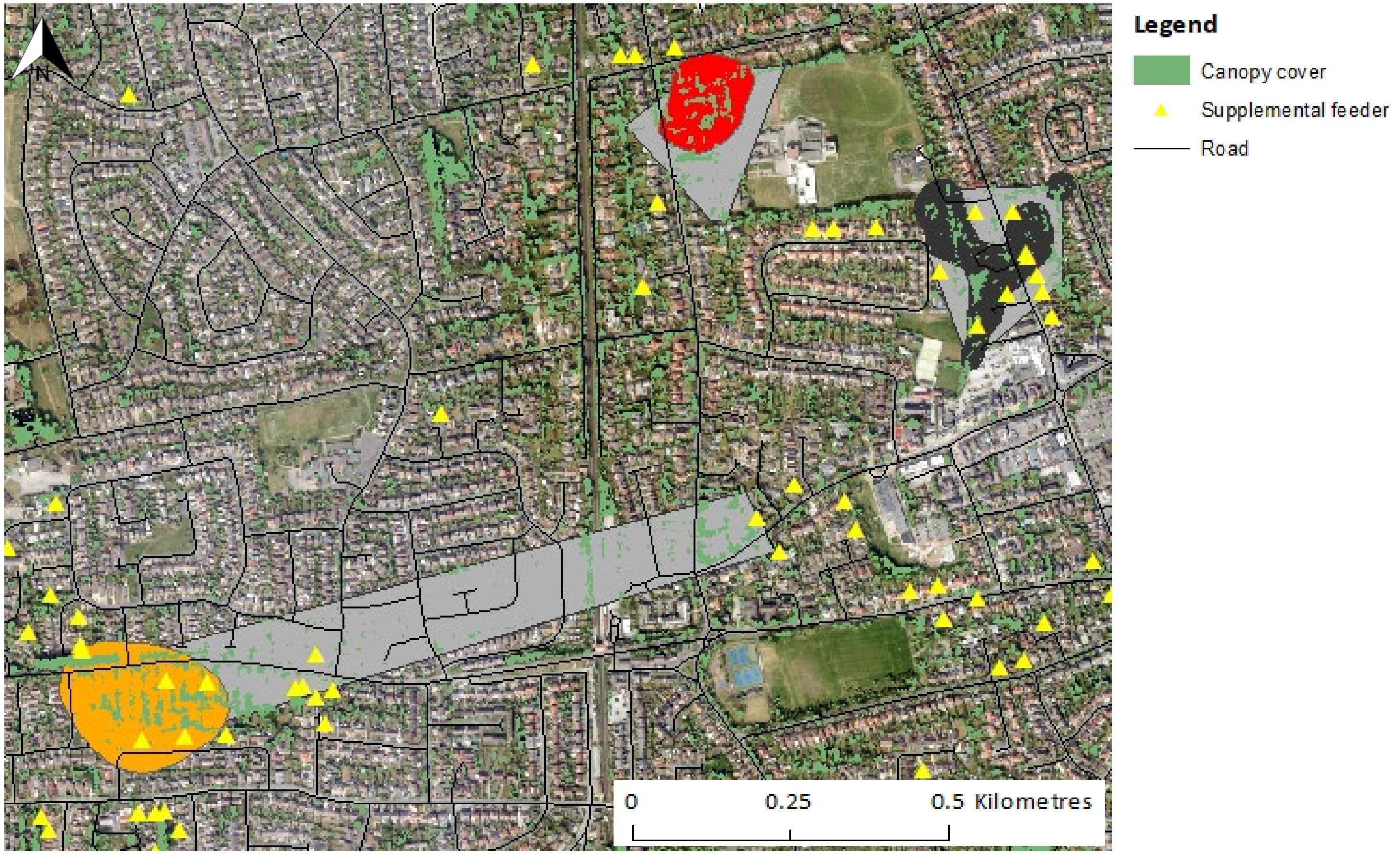


Figure 4.6. 100% MCP home ranges (grey polygons) and 70% core areas (coloured polygons, with different colours representing different individuals) in the urban area in 2018. Canopy cover, locations of supplemental feeders, and roads are also mapped (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Aerial Digimap Service (2019)).

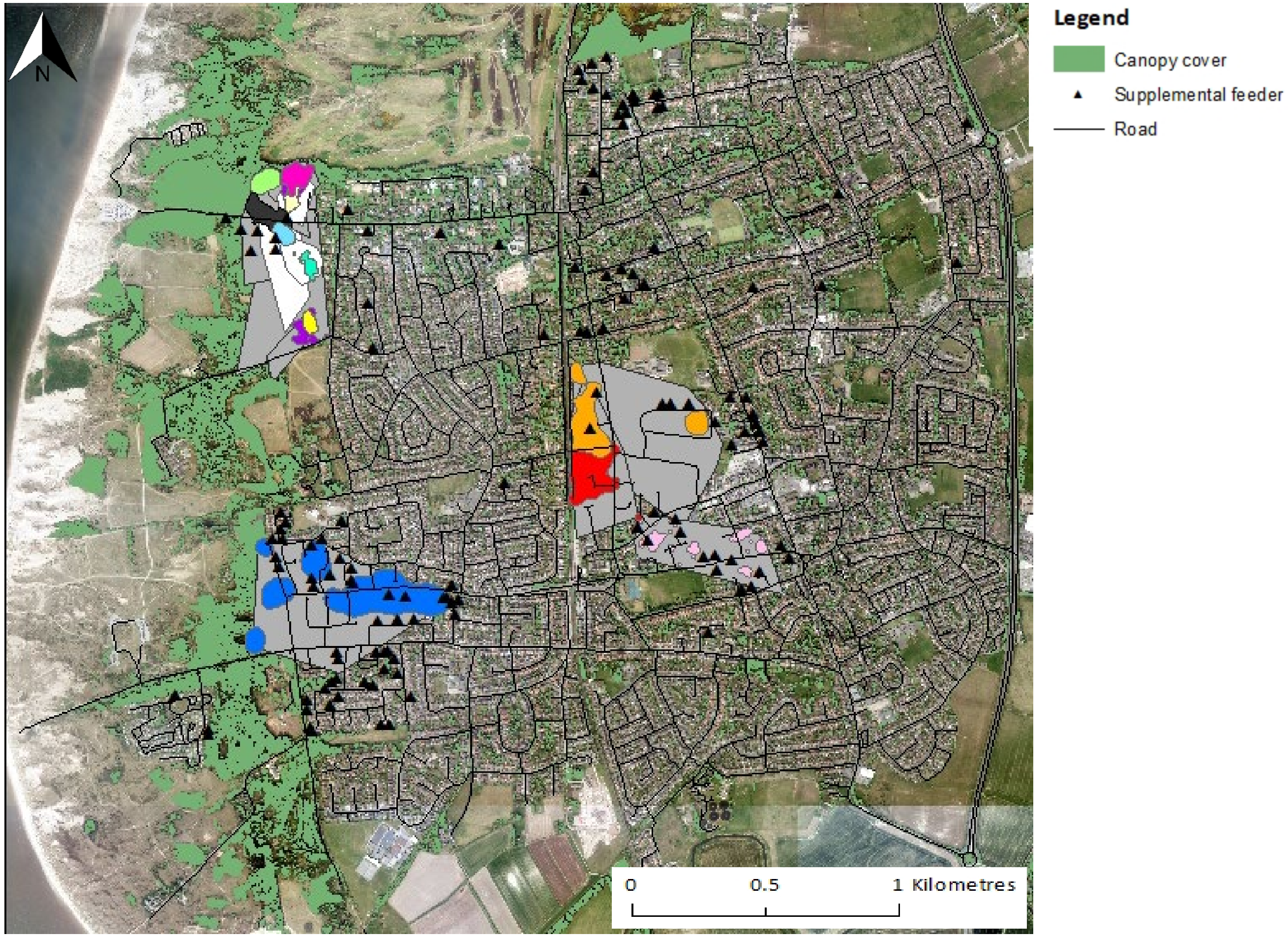


Figure 4.7. Overview of the 100% MCP home ranges (grey polygons for males and white polygons for females) and 70% core areas (coloured polygons, with different colours representing different individuals) in 2019. Canopy cover, locations of supplemental feeders, and roads are also mapped (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Aerial Digimap Service (2019)).

Figure 4.8. 100% MCP home ranges (grey polygons for males and white polygons for females) and 70% core areas (coloured polygons, with different colours representing different individuals) in the northern woodland in 2019. Due to the number of radio-collared individuals, the visualisations have been split across two maps. Canopy cover, locations of supplemental feeders, and roads are also mapped (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Aerial Digimap Service (2019)).

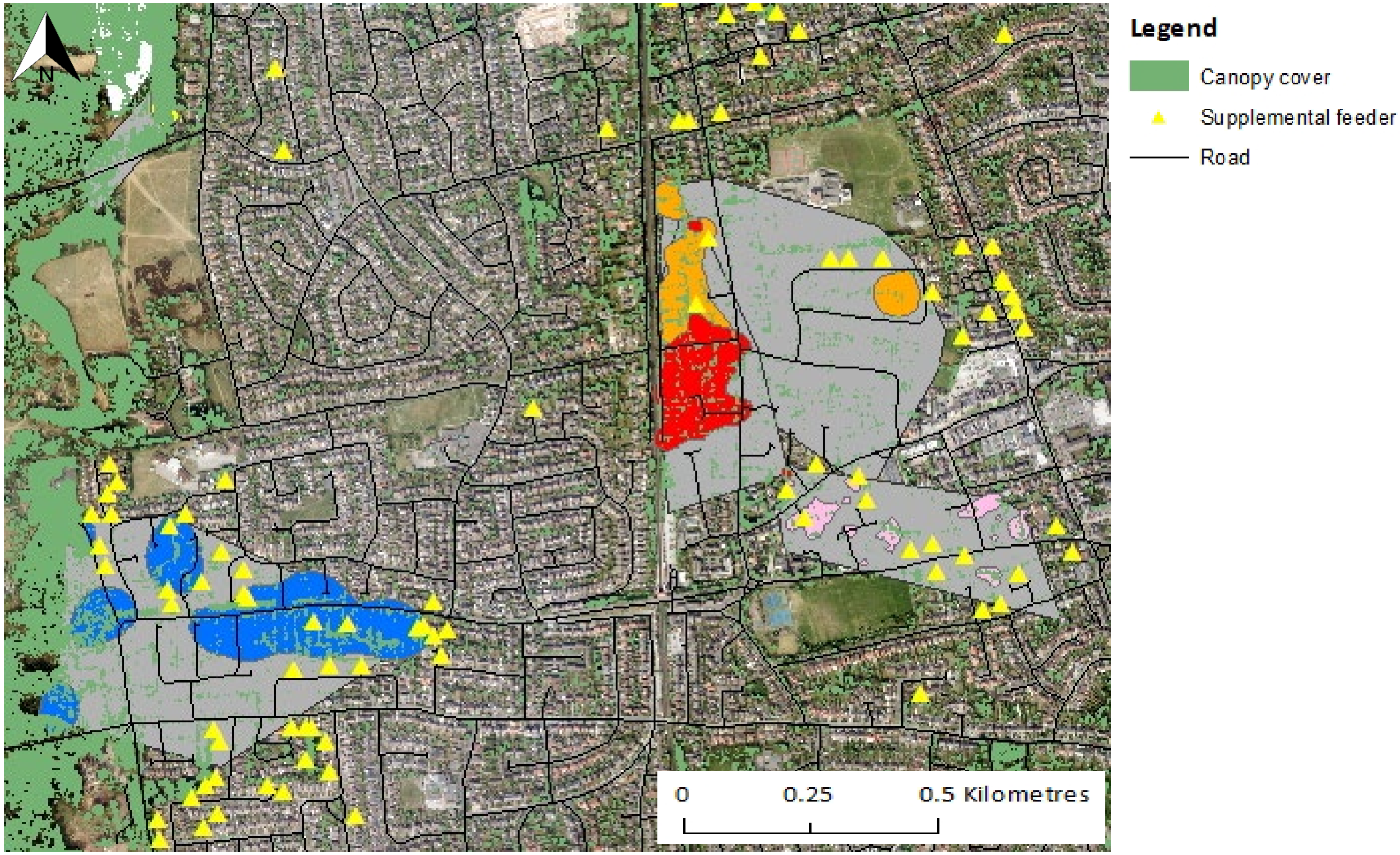


Figure 4.9. 100% MCP home ranges (grey polygons) and 70% core areas (coloured polygons, with different colours representing different individuals) in the urban area in 2019. Canopy cover, locations of supplemental feeders, and roads are also mapped (created in ArcGIS (v10.5.1, ESRI 2017) and base map obtained from EDINA Aerial Digimap Service (2019)).

4.3.1. Home Range Analysis

The mean (± SD) 100% MCP home range size (ha) for the radio-collared red squirrels inhabiting the urban area was 12.33 ± 7.99 (only males) and for those inhabiting the woodland was 2.80 ± 3.82 (M: 3.75 ± 4.79, F: 1.58 ± 1.64). The mean (± SD) 70% KDE_{LSCV} core area size (ha) for the radio-collared red squirrels inhabiting the urban area was 3.80 ± 2.57 (only males) and for those inhabiting the woodland was 0.81 ± 0.44 (M: 0.79 ± 0.49, F: 0.34 ± 0.15).

There was no significant difference in 100% MCP home range size between males ($\tilde{x}$ = 1.86 ha) and females ($\tilde{x}$ = 1.05 ha) in the woodlands (Mann Whitney U test: W = 21, p = 0.30; Fig. 4.10a). However, there was a significant difference in 100% MCP home range size of males between habitats (Mann Whitney U test: W = 55, p = 0.012; Fig. 4.10b), with males in the woodlands having smaller home ranges ($\tilde{x}$ = 1.86 ha) compared to those in the urban area ($\tilde{x}$ = 9.75 ha).

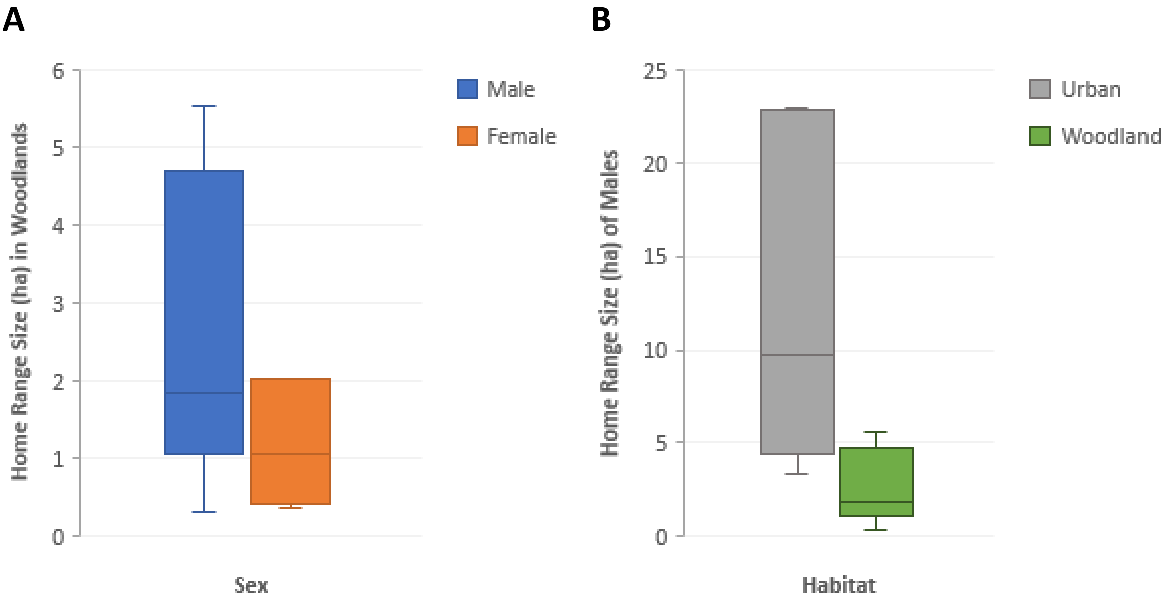


Figure 4.10. Comparison of 100% home range sizes between (A) male and female red squirrels in the woodlands and (B) males inhabiting the urban area and woodlands.

The gamma GLM with inverse link function (Table 4.4) showed significant negative effects of canopy cover and mean tree height (m) on 70% core area size (ha), with increased canopy cover and tree height both resulting in smaller core areas (Fig. 4.11 and 4.12). There was also a significant effect of sex on core area size, with males having larger core areas ($\bar{x} \pm SD$ = 2.11 ha ± 2.27) than females ($\bar{x} \pm SD$ = 0.34 ha ± 0.15). There was no significant effect of distance to the nearest road (m; Fig. 4.13) or nearest supplemental feeder (m; Fig. 4.14). Overall model fit showed a pseudo R^2 = 0.83.

Table 4.4. Summary of gamma GLM to model core area size (ha) of radio-tagged red squirrels.

Model Parameter	Estimate	SE	*P*
Intercept	2.115	0.617	< 0.01
Canopy cover	1.788	0.658	< 0.05
Mean tree height	-0.091	0.038	< 0.05
Distance to nearest road	0.009	0.010	0.372
Distance to nearest feeder	0.004	0.004	0.287
$Sex_{(Male)}$	-1.653	0.599	< 0.05

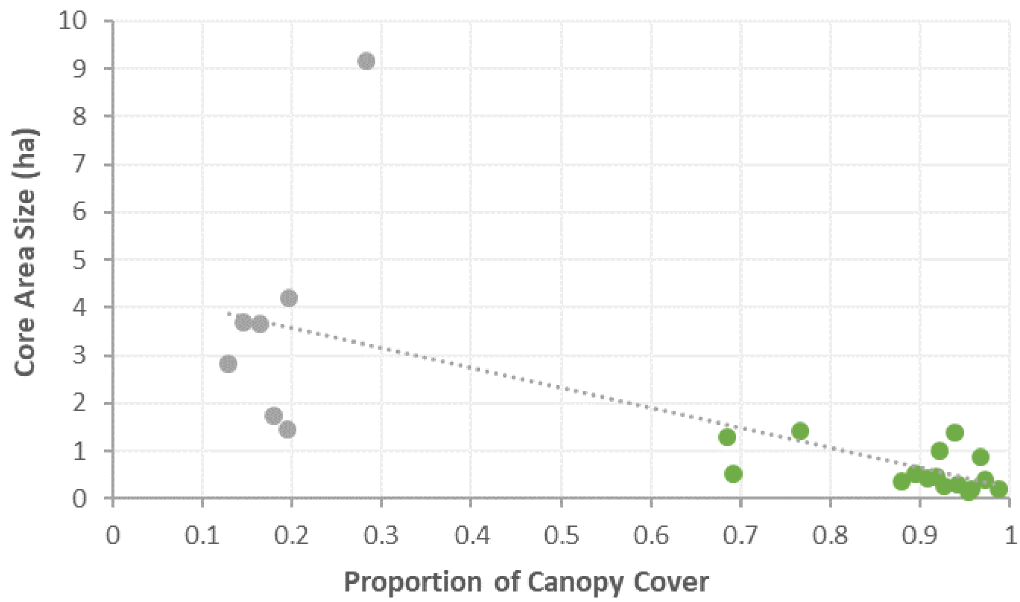


Figure 4.11. The effect of the proportion of canopy cover on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

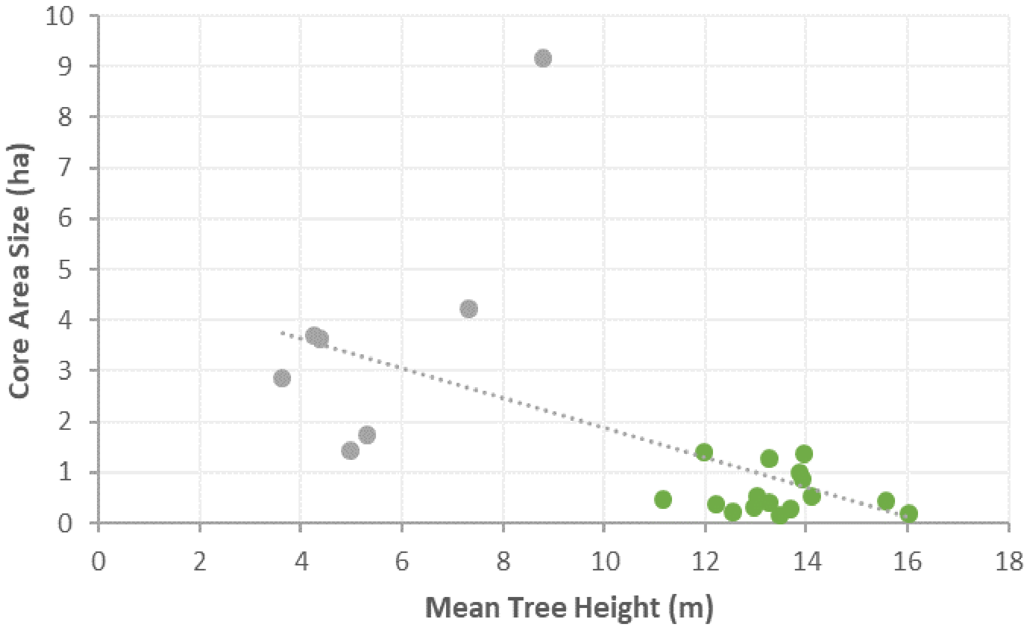


Figure 4.12. The effect of mean tree height (m) on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

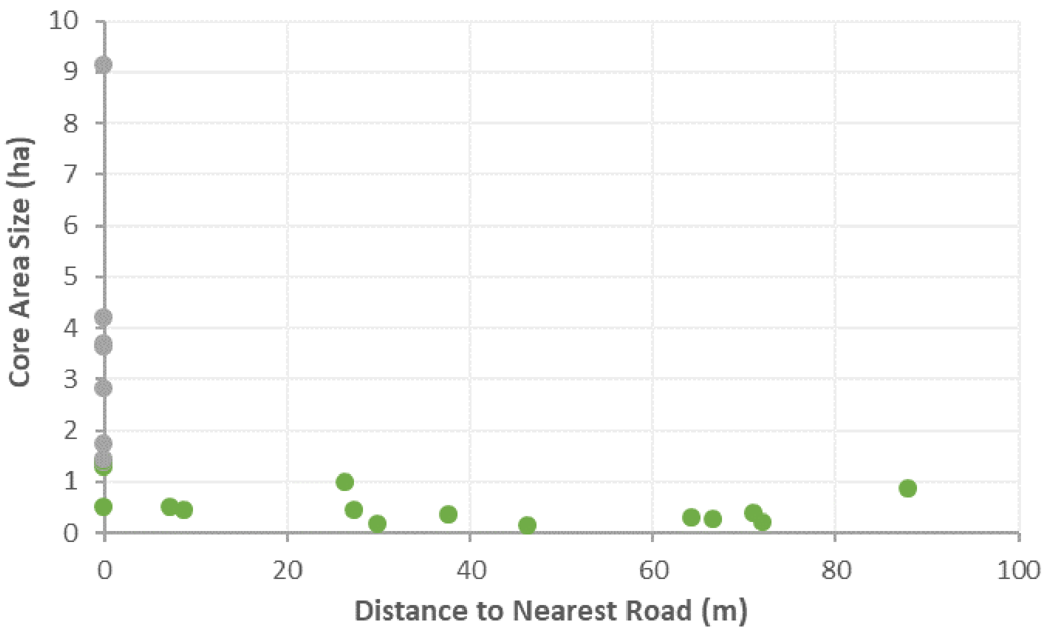


Figure 4.13. No effect of the distance to the nearest road (m) on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

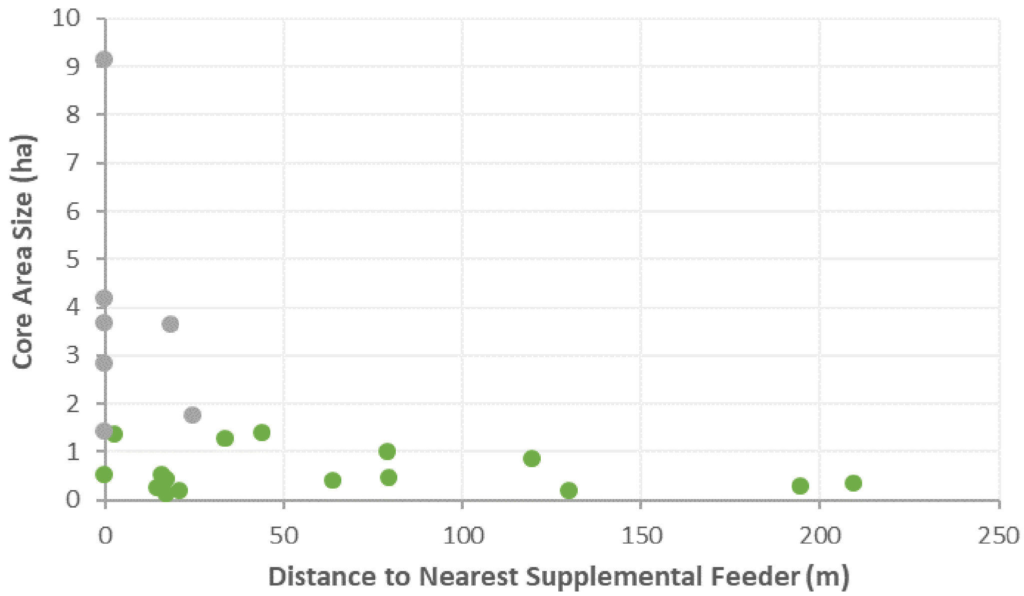


Figure 4.14. No effect of the distance to the nearest supplemental feeder (m) on the size (ha) of the red squirrels' 70% core areas, with those inhabiting the urban area highlighted by grey points and those in the woodlands highlighted by green points.

4.4. DISCUSSION

The target sample size of 20 squirrels per research season was not attained due to a lack of suitable individuals in the urban area, as a result of lower trapping success (see section 2.3.1, p. 59) and a higher proportion of breeding females that could not be radio collared. Only one adult, non-breeding female was trapped in the urban area (individual A9C5), who was later excluded from the data analyses due to an insufficient number of radio-tracking fixes as she died in the SQPV outbreak. Therefore, although more individuals could have been radio collared in the woodlands, this was restricted by the number that could be radio collared in the urban area to maintain approximately equal sample sizes in the two areas within the study site.

4.4.1. Sex and Home Ranges

The findings from this study partially support the published literature, in that the males had larger 70% core areas than the females, which is associated with increasing mating opportunities (Wauters & Dhondt 1992, Andrén & Delin 1994, Lurz et al. 2000, Wauters et al. 2001b, Verbeylen et al. 2009, Krauze-Gryz et al. 2021). Males also typically overlap with multiple females for which there was some evidence in this study, e.g. individuals 5B53 and 5EA6 (bright purple and pale pink respectively in the right image in Fig. 4.5), 5B57 and 67C2 (bright purple and pale yellow respectively in the left image in Fig. 4.8), and 3C42 and 5FE8 (black and blue respectively in the right image in Fig. 4.8), although this couldn't be statistically determined as not all individuals within a given location (i.e. with potentially overlapping ranges) were radio-collared. However, in contrast to the literature, there was no significant difference in the 100% home range size between the woodland males and females. This may have been due to the small sample size (particularly the lack of radio-collared females in the urban area, so that only the woodland individuals could be analysed), or due to the high-quality habitat throughout the woodlands (see section 3.3.2, p. 116) reducing the need for exploratory forays for resources.

In addition, in contrast to the published literature that reports that females have a high degree of intrasexual territoriality and so their core areas are strongly spaced apart (Wauters & Dhondt 1992, Lurz et al. 2000, Pierro et al. 2007), there was an example in this study of two females whose core areas almost completely overlapped. Individuals 982B and AAD4 (bright pink and pale purple respectively in the left image in Fig. 4.5) were located in a mixed patch of beech and pine immediately adjacent to 'Squirrel Walk' in the northern woodland. This was similarly observed by Lurz et al. (2000), who found that higher habitat quality resulted in smaller core areas with higher intrasexual overlap, which suggests that the high-quality woodland habitat was sufficient to reduce the need for intrasexual territoriality. It is also interesting to note that they were never radio-tracked to the nearby supplemental feeders on 'Squirrel Walk', despite radio-tracking taking place at different times each day including during feeding time, which suggests that their nutritional requirements may have been met through the natural food availability without the need for supplemental food.

4.4.2. Habitat and Home Ranges

Other findings from this study also contrast with the published literature. Firstly, that the home ranges and core areas of the radio-collared squirrels were smaller in the peri-urban woodlands compared with the urban centre of Formby (Thomas et al. 2018, Krauze-Gryz et al. 2021). Furthermore, the home ranges and core areas of the urban squirrels were larger in comparison with published studies conducted in rural woodlands, whereas the home ranges and core areas of the peri-urban woodland squirrels were smaller in comparison with these studies (Wauters & Dhondt 1992, Wauters et al. 2001b, Verbeylen et al. 2009). The first two findings contrast with the trend that ranges are typically smaller in more urbanised environments compared with more rural habitats, although the third finding does adhere to this trend (O'Donnell & delBarco-Trillo 2020). However, much of the published literature regarding the home ranges of red squirrels does not specify the bandwidth used when estimating the core areas, which makes it difficult to compare core area sizes between studies.

Rather than widespread and abundant supplemental feeding in the urban area allowing the squirrels to maintain higher body masses and meet their nutritional requirements with less foraging effort (Turner et al. 2017, Thomas et al. 2018), instead the lower body masses (see section 2.4.2.2, p. 73) and larger ranges of the urban squirrels in this study indicate an increased foraging effort. In other words, despite the availability of supplemental food, the squirrels require natural food sources to meet their nutritional requirements and so had to move further to exploit the remaining greenspaces scattered throughout the urban environment. On the other hand, the woodland squirrels had small home ranges as they did not have to travel far for resources within the high-quality woodland habitat. This is supported by the results of the GLM that found significant negative effects of canopy cover and mean tree height on core area size (i.e. lower habitat availability and quality result in larger core areas, and vice versa) but no effect of distance to the nearest supplemental feeder.

It has been suggested that urban wildlife populations may have a source-sink dynamic, where the sources are higher quality habitat patches with abundant food and the sinks are lower quality urban areas (McCleery 2010), which has been similarly suggested for red squirrel populations inhabiting fragmented woodland habitats (Verboom & Apeldoorn 1990, Celada et al. 1994). This study highlights that this may be the case in Formby, with the source being the higher quality peri-urban woodlands and the sink being the urban area consisting of scattered greenspaces embedded within a lower-quality landscape. The findings suggest that, without the woodland source population, the habitat quality in the urban area may be below a 'critical minimum' necessary for the long-term red survival of the red squirrel population (Thomas et al. 2018) and that supplemental feeding may not be sufficient to offset a lack of natural food sources in the urban area (Shuttleworth 1997).

In addition, although Fey et al. (2016) suggested that urban red squirrels appear to avoid crossing roads during daily routine activities within their home ranges, this study found that there was no significant effect of roads on core area size and there were several examples of busy roads passing through the home ranges of the radio-collared red squirrels (e.g. C4CF and CC0B, orange and black respectively in Fig. 4.6, and E963 and 8BE9, blue and pink respectively in Fig. 4.9). However, Fey et al. (2016) also highlighted that red squirrels perceive larger roads with more daily traffic as more dangerous than smaller roads, so they may be willing to regularly cross the minor roads throughout the town in order to access resources.

Overall, these findings contrast with the suggestion that the smaller home ranges of urban individuals may be due to occupying the limited, remaining habitat fragments with available food sources, or due to barriers to movement (Rondinini & Doncaster 2002, Tigas et al. 2002, Mitsuhashi et al. 2018, Thomas et al. 2018, Bauder et al. 2020, Bista et al. 2022). Instead, the findings support the suggestion that smaller home ranges and core areas are due to the increased availability of high-quality natural food sources (Lurz et al. 2000, Wauters et al. 2001b, 2005, Pierro et al. 2007, Reher et al. 2016), as (1) the home ranges and core areas were smaller in the peri-urban woodlands compared to the urban area (i.e. no restriction to habitat fragments), (2) roads appeared not to act as a barrier to movement, and (3) there was a significant effect of canopy cover and mean tree height (as indicators of food abundance) on core area size, whereas this was not the case with supplemental food availability.

However, with regard to the spatial analyses, not all supplemental feeders across the study site would have been mapped, as it was unfeasible to collect these data from all residents. In addition, the National Tree Map^{TM} data, which is a robust and high-quality dataset, was from 2015 but ongoing woodland management and urban intensification may have altered the available habitat by 2018/19. Furthermore, due to the correlation between canopy cover and mean tree height, it is difficult to determine the relative contributions of these variables to core area size; however, both habitat availability and quality are clearly important to red squirrels' patterns of space use.

4.4.3. Source-Sink Population Dynamic

During radio-tracking, several individuals appeared to undertake extensive exploratory forays and, in some cases, establish a new core area. For example, individual 5B57 (dark purple in the left image in Fig. 4.8 in section 4.3) was initially trapped and radio-tracked north of Victoria Road in 2019, then they gradually moved south through the woodlands before settling just north of Blundell Avenue. This behaviour was more frequent in 2019 (*pers. obvs.*), which may have been due to the SQPV outbreak that was fortunately mainly contained in the south-west of the study site including the southern woodlands below Blundell Avenue. For instance, one individual (A9C5, who was excluded from the analyses due to the insufficient number of fixes) was initially trapped and tracked in the north of the urban area (Victoria Road) before moving down to the south-west (Kirklake Road), shortly after which they died from SQPV. Another individual in the northern woodland (3975, bright yellow in the right image in Fig. 4.8) also conducted exploratory forays south towards Blundell Avenue before dying from SQPV. As red squirrels will claim high-quality ranges should they become vacant (Wauters & Dhondt 1992) and SQPV can be spread between conspecifics (Chantrey et al. 2014), this suggests that a source-sink dynamic may become established during a SQPV outbreak, in a similar manner to mange outbreaks in red fox populations (Gosselink et al. 2007), whereby individuals from outside the outbreak area move in to occupy vacant ranges from those that have perished and then catch the disease themselves. This cycle may drive a more severe disease outbreak resulting in higher population mortality, unless management can be implemented to either discourage movement into vacant territories or, more feasibly, to reduce conspecific interactions by, for example, taking down supplemental feeders and removing infected or dead individuals from the environment as soon as possible.

4.5. CONCLUSION

These findings, in conjunction with the findings from Chapters Two and Three, suggest that the small home ranges and high population density in the peri-urban woodlands are due to the high-quality habitat in the woodlands providing abundant natural food sources. The fact that the urban squirrels had larger home ranges than expected when compared with both the woodland squirrels and the published literature suggests that the overall habitat quality in the urban area may be lacking. It is highly likely that the widespread supplemental feeding is also supporting the population, and that squirrels will switch their diet between natural and supplemental food sources based on their nutritional requirements, but supplemental feeding may not be sufficient to offset a lack of greenspaces and associated natural food sources in the urban area. Therefore, these findings provide evidence of a source-sink population dynamic, with squirrels dispersing out of the high-quality woodland habitat into the lower quality urban area where they must move further to forage within the remaining greenspaces.

Furthermore, the squirrels are likely to have the ability to disperse throughout the study site, as roads appear not to be a barrier to their movement. Therefore the fact that they are absent from certain parts of the urban area, but present in others, is likely to be due to the availability of habitat and the associated natural food sources rather than an inability to disperse.

These conclusions highlight that it is essential to maintain and ideally increase the available greenspaces in the urban area, amidst concerns regarding urban intensification in the study site. This would help to increase food availability and encourage dispersal, so that the red squirrels could occupy parts of the town where they are currently absent.

5.0. Chapter Five: Discussion

The England Red Squirrel Action Plan (UK Squirrel Accord 2023) specifies that two of its four core aims are to 'protect…and strengthen red squirrel populations across the current range' and to 'expand the current range of red squirrels'. Similarly, on a more local scale, the LWT Red Squirrel Project aims for the red squirrel population to recover across North Merseyside and West Lancashire (The Lancashire Wildlife Trust 2020). As such, this chapter aims to collate and critically evaluate the data analysed in Chapters Two to Four to make conservation management recommendations to benefit the red squirrels across Merseyside and other UK strongholds, as well as urban wildlife more broadly.

5.1. Importance of Urban Areas for Wildlife Conservation

McKinney (2006) suggested that increasing urbanisation leads to 'biotic homogenisation', as the same urban-adaptable and often non-native species become locally abundant in urban areas across the globe. As a result, urban residents become increasingly disconnected from nature, in particular native species, which has been termed the 'extinction of experience' and can lead to disinvestment in protecting the natural world (Miller 2005). Therefore, it is crucial to target conservation efforts in urban areas to encourage meaningful interactions with nature and increase public awareness, in order to promote effective conservation of native species as well as to improve human physical and mental well-being (Miller & Hobbs 2002, Miller 2005, McKinney 2006, Otto & Pensini 2017, Richardson & McEwan 2018). In other words, positive encounters with charismatic and potentially rare native species can inspire urban residents to engage in wildlife conservation, especially within their local area, either through providing resources (e.g. volunteering their time, donating money to projects) or political pressure (e.g. voting for particular parties, signing petitions). In this way, volunteers are vital contributors to conservation efforts, for example through population monitoring or invasive species control (e.g. Sullivan et al. 2016, Mueller et al. 2019, Turner et al. 2022, Parris et al. 2023). For instance, Dunn et al. (2021) found that people living alongside red squirrels valued the species more, were more aware and knowledgeable about squirrels and their management, and were more accepting of grey squirrel control than the wider population. This was evident in the study site, with numerous houses, roads, and businesses incorporating red squirrels into their names or logos and many residents engaging with the LWT Red Squirrel Project through reporting squirrel sightings and hosting live-capture traps for grey squirrel control (*pers. obvs.*). As such, red squirrels were embedded as part of the identity of Formby and so benefitted from local engagement with conservation efforts.

5.2. Conservation Management Recommendations

5.2.1. Provision of Supplemental Food Sources

Supplementary feeding offers both positive and negative effects for wildlife, and so is a complex issue (Robb et al. 2008, Murray et al. 2016). Reliable supplemental food sources can aid population recovery (e.g. of endangered bird species such as the Spanish imperial eagle (*Aquila adalberti* Brehm 1861), Mauritius pink pigeon (*Nesoenas mayeri* Prévost 1843), and New Zealand kākāpō (*Strigops habroptilus* Gray 1845); Elliott et al. 2001, González et al. 2006, Edmunds et al. 2008) and support higher density populations, particularly in urban areas (e.g. of medium-sized carnivores such as the red fox and badger (*Meles meles* Linnaeus 1758); Bateman & Fleming 2012, Šálek et al. 2015), which in turn can lead to positive interactions with the public (e.g. such as with the San Joaquin kit fox (*Vulpes macrotis mutica* Merriam 1902); Bjurlin & Cypher 2005). This research has highlighted that this was the case in the study site, where widespread and abundant supplementary feeding has helped to support higher numbers of red squirrels that regularly visit local residential gardens. Supplemental feeding of the red squirrels and birds was intertwined, and so may also benefit the local bird population (Robb et al. 2008).

However, widespread supplemental feeding can influence interspecific competition and trophic cascades, ultimately impacting wider ecological communities. For example, it has been suggested that supplemental feeding has resulted in an increased abundance of generalist blue tits (*Cyanistes* *caeruleus* Linnaeus 1758) and great tits (*Parus major* Linnaeus 1758), which can exploit this food resource. These species are socially dominant and subsequently out-compete the more specialist marsh tits (*Poecile palustris* Linnaeus 1758) and willow tits (*Poecile montanus* Conrad von Baldenstein 1827), which do not tend to use bird feeders as frequently, for natural food sources and so has contributed to their population decline across the UK (Shutt & Lees 2021). Supplementary feeding has also been widely implicated in the increased risk of disease transmission (e.g. bovine tuberculosis (*Mycobacterium bovis*) in white-tailed deer (*Odocoileus virginianus* Zimmermann 1780), chronic wasting disease in moose (*Alces alces* Linnaeus 1758), and trichomonosis (*Trichomonas gallinae*) in greenfinches (*Chloris chloris* Linnaeus 1758); Sorensen et al. 2014, Murray et al. 2016, Broughton et al. 2022). This is a substantial concern for the high-density red squirrel population in the study site with regard to the spread of SQPV (Chantrey et al. 2014) and other diseases (e.g. adenovirus; Everest et al. 2014), particularly considering the lack of suitable feeder hygiene practices. Furthermore, extensive supplementary feeding appears to be discouraging the squirrels from dispersing out of Formby into the wider stronghold, which has been similarly observed in migratory birds over-wintering in northern cities in Finland rather than migrating south (Jokimaki et al. 1996, as cited in Robb et al. 2008). In addition, supplemental feeding on its own cannot compensate for a lack of high-quality natural food sources (Shuttleworth 1997). This is also worth noting with regard to the impact of feeding peanuts: although the red squirrels currently do not appear to be malnourished as a result of consuming peanuts (see Chapter Three), this may be offset by the availability of natural food sources allowing the squirrels to switch their diet and compensate for any nutritional deficiencies (Thomas et al. 2018). Therefore, if urban greenspaces continue to be lost (see Chapter Three; Pauleit et al. 2005) and the squirrels are unable to switch their diet to natural food sources, this may result in nutritional deficiencies and associated diseases (e.g. metabolic bone disease; Bosch & Lurz 2012) in the future. Similar nutritional deficiencies caused by anthropogenic feeding have been observed in other species, such as 'angel wing' in waterfowl caused by high-calorie diets lacking in vitamins and minerals or potassium deficiencies in rock iguanas (*Cyclura cychlura* Cuvier 1829; Flinchum 1997 and Knapp et al. 2013, as cited in Murray 2016).

Therefore, it would be beneficial to encourage residents to: (1) reduce the amount of supplemental food being provided (e.g. fill up feeders less frequently and have some days where they are left empty, as well as only feeding during spring and summer when natural food sources and scatter-hoarded cache supplies are scarce); (2) feed a wider variety of food items, particularly those found naturally in the UK such as hazelnuts, walnuts, and pine nuts, whilst reducing the provision of peanuts; (3) regularly clean feeders with disinfectant, or alternatively scatter-feed on the ground; and (4) promptly remove feeders when SQPV cases are identified. It may be necessary to implement a public awareness campaign (e.g. via local newspapers, leaflets, talks, etc.) to alter the perception that the squirrels will starve without supplementary feeding. It may also be appropriate to feed non-shelled items, or at least encourage regular removal of accumulated debris from under the feeders, to reduce the environmental disease transmission risk both between conspecifics (Bruemmer et al. 2010) and with other rodents (e.g. rats and mice; Everest et al. 2014, Fountain et al. 2021). Although the provision of peanuts does not currently appear to be causing malnutrition, it may still be beneficial to supply additional sources of calcium (e.g. deer antlers, cuttlefish bones) for both the squirrels and birds (Reynolds & Perrins 2010).

In addition, some wildlife species (e.g. raccoons (*Procyon lotor* Linnaeus 1758), striped skunks (*Mephitis mephitis* Schreber 1776), a wide variety of bird species, as well as red squirrels) have been shown to be aware of and attracted to newly-installed feeders (Cooper & Ginnett 2000, Galbraith et al. 2015, Bandivadekar et al. 2018, Starkey & delBarco-Trillo 2019). As such, in conjunction with reducing the amount of supplementary feeding within Formby, feeders could be strategically installed in suitable habitat corridors and woodlands on the outskirts of the town and out in the wider stronghold. This would help to both reduce the high-density population within Formby, thus reducing the disease outbreak risk, and encourage the squirrels to disperse across the Merseyside stronghold, thus contributing to their range expansion. However, it would be crucial to maintain grey squirrel control to prevent an influx into the area through the same habitat corridors. These conservation management recommendations highlight how supplemental feeding can be both advantageous (e.g. through encouraging dispersal) and disadvantageous (e.g. through increasing disease transmission risk) for urban wildlife populations, so any use of supplemental feeders needs to be carefully managed (e.g. through appropriate hygiene practices) to mitigate the potential negative impacts.

5.2.2. Habitat Availability and Quality

Although red squirrels are not hindered in moving through the urban environment by buildings or roads, this research has highlighted a lack of available habitat in some areas of the town and consequently an absence of squirrels from those areas (see Chapter Two). The importance of the remaining fragmented urban greenspaces has also been highlighted by the spatial patterns of the radio-collared squirrels inhabiting the urban area, with individuals travelling further across larger home ranges and core areas to exploit the available habitat (see Chapter Four). However, urban intensification is leading to a loss of these remaining greenspaces, including residential gardens through building new houses and expanding driveways (Pauleit et al. 2005, Sainsbury & Slater 2023). Therefore, it is critical to prevent any further habitat loss and ideally increase the availability of high-quality greenspaces across the study site (Alvey 2006), both to encourage the red squirrels to disperse into currently unoccupied parts of the town (which would lead to positive encounters with the public) and to provide natural food sources to mitigate the potential negative impacts of supplemental feeding, as well as benefitting other urban wildlife species. This should also include engaging with residents to encourage ‘wildlife-friendly’ gardening practices, as gardens form a substantial component of the greenspaces within the urban landscape (Goddard et al. 2010) and contribute to improved physical and mental well-being (Chalmin-Pui et al. 2021).

In addition, the peri-urban woodland should be managed to ensure the long-term availability of natural food sources. The Sefton Coast Woodlands Working Plan (The Mersey Forest 2013) identified that the current age structure of the woodland means that the pine trees are becoming over-mature and, if no management action is taken, this would result in little or no healthy cone crop within the next 60 years. Therefore, urgent forest management is required to provide a mosaic of age structure, to ensure continuous cone production. This is critical considering that the woodland appears to support the high-density source population for Formby (see Chapter Four) and so, if the habitat quality declines over the coming decades, this may impact the long-term survival of the whole stronghold population.

5.2.3. Provision of Water Sources

Although water sources appear to be available in many of the residential gardens, more widespread provision of water sources in the woodlands, particularly in the north where the highest squirrel population density was located, may be beneficial. Additional water sources may be particularly important in drought years when the seasonal ponds dry up, which is likely to become more prevalent with climate change (Brooks 2009). These could consist of additional dog water fountains stationed around 'Squirrel Walk' and other pathways, which could also be used by local dog walkers; the creation of artificial ponds, which may also benefit the rare natterjack toad (*Epidalea calamita* Cope 1864; Denton et al. 1997) that is present in the National Trust reserve; or even the establishment of a reflection pool and associated camera hide, which may also be beneficial for public engagement, income generation, and as a water source for other wildlife (e.g. birds).

There are potential zoonotic disease risks from squirrels sharing water sources with domestic dogs and cats, such as *T. gondii* (Jokelainen & Nylund 2012) and *Cryptosporidium* (Bujila et al. 2021). It would be difficult to prevent squirrels and domestic pets from accessing specific water sources, as both are likely to use any sources that are available to them; for example, raised water bowls could be installed on trees, out of reach of domestic pets, but squirrels are still likely to exploit available pet water bowls or garden ponds. However, most recorded cases of zoonotic disease transmission from red squirrels have occurred via captive populations in zoos or as exotic pets (Deng et al. 2020, Allendorf et al. 2021) rather than wild populations.

5.2.4. Prevention of SQPV Outbreaks and Road Traffic Casualties

This research has provided evidence for a population density threshold, at which the population may be prone to future disease outbreaks (see Chapter Two). Similar occurrences have been observed in Tollymore Forest Park, in County Down in Northern Ireland, and Drumlanrig Castle Estate, in Dumfries & Galloway in the south of Scotland, where high density red squirrel populations have suffered from SQPV outbreaks despite the low abundance of grey squirrels (McInnes et al. 2013). In addition, this research has highlighted a potential source-sink population dynamic during a SQPV outbreak (see Chapter Four). Similar associations between movement patterns and disease spread have been observed during mange outbreaks in red foxes (Gosselink et al. 2007) and tuberculosis outbreaks in European badgers (Rogers et al. 1998).

Therefore, conservation management should aim to: (1) reduce and maintain the population density within Formby below the population density threshold (approximately 6 individuals/ha), through encouraging dispersal into the wider stronghold; (2) prevent the initiation of a SQPV outbreak through ongoing grey squirrel control, both within the red-only area centred around Formby and the wider Merseyside stronghold (Chantrey et al. 2014), which would also help to create available habitat in the wider stronghold into which the red squirrels can disperse; and (3) minimise disease transmission risk between red squirrels. Some potential strategies to achieve these have been previously discussed in section 5.2.1, but it may also be beneficial to implement a disease monitoring scheme using a non-invasive surveillance strategy (e.g. using tail-hair samples that can be collected via sticky patches on supplemental feeder boxes; Everest et al. 2019) to rapidly identify and remove any infectious individuals (Fiegna et al. 2016). However, this could be costly to run on a permanent basis in terms of both time and resources, so could instead be implemented following any grey squirrel sightings within the red-only stronghold and/or when the red squirrel population is approaching the proposed population density threshold (i.e. when the risk of a SQPV outbreak is at its highest).

Although grey squirrels and the spread of SQPV are the main threats for red squirrel populations, the high number of road traffic casualties in urban areas (see Chapters One and Three) are also a concern as this may put vulnerable populations under additional pressure. Therefore, it may be feasible to install aerial bridges at one or more of the identified road traffic mortality ‘hotspots’ to provide the squirrels with an alternative crossing route and reduce the number of road traffic casualties (Magris & Gurnell 2002, White & Hughes 2019). Alternatively, mature trees lining the roads may provide crossing routes via their canopy cover, whilst simultaneously increasing the habitat availability and quality within the urban area.

As previously discussed, it has been suggested that the population has a source-sink dynamic, where the source is the higher quality peri-urban woodlands and the sink is the lower quality urban area. In this way, ‘surplus’ individuals (i.e. unsettled subordinate adults and sub-adults, known as ‘floaters’; Wauters et al. 2001) from the woodlands disperse into the urban area, where a disproportionate number are then killed by road traffic (McCleery 2010). This is supported by the evidence that the main road traffic mortality ‘hotspots’ were identified on Victoria Road near the entrance to the National Trust reserve, where the red squirrel population density is highest, and further eastwards at the intersection with Gores Lane (see Chapter Three). Similarly, Shuttleworth (2001) found a positive correlation between the number of road traffic casualties and the red squirrel population density in the National Trust reserve during his study in Formby. This source-sink dynamic may also be a contributing factor to the lack of dispersal out of Formby, with the urban area acting as an ‘ecological trap’ between the woodlands in the west and the wider stronghold out to the east (Verbeylen et al. 2003a). Therefore, it may be necessary to provide a habitat corridor connecting the woodlands through to the eastern side of town, so that Formby could act as a source population for the wider stronghold (Gosselink et al. 2007).

5.3. Future Research

This research has highlighted that there are potential impacts of different degrees of urbanisation (e.g. city centres through to suburban areas with isolated greenspaces and peri-urban forests) on the behavioural ecology of wildlife populations. Therefore, it would be beneficial for comparative research across the whole urban-rural gradient for both urban-adaptor and urban-avoider species, to help inform urban management for wildlife conservation.

It is somewhat concerning that several individuals with suspected SQPV (i.e. with visible suspected symptoms, such as lesions) later tested negative for the disease. This suggests that they were potentially infected with a visually similar disease such as *Staphylococcus aureus*-associated FED, which has been highlighted as a cause for concern in other red squirrel populations (e.g. on Jersey and the Isle of Wight; Simpson et al. 2010, 2013b, Blackett et al. 2018, Fountain et al. 2021) but has yet to be identified in the Formby population. It would be beneficial to test for FED in an attempt to identify this currently unknown disease in the Formby population, as FED has the potential to cause substantial mortality particularly in isolated populations (Blackett et al. 2018).

As the provision of supplemental food for wildlife is so widespread in the UK, it is difficult to conclusively determine the impact of feeding peanuts to red squirrels; however, as nutritional deficiencies have been identified in other supplementary-fed wildlife species (Murray et al. 2016), this warrants further investigation. Further comparative analyses with other red squirrel populations from mainland Europe, where peanuts appear to be less preferred by red squirrels (Kostrzewa & Krauze-Gryz 2020), may be beneficial. Alternatively, an experimental study could be conducted by feeding different quantities of supplemental food items to captive grey squirrels and repeating the bone strength and mineral content analyses used in this research.

5.4. Conclusion

This research, in addition to providing a suitable model for studying other urban wildlife species, has demonstrated that urban environments can be suitable habitats and may aid in conservation efforts for the endangered, native red squirrel, as long as they are appropriately managed. The red squirrel population in the study site of Formby, Merseyside, is currently at risk of urban intensification, leading to the loss of the remaining greenspaces and associated natural food sources, as well as future SQPV outbreaks. Widespread supplemental feeding has helped to support the population but, on its own, cannot compensate for the requirement for high-quality habitat patches embedded throughout the urban landscape. Supplemental feeding may also be a beneficial tool for encouraging the squirrels to disperse into the wider Merseyside stronghold, thus contributing to their range expansion, but needs to be carefully managed to mitigate any negative impacts regarding increased disease transmission risk. Instead, it is essential to manage the availability and quality of the urban greenspaces to ensure the availability of reliable natural food sources and suitable nesting sites, as well as habitat corridors (either via aerial rope bridges or the canopy cover of mature trees) to reduce road traffic mortality. It would also be beneficial to maintain, and ideally improve, the habitat quality in the adjacent peri-urban woodlands, as this supports the main source population for the local area and potentially for the wider stronghold. In addition, it is vital to continue grey squirrel control across the stronghold, both to protect the red-only population in Formby but also to provide space in the wider stronghold into which they can disperse. Through more sympathetic management of urban areas, this would benefit wildlife conservation efforts amidst the current biodiversity crisis as well as the mental and physical well-being of the human inhabitants.

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Appendices

APPENDIX I: Fingland K, Ward SJ, Bates AJ, Bremner-Harrison S (2022) A systematic review into the suitability of urban refugia for the Eurasian red squirrel *Sciurus vulgaris*. *Mammal Review* 52: 26–38.

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REVIEW

A systematic review into the suitability of urban refugia for the Eurasian red squirrel *Sciurus vulgaris*

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ABSTRACT

1. Urban growth and intensification are projected to increase as the global human population increases. Historically, urban areas have been disregarded as suitable wildlife habitat, but it is now known that these areas can be biodiverse and that wildlife species can adapt to the environmental conditions. One such urban-dwelling species is the Eurasian red squirrel *Sciurus vulgaris*, which has suffered population declines in several countries throughout its range in recent decades.
2. The current published literature was systematically reviewed to determine whether or not urban habitats are suitable refugia for red squirrels, through identifying and discussing key topics regarding the urban ecology of red squirrels.
3. Urban environments can support higher population densities of red squirrels than rural areas, probably due to the widespread and reliable provision of anthropogenic supplemental food alongside natural food sources. The availability and quality of urban greenspaces are important determinants of the suitability of urban habitats for red squirrels, as they provide natural food sources and nesting sites. Despite the barriers present in urban landscapes (e.g. roads), red squirrels can still disperse and maintain gene flow at the population level.
4. Road traffic accidents appear to be a significant cause of mortality in some urban red squirrel populations, and seasonal peaks of mortality occur during the autumn months. Diseases (e.g. squirrelpox virus) can also be a significant cause of mortality, although effects differ between populations and depend on whether grey squirrels *Sciurus carolinensis* are present. Many of the predation events that affect red squirrels appear to be due to free-ranging domestic and feral cats *Felis catus*, although there is currently little evidence to suggest that predation is a limiting factor for urban red squirrel populations.
5. We conclude that urban areas can be suitable refugia for red squirrels, provided that high-quality greenspaces are maintained. Mitigation measures may also be necessary to reduce population mortality and to prevent disease outbreaks.

26 Mammal Review **52** (2022) 26–38

INTRODUCTION

Currently, 55% of the global human population inhabits urban areas; the percentage is projected to increase to 68% by 2050 (United Nations 2019). The resultant urban growth and intensification are dramatic forms of habitat alteration, which present substantial challenges to wildlife conservation (McCleery et al. 2014). For instance, urban areas can lack sufficiently large and connected greenspaces to provide foraging resources, nesting sites, and dispersal for wild animals (Marzluff & Ewing 2001). The presence of roads has been identified as a mortality risk and a potential barrier to movement (Rondinini & Doncaster 2002). The urban environment may also support an increased abundance of predators, particularly free-ranging companion animals such as cats *Felis catus* and dogs *Canis lupus familiaris*, associated with higher numbers of human inhabitants (Baker & Harris 2007).

Urban developments have historically been ignored as potential wildlife habitat (McCleery et al. 2014). However, it has been demonstrated that urban areas can be biodiverse and, in some cases, support populations of endangered species (Alvey 2006). Environmental action plans now include the development of urban areas, particularly identifying the importance of greenspaces (e.g. DEFRA 2018), which highlights the urgent need to advance our understanding of urban wildlife ecology to inform appropriate management. Resources can be plentiful in urban habitats, resulting in higher population densities of wild animals than in rural locations. For example, peregrine falcons *Falco peregrinus* have significantly higher clutch sizes, brood sizes, and fledging success in urban areas (Kettel et al. 2018). Some species have the behavioural flexibility to adapt to the urban environment, resulting in urban populations having adaptations that are not shared by their rural counterparts. For instance, some urban mammals have been shown to alter their foraging patterns temporally and spatially, to avoid periods of peak human activity (Lowry et al. 2013).

This study focusses on the Eurasian red squirrel *Sciurus vulgaris* (hereafter referred to as the red squirrel), which is a diurnal, arboreal rodent that currently remains widespread throughout most of Eurasia. In the UK, the population has declined following extensive habitat loss and the introduction of the Eastern grey squirrel *Sciurus carolinensis* (hereafter referred to as the grey squirrel) from North America in the late 19th century (Bosch & Lurz 2012). Grey squirrels out-compete the red squirrel for resources (Wauters et al. 2002) and spread the squirrelpox virus (SQPV), which they carry asymptomatically but is often fatal to red squirrels (Rushton et al. 2006). Following the more recent introduction and subsequent spread of grey squirrels in Italy, the continental population of red squirrels is now also threatened (Bertolino et al. 2008, 2014).

In the UK, red squirrels are part of the natural heritage and an iconic species, which tourists specifically travel to reserves to see (Shuttleworth 2001). Red squirrels are highly charismatic, and interactions with them can encourage people to connect with nature and develop a wider interest in wildlife. This is particularly important in urban areas, where access to greenspaces may be limited, as contact with local wildlife has been shown to provide environmental education and improve mental well-being (Dearborn & Kark 2009, Irvine et al. 2010). People who live alongside red squirrels tend to have positive feelings towards the species and are aware of the conservation issues affecting the squirrels (Rotherham & Boardman 2006). Therefore, red squirrels have a high cultural value (Gurnell & Pepper 1991), but need conservation management in the UK and in other countries where populations are declining (e.g. Turkia et al. 2018). Towns and cities could potentially act as refugia for red squirrels, so understanding the species' urban ecology would help to inform effective management.

Review objective

Systematic reviews using strict methodologies were pioneered in the field of medicine to overcome the selection and interpretation biases that can occur in traditional literature reviews (Haddaway et al. 2015). In recent years, systematic reviews have been promoted within conservation biology, due to the associated benefits of increased transparency and objectivity, in order to inform evidence-based management decisions (Cook et al. 2013).

This systematic review aimed to evaluate the current published literature regarding the urban ecology of red squirrels, in order to determine whether urban areas are suitable refugia for red squirrels. The initial broad literature search aimed to ensure an objective approach to the review and to overcome any potential selection bias. Following the screening process and based on the final dataset of articles, this review aimed to identify and synthesise key topics regarding the urban ecology of red squirrels.

METHODS

Definitions

Interpretation of the term 'urban' is complicated, with definitions based upon different factors (e.g. density of buildings or the human population) and varying between scientific disciplines (McIntyre et al. 2000). Therefore, for the purpose of this review, 'urban' is broadly classified as any area characterised by a collection of buildings, including houses and shops, and associated infrastructure, such as gardens, roads, and parkland (Baker & Harris 2007).

In the context of this study, we define anthropogenic food sources as food provided by humans for wild animals; this includes supplemental feeding, which is the deliberate provision of food (e.g. through bird feeders or squirrel boxes), as well as the accidental provision of food (e.g. through garden allotments or rubbish, which can be scavenged). In addition, we define natural food sources as food that would be available without human intervention, regardless of whether those items are available through artificial planting and management by humans. For example, in the context of squirrels, this would include a range of coniferous and broad-leaved tree species growing naturally in rural woodlands, as well as those being managed by humans in forestry plantations and urban areas.

Literature search

A systematic literature review was undertaken in December 2019 following the PRISMA protocols (Moher et al. 2009). The PRISMA protocols aim to improve reporting of systematic reviews by following a process of screening and assessing the identified literature using clearly defined inclusion and exclusion criteria.

A literature search was conducted on Scopus, Web of Science, and Google Scholar using the following search terms: ('red squirrel*' OR 'Eurasian red squirrel*' OR 'European red squirrel*' OR 'Sciurus vulgaris') AND (urban* OR town OR city). All the results listed on Scopus and Web of Science were collected. Only the first 200 articles of Google Scholar were collected, following the threshold used in other reviews (e.g. Lisón et al. 2020); after this point, the results become less relevant. There were no restrictions regarding the year of publication.

Inclusion criteria

Articles were excluded if they were not published in a peer-reviewed journal or not written in English. The articles were then screened for inclusion using the following criteria: 1) the title, abstract, or keywords specified '(Eurasian) red squirrel' or '*Sciurus vulgaris*', and 2) the keywords included, e.g. 'urban ecology' or 'urbanisation/urbanization', or 3) the abstract specified that the study was conducted in an urban environment, including comparisons with rural areas, or 4) the abstract specified that the study investigated the impact of an anthropogenic activity (e.g. road traffic mortality, supplemental feeding). Any articles that did not meet the screening criteria were excluded from the review.

For the articles that passed the initial screening process, the full text was then assessed for eligibility. The articles were required to meet all three of the following inclusion criteria; otherwise, they were excluded: 1) Eurasian red squirrels were the focal study species; 2) the research had been conducted on an urban population, including if compared with a rural population; and 3) the study investigated at least one aspect of red squirrel biology (e.g. behavioural ecology, genetics, population mortality) or conservation (e.g. reintroductions). Assessment of the scientific quality (e.g. identification of causal relationships, empirical evidence, replication, and wider application) was not used to exclude articles at this stage, but instead informed later evaluation in this systematic review. Only the key findings from the final dataset of articles were collated and reported.

RESULTS

The initial literature search returned 226 articles once duplicates were removed (Fig. 1). During the screening process and eligibility assessment, 201 articles were excluded in total, resulting in a final dataset of 25 articles

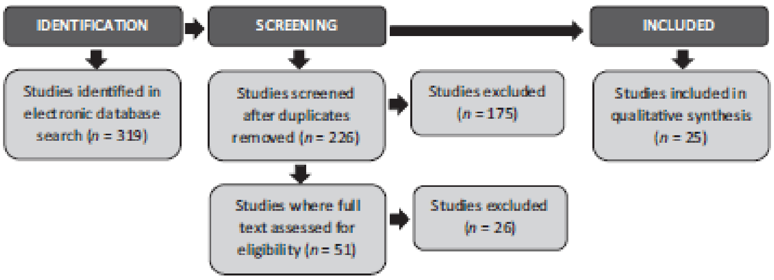

Fig. 1. PRISMA literature search and screening flow diagram of articles included and excluded from systematic analysis. Each article describes one study.

describing 25 studies (Appendix S1). Of the 25 articles, 76% were published since 2014, whilst the remaining articles were published between 1986 and 2009, often with large gaps between years (Fig. 2). The majority of the published research was conducted in mainland Europe (n = 16); five studies were conducted in the UK and Republic of Ireland, three studies were conducted in Japan, and one study was carried out in both Poland and the UK (Appendix S1).

Of the 25 articles identified for this review, 84% provided evidence that urban areas can be suitable habitat for red squirrels, whilst the remaining 16% highlighted the potential risks of urban environments, such as mortality threats (Appendix S1). Five broad topics regarding the urban ecology of red squirrels were categorised from the studies, with each article evaluating at least one of the identified topics (Fig. 3).

Supplemental feeding

Supplemental feeding appears to be widespread in urban areas throughout the red squirrel's geographic range (Appendix S1) and can help to increase the viability of small, isolated populations (Bertram & Moltu 1986, Rézouki et al. 2014, Vieira et al. 2015). Reher et al. (2016) found that red squirrels' home ranges in a cemetery in Hamburg,

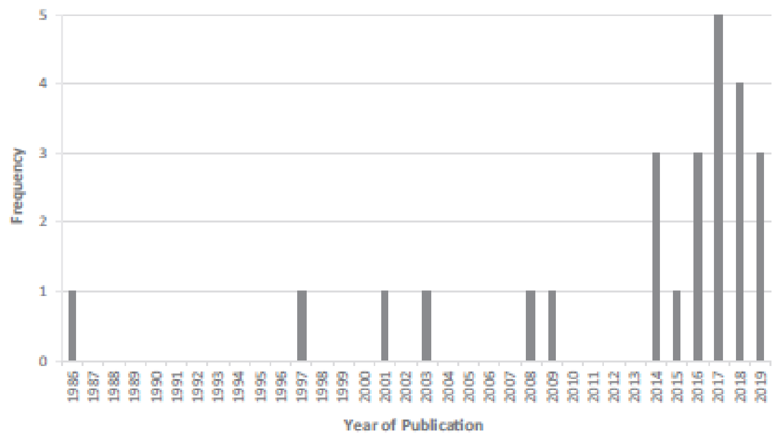

Fig. 2. The number of red squirrel urban ecology studies, based on the final dataset of articles (n = 25), published each year from 1986 to 2019.

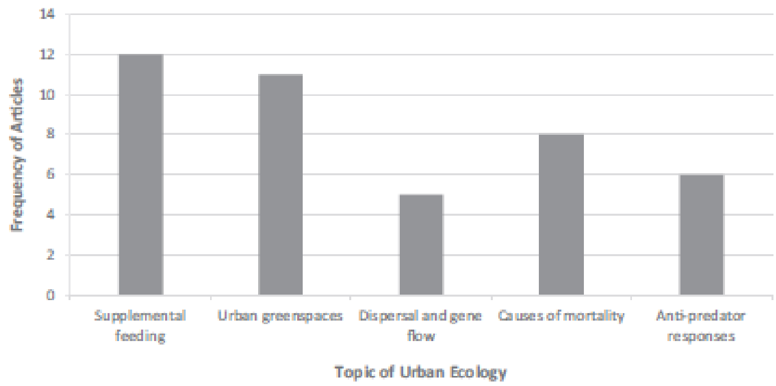

Fig. 3. The number of articles evaluating each topic regarding the urban ecology of red squirrels, as identified and categorised by this review. Each article can include information on more than one topic.

Germany, always incorporated stable natural food sources, but squirrels responded to seasonal changes in the provision of supplemental food by relocating their core areas. Core areas were shifted closer to reliable supplemental food sources in the autumn, when availability was highest, and moved to locations with natural food supplies in spring and summer when supplemental food became limited. Therefore, anthropogenic food sources had a measurable effect on patterns of space use, despite the abundance of natural food (Reher et al. 2016).

Supplemental feeding can support high red squirrel population densities in urban areas (Babińska-Werka & Żółw 2008, Kopij 2014, Jokimäki et al. 2017), as urban home ranges were found to be smaller, with a greater degree of overlap, than those in more rural populations (Reher et al. 2016, Thomas et al. 2018). Thomas et al. (2018) also reported that the urban squirrels in Hamburg city centre were less active throughout the day (mean activity rate of 25% per one-hour time slot) than the individuals inhabiting a nearby semi-natural site (mean activity rate of 58%). Furthermore, Turner et al. (2017) found that the red squirrels' body mass in the same cemetery site in Hamburg was relatively constant throughout the year, with only minimal physiological changes (e.g. metabolic rate) in response to seasonal fluctuations. These studies indicate that the widespread and abundant supplemental food sources available in urban environments allow red squirrels to satisfy their energy requirements whilst minimising energy expenditure, by reducing the need to travel as far or as often for foraging excursions (Reher et al. 2016, Turner et al. 2017, Thomas et al. 2018). Supplemental feeding may further increase population sizes, by allowing females to start breeding at the earliest opportunity and enhancing juvenile survival (Rézouki et al. 2014).

However, there are also risks associated with encouraging squirrels to travel between gardens whilst foraging and caching human-provided food items. Red squirrels have been shown to spend more time foraging on the ground when supplemental food is available, which may lead to more encounters with road traffic or predators; red squirrels usually spend 67–90% of their active time foraging for natural food in the canopy, but this is reduced to 50–53% when foraging for supplemental food (Shuttleworth 2001). Reproductive rates are density-dependent, so artificially high population densities can reduce fecundity in some females and therefore decrease breeding success (Kopij 2014, Stirkė 2019). Excessive consumption of supplemental food (e.g. peanuts) can also cause nutritional deficiencies, which may have health implications in highly urbanised environments where squirrels may not be able to compensate for such malnutrition if there is limited availability of natural food sources (Thomas et al. 2018). Finally, feeders may facilitate the spread of parasites and diseases, such as SQPV, by encouraging interactions both between red squirrels and with grey squirrels (Chantrey et al. 2014, Kopij 2014, Stirkė 2019).

Size, quality, and connectivity of urban greenspaces

Of the articles identified for this review, 44% highlighted the importance of size, quality, and/or connectivity of urban greenspaces for the persistence of red squirrel populations (Appendix S1). For example, Verbeylen et al. (2003) found that woodland patches within an urban landscape had a higher chance of red squirrel occupation if they were larger (>5 ha), higher quality, and well connected to nearby populations.

Using visual transects, Babińska-Werka and Żółw (2008) and Kopij (2014) recorded higher densities of red squirrels in city parks (1.8 and 0.26 individuals/ha, respectively) than in woodlands located on the outskirts of the cities (0.004–0.033 and 0.01 individuals/ha, respectively). Similarly, Jokimäki et al. (2017) found that relative squirrel abundance was higher in urban habitats (4.24 individuals/10 km transect) than in forests (1.43 individuals/10 km transect).

Babińska-Werka and Żółw (2008) and Kopij (2014) also found a significant positive correlation between the density of red squirrels and the size of the urban park, with higher densities in the larger parks, where diverse and mature trees provided ample natural food sources and which were connected to other nearby parks via habitat corridors. Conversely, smaller and more isolated urban parks with immature and few tree species had lower squirrel densities. For example, Kopij (2014) reported densities of 0.31–1.0 individuals/ha in the larger urban parks of 15 to 75 ha, compared with 0.0–0.4 individuals/ha in the parks less than 12 ha in size. Kopij (2014) also reported that parks that were close to allotment gardens, which provided anthropogenic food sources, supported higher densities of squirrels (0.55 individuals/ha) than similar-sized parks that were not bordered by allotments (0.1 individuals/ha).

The importance of high-quality habitat, specifically the availability and diversity of natural food sources, was further emphasised by Wauters et al. (1997), Vieira et al. (2015), and Jokimäki et al. (2017). Wauters et al. (1997) found that female reproductive rates and subsequent juvenile recruitment improved as the tree seed crop abundance increased. Jokimäki et al. (2017) also found that squirrel abundance increased as the spruce *Picea* cone crop increased. Vieira et al. (2015) reported that two red squirrel populations, which had been reintroduced into urban parks, were suffering from long-term declines due to the poor-quality habitat. Tree species composition in urban greenspaces is also important for providing suitable nesting sites (Kopij 2009, Stirkė 2019).

Although red squirrels seem to prefer areas with more trees available than in the surrounding urban landscape, they have been observed using very small groups or even lone trees, encircled by buildings and with no connectivity to other greenspaces, for dispersal and exploration (Hämäläinen et al. 2018). Urban red squirrels were found to travel in closer proximity to buildings than would be expected at random, with some instances of squirrels travelling along roofs and using buildings as nesting sites. This indicates that they are not confined to using only the available greenspaces (Hämäläinen et al. 2018, 2019).

Dispersal and gene flow

Fey et al. (2016) found that dispersing and non-dispersing red squirrels responded differently to the presence of roads. Non-dispersing individuals seemed to avoid roads during routine daily activities (e.g. foraging) within their home ranges, and perceived large roads (average daily traffic of 2000–7000 vehicles) as more dangerous than smaller roads (average daily traffic of 500–2000 vehicles). Conversely, roads did not act as barriers for dispersing individuals, who crossed them regardless of their size. Hämäläinen et al. (2019) also found that the landscape structure typically did not affect red squirrels' final straight-line dispersal distances, although it did alter their movement paths, with individuals favouring woodland patches whilst attempting to avoid more unsuitable habitats (e.g. open fields or buildings). In other words, individuals were likely to bypass barriers by travelling longer distances, but this did not impact how far they settled from their natal site. However, the overall dispersal distances of urban individuals were shorter than those of rural individuals (Fey et al. 2016, Selonen et al. 2018, Hämäläinen et al. 2019).

Despite shorter dispersal distances in urban populations, Fey et al. (2016) and Hämäläinen et al. (2019) suggest that the dispersal process still maintains the potential for gene flow at the population level. This is supported by Rézouki et al. (2014), who found that a red squirrel population in an urban park had relatively high levels of genetic diversity and minimal levels of inbreeding, despite being comparatively small, due to immigration from the surrounding woodlands. Similarly, Selonen et al. (2018) found no evidence to suggest that squirrels in their urban study site had been genetically isolated from the adjacent rural population.

Causes of mortality

Of the articles identified for this review, 24% discussed causes of recorded deaths. Instances of road traffic accidents were reported in 16% of the articles, ranging from 20% (n = 10; Bertram & Moltu 1986) and 33% (n = 12; Wauters et al. 1997) to 51% (n = 337; Blackett et al. 2018) and 65% (n = 188; Shuttleworth 2001) of recorded deaths. In some individual habitats, 88% of recorded deaths were attributed to road traffic accidents (Shuttleworth 2001). Further in-depth analysis of road traffic mortality by Shuttleworth (2001) determined that there was a distinct seasonal pattern, with a clear peak in the autumn months accounting for 54% of the total number of recorded deaths. Although there was no overall significant difference in the sex ratio of road traffic casualties, breeding adult males were more likely to be killed during the winter months.

Predation incidents were reported in 20% of the articles, ranging from 5.3% (n = 188; Shuttleworth 2001), 7.1% (n = 337; Blackett et al. 2018), and 10% (n = 10; Bertram & Moltu 1986) to 22% (n = 32; Fey et al. 2016) and 25% (n = 12; Wauters et al. 1997) of recorded deaths. Many of the mortality events were attributable to predation by free-ranging domestic or feral cat attacks (Bertram & Moltu 1986: 10%, Wauters et al. 1997: 8.3%, Blackett et al. 2018: 5.0%). Shuttleworth (2001) and Fey et al. (2016) did not specify the predator species, although the latter suggested they were most likely to be free-ranging cats and red foxes *Vulpes vulpes*.

Only two of the studies discussed the potential impact of accidental anthropogenic poisoning on red squirrel populations. Blackett et al. (2018) reported that 1.2% of red squirrels found dead displayed signs of poisoning, most likely by anticoagulant rodenticides. Lurz et al. (2017) conducted a pilot study investigating levels of industrially produced mercury in an urban red squirrel population in the city of Warsaw, Poland, and in two rural populations on the islands of Arran and Brownsea, UK. The results indicated that red squirrels have high levels of mercury, even individuals from rural UK islands where industrial activities are minimal, with currently unknown health implications. However, there is little evidence to suggest that accidental anthropogenic poisoning is a limiting factor for urban red squirrel populations.

Finally, Blackett et al. (2018) reported that 34% of recorded casualties on Jersey in the Channel Islands, UK, were attributed to diseases and that many individual red squirrels found dead were suffering concurrently with multiple conditions. The confirmed diseases included the following: amyloidosis (19%), where deposits of an abnormal protein can result in renal or hepatic failure; fatal exudative dermatitis (FED) associated with *Staphylococcus aureus* infection (15%); and parasitic infections of *Capillaria hepatica* (34%), *Hepatozoon* species (16%), and *Toxoplasma gondii* (2.1%). Amyloidosis and FED were determined to be major contributors to red squirrel mortality on the island. Amyloid deposits were often found alongside co-existing FED or *Capillaria hepatica* infections, suggesting there may be some association between the diseases. In squirrels on Jersey, there was no evidence of SQPV, which is visually similar to and often confused with FED (Blackett et al. 2018). SQPV can be a significant cause of red squirrel mortality, as occurred in Merseyside, UK, where the red squirrel population was reduced by over 80% (Chantrey et al. 2014). Despite this, Chantrey et al. (2014) found that 8.4% of red squirrels exposed during an SQPV outbreak survived the infection. Analyses supported the prediction that grey squirrels were responsible for initiating the outbreak, but once the disease had been introduced, the main driver of SQPV infections was red squirrels transmitting the disease to each other (Chantrey et al. 2014).

Anti-predator responses

Urban red squirrels had significantly shorter flight initiation distances and vertical escape distances than their rural conspecifics when approached by humans, which indicates strong habituation (Uchida et al. 2016, 2017). It is not clear whether urban squirrels are better at assessing risk (i.e. they tolerate human presence, until humans are in close proximity) or have reduced vigilance levels; if the latter, then they may not respond appropriately to predation threats (Uchida et al. 2016). As described by Uchida et al. (2017), alert distance (the distance at which an animal first detects a potential threat) represents vigilance, whilst flight initiation distance and vertical escape distance represent risk assessment. Uchida et al. (2019) found that both alert distance and flight initiation distance were significantly shorter in urban squirrels than in their rural conspecifics, which implies that, although urban individuals have reduced vigilance, they are also able to evaluate risk levels.

When reintroduced into an urban park, red squirrels that were used to human disturbance had a higher probability of survival and breeding than individuals initially taken from rural woodlands (Wauters et al. 1997). Wauters et al. (1997) suggested that squirrels that are familiar with receiving food from humans might adapt better to supplemental feeding, although red squirrels translocated from rural Fife in Scotland to Regent's Park in London still habituated to human disturbance and successfully adapted to using supplemental food hoppers (Bertram & Moltu 1986). However, these individuals had a period of captivity before release (Bertram & Moltu 1986), whereas the individuals described by Wauters et al. (1997) were released on the day of capture.

Another study monitored the impact of visitors to Fota Wildlife Park, Ireland, on the red squirrel population and found temporal avoidance of public areas (Haigh et al. 2017). The authors observed that, even though large numbers of squirrels continued to use the park, the squirrels only moved into the public areas when the park was closed, and instead were significantly more active in the non-public areas of the park when it was open. Despite this, there was no significant association between faecal cortisol metabolite levels (commonly used as a measure of stress) and visitor abundance.

DISCUSSION

The literature search highlighted the gradual increase in articles regarding urban ecology of red squirrels since 2014, which suggests that this is an area of growing research interest and most likely reflects the wider realisation of the need to understand the impact of increasing urbanisation on wildlife ecology (McCleery et al. 2014). Red squirrels have successfully adapted to urban environments, where their behavioural flexibility allows them to exploit the resources available whilst avoiding or adapting to the risks present.

Risks in the urban environment

Of the reviewed studies that reported instances of road traffic mortality, only Blackett et al. (2018) compared different causes of death. However, other comparative studies have also found that road traffic accidents accounted for 42% (n = 163; Simpson et al. 2013b), 43% (n = 245; LaRose et al. 2010), and 48% (n = 119; Shuttleworth et al. 2015) of recorded red squirrel deaths, comparable to the 51% reported by Blackett et al. (2018). This suggests that road traffic accidents are a significant cause of mortality in some urban red squirrel populations, although there are opposing arguments regarding whether records tend to be over- or underestimated (Shuttleworth 2001). Road kills tend to be more conspicuous, and may be more likely to be reported, than other causes of mortality (e.g. predation). On the other hand, cases could go unreported due to the carcasses being disposed of by roadkill removal services, eaten by scavengers, or degraded by road traffic and the weather. In some instances, squirrels injured by road traffic accidents shelter under nearby vegetation or in dreys before dying.

The seasonal pattern in road traffic mortality may be due to a combination of reasons. Red squirrels spend more of their active time engaging in foraging and scatter-hoarding behaviours on the ground, increasing from 32% in the summer months to 44% in the autumn due to the increased food availability, which could result in squirrels crossing roads more often as they travel to find and cache food items (Shuttleworth 2000, 2001). There are also typically lower red squirrel numbers in late spring and early summer, followed by a post-breeding increase in numbers in autumn and early winter, when the young are weaned (Bosch & Lurz 2012). As the juveniles then disperse, they are potentially more likely to encounter road traffic, although Shuttleworth (2001) found that the majority of autumnal road traffic casualties were adults. Similarly, Blackett et al. (2018) reported that only 2.9% of the 171 identified road traffic casualties were juveniles and 8.2% were subadults, although there was no further investigation into seasonal patterns of mortality. The male-biased winter mortality peak may be due to the breeding males searching for sexually active females (Shuttleworth 2001), as the start of the reproductive period tends to fall in December or early January (Bosch & Lurz 2012).

The presence of predators in urban environments appears to vary depending on the species and potentially the extent of urbanisation. Some studies suggest that predator abundance is decreased compared with rural areas, whereas others have suggested higher densities of predators in urban areas (e.g. Shochat et al. 2006, Bateman & Fleming 2012, Jokimäki et al. 2017). Similarly, predation risk varies between studies, ranging from less than 10% (Shuttleworth 2001, Blackett et al. 2018) to over 20% of recorded deaths (Wauters et al. 1997, Fey et al. 2016). When predation of red squirrels in urban environments does occur, it appears that free-ranging domestic and feral cats are generally responsible (Fey et al. 2016, Jokimäki et al. 2017, Blackett et al. 2018).

From the reviewed articles, there is currently little evidence to suggest that predation has significantly contributed to the decline of the red squirrel; this is consistent with other published studies (e.g. Petty et al. 2003, Turkia et al. 2018). However, the additional mortality pressure could have localised impacts in areas where predator densities are particularly high, as is the case with cats in urban environments, especially where red squirrel populations are already vulnerable. In addition, predation events may be underestimated as they can be difficult to identify, for instance if the squirrel carcass is mostly consumed or carried away by the predator. This implies that the impact of predation, particularly by free-ranging domestic and feral cats, on urban red squirrel populations may be greater than previously estimated and so may benefit from further investigation.

Red squirrels are susceptible to a range of diseases, some of which could potentially limit populations, such as SQPV (Chantrey et al. 2014). High concentrations of viral DNA have been found in the oral mucosa and exudative skin lesions of SQPV-infected individuals, which suggests that red squirrels may transmit the disease amongst themselves via social interactions (e.g. fighting, grooming, drey sharing) and scent marking (Fiegna et al. 2016). Local disease outbreaks may be exacerbated in urban environments, where red squirrel populations can often reach higher densities and the presence of supplemental feeders may act as sources for the spread of infection. Therefore, there is a need to identify any infectious individuals rapidly (Fiegna et al. 2016), ideally using non-invasive surveillance strategies such as those developed by Everest et al. (2019), to remove those individuals and reduce transmission of the disease.

There has been growing evidence that adenovirus may present a significant threat to red squirrel populations (e.g. Everest et al. 2014, 2018). Blackett et al. (2018) did not fully explore the potential for adenovirus infections within the Jersey population, having only tested 12 out of 337 individuals; however, 42% of those 12 tested positive for adenovirus. Another emerging disease is squirrel leprosy *Mycobacterium lepromatosis*, for which clinical cases have been diagnosed across the UK (e.g. Simpson et al. 2015, Avanzi et al. 2016), but which was not identified on Jersey (Blackett et al. 2018). In addition, there was no evidence of SQPV on Jersey (Blackett et al. 2018), which is likely due to the absence of grey squirrels on the island (McInnes et al. 2009), but the disease is likely to be a significant cause of red squirrel mortality where grey squirrels are present (Chantrey et al. 2014).

Blackett et al. (2018) highlighted FED and amyloidosis as causes of concern on Jersey. FED has also been identified as a potential cause of concern on the Isle of Wight (Simpson et al. 2010), although to a lesser extent than on Jersey, and there has been at least one possible case on Anglesey in North Wales (Shuttleworth et al. 2015). There have been no other published reports of amyloidosis, apart from one potential case on the Isle of Wight (V Simpson, unpublished observations, as cited in Blackett et al. 2018) and occasional cases in Lancashire, UK (J Chantrey, personal communication, as cited in Blackett et al. 2018). The origins and development of both FED and amyloidosis are currently unclear, although some evidence suggests that genetic predisposition (i.e. a heritable allele that increases an individual's susceptibility to a disease) and stress may be factors for amyloidosis (Caughey & Baron 2008, Simpson et al. 2013a). This may explain why the disease is so prevalent on Jersey, where the red squirrel population is small and isolated, and stresses associated with traffic, pets, and high local densities of squirrels lead to agonistic interactions at food sources (Blackett et al. 2018). As urban populations of red squirrels are often isolated and exposed to similar stressors, the impact of FED and amyloidosis requires further investigation in other locations.

Resources in the urban environment

Urban wildlife populations are reported to have higher numbers of bold individuals than rural populations (e.g.

Møller 2012, Díaz et al. 2013, Lowry et al. 2013), as demonstrated in urban red squirrel populations (Uchida et al. 2016, 2017, 2019), allowing them to exploit the available resources. Boldness is thought to result from repeated exposure to non-lethal stimuli from frequent human disturbance, resulting in a 'transfer of habituation' to other predator stimuli and an overall reduction in anti-predator response (McCleery 2009). This adaptive response helps to prevent urban animals repeatedly fleeing and expending energy unnecessarily, instead allowing them to spend more time foraging (Sol et al. 2013). Furthermore, Uchida et al. (2019) suggested that the capacity to assess varying risk levels effectively and respond accordingly may reflect higher cognitive abilities, which supports the proposition that learning in urban animals may be improved by the environmental complexity and unpredictability associated with urban areas (Griffin et al. 2017).

Urban environments appear to support higher population densities of red squirrels than rural areas (e.g. Babińska-Werka & Żółw 2008, Kopij 2014, Jokimäki et al. 2017). However, as highlighted by Jokimäki et al. (2017), the use of visual transects to count red squirrels in urban environments and woodlands may be affected by differences in habitat and habituation to people. For example, detectability may be higher in urban areas due to the increased visibility in more open greenspaces (e.g. parks) and more bold individuals (e.g. Uchida et al. 2016). On the other hand, detectability may be reduced due to obstructions by buildings (Jokimäki et al. 2017). The use of capture–mark–recapture can provide more accurate and reliable estimates of abundance (e.g. Turlure et al. 2018), but this invasive method requires time, resources, and experience. Alternatively, the use of baited visual transects may be a more effective method in future studies to improve detectability whilst maintaining the benefits of this non-invasive technique (Gurnell et al. 2011).

Many of the studies in this review have emphasised the importance of the availability and quality of urban greenspaces for red squirrel populations. For example, it is crucial to manage the tree species composition in urban greenspaces to ensure the availability of natural food sources (Vieira et al. 2015, Reher et al. 2016, Jokimäki et al. 2017) and the provision of suitable nesting sites (Kopij 2009, Stirkė 2019). However, it is difficult to unpick the relative importance of natural and supplemental food sources and habitat quality, as there often tends to be supplemental feeding wherever red squirrels are present in urban areas, as well as the availability of other anthropogenic food sources. For example, city parks with diverse and mature tree species had high densities of red squirrels, but of those parks, those that bordered allotments with anthropogenic food sources had the highest population densities (Kopij 2014). Clearly, high-quality natural food sources can support higher population densities, but it appears that the availability of supplemental food further increases the habitat quality. This is supported by the fact that supplemental feeding directly impacts patterns of space use, even when natural food sources are abundant (Reher et al. 2016).

Supplemental food is also typically available throughout the year and so can be relied upon when natural food is seasonally scarce, although supplemental food may not be nutritionally ideal: many people provide peanuts, which are high in fat and desirable to the squirrels, but can result in calcium deficiencies (Bosch & Lurz 2012). Natural food sources can help to provide a more nutritionally balanced diet and compensate for any malnutrition caused by eating supplemental food (Thomas et al. 2018).

Thomas et al. (2018) suggested that the smaller and overlapping home ranges observed in urban individuals may be because suitable habitat is sparse and movement between fragments is energetically costly, so red squirrels may simply occupy the remaining habitat fragments with available food sources. This is supported by Wauters et al. (1994), who found that the core areas of red squirrel home ranges in fragmented woodlands were smaller than those in larger, continuous woodlands, which suggests that home range size can be limited by the size of the woodland fragment.

The higher population densities in urban areas are likely to be due to the distribution and abundance of supplemental food, combined with the availability of natural food sources in the remaining greenspaces throughout the urban landscape, resulting in smaller home ranges with a greater extent of overlap between individuals (Reher et al. 2016, Thomas et al. 2018). This is supported by other studies that suggest that food availability is the main factor limiting abundance (Petty et al. 2003) and that squirrels alter their patterns of space use in response to changes in the distribution and abundance of food in rural woodlands (Wauters et al. 2005). The increased food availability also means that urban individuals can quickly attain the minimum body weight required to come into oestrus (Wauters & Dhondt 1989a), further increasing population sizes by allowing females to start breeding at the earliest opportunity (Rézouki et al. 2014), and can maintain stable body masses throughout the year (Turner et al. 2017). This differs in comparison with rural populations, where body weight is at a minimum in late summer and reaches a maximum in late winter, due to the limited provision or absence of supplemental food and the large seasonal changes in ambient temperature that affect natural food availability (Wauters & Dhondt 1989b).

Wauters et al. (2010) suggested that increasing habitat fragmentation when fragments are surrounded by an unsuitable or hostile matrix (e.g. barriers or urbanised areas) may inhibit dispersal, whereas when the matrix is not hostile (e.g. farmland or rural villages that are unsuitable for settling but not for movement), different degrees of habitat fragmentation appear not to affect dispersal behaviour. Contrary to the suggestion that urban areas could be considered a hostile matrix, the findings from this review indicate that movement ability does not appear to be restricted by built structures within the urban landscape. Instead, the shorter dispersal distances of urban individuals appear to be due to the stable resource availability reducing the need to disperse further (Fey et al. 2016, Selonen et al. 2018, Hämäläinen et al. 2019). This is supported by the fact that urban red squirrels travel in closer proximity to buildings than would be expected at random, as they move through the urban landscape to exploit supplemental food (Jokimäki et al. 2017, Hämäläinen et al. 2019).

Habitat loss and fragmentation are closely correlated with urbanisation (Liu et al. 2016) so it can be difficult to distinguish between the impacts of urbanisation and habitat fragmentation on red squirrel populations, as both can result in similar behavioural patterns. For example, similar effects of habitat fragmentation on spatial organisation and dispersal have been identified in both urban and rural woodland squirrel populations (Wauters et al. 1994, 2010, Thomas et al. 2018, Hämäläinen et al. 2019). However, the findings from this review suggest that habitat loss due to urbanisation has a greater impact on red squirrel populations than fragmentation, which is supported by Fahrig (1997). Therefore, increasing the availability and quality of greenspaces would be of most benefit to red squirrel conservation in urban environments, rather than improving connectivity between greenspaces.

CONCLUSION

The findings from this systematic literature review indicate that urban habitats can be suitable refugia for red squirrels, as their behavioural flexibility has allowed them to adapt successfully to the urban environment. However, it is desirable to manage urban habitats more effectively for both wildlife and people. For instance, the management of sufficiently large greenspaces and their tree species composition would ensure the availability of high-quality, reliable natural food sources and appropriate nesting sites for red squirrels, as well as benefitting the mental and physical well-being of the human residents. Furthermore, the provision of supplemental food can have substantial benefits, although mitigation measures may be required to minimise any negative impacts. For example, public engagement regarding suitable food items and appropriate hygiene practices may help to reduce nutritional deficiencies in the squirrels' diets and prevent disease outbreaks from sharing feeders. As is the case with existing red squirrel reserves, grey squirrel control within and around urban refugia would help to reduce SQPV outbreaks.

Mitigation measures may also help to reduce mortality; for instance, the use of non-invasive surveillance strategies could help to identify potential disease outbreaks, or rope bridges could be constructed to provide crossing points over busy roads to reduce road traffic casualties. In addition, the current evidence regarding the impact of predation by free-ranging domestic and feral cats is limited, but would benefit from further research to evaluate whether it has been previously underestimated. Mitigation measures may help to reduce instances of predation by pets; for example, owners could keep their cats inside during early morning when squirrels are most active and likely to be predated, but this would not address any potential impact of feral cats.

Red squirrels are well adapted to inhabiting urban areas, becoming strongly habituated to human presence, and being able to move through the built landscape to exploit the available resources. Therefore, urban refugia, if appropriately managed, may aid conservation efforts for this declining, native species whilst simultaneously benefitting the human inhabitants.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

Appendix S1. Key findings from the final dataset of published articles (n = 25), determined from the Abstracts and Discussions, and conclusions drawn regarding whether or not urban habitats are feasible refugia for red squirrels.

APPENDIX II: *Mammal Review* Supporting Information Appendix S1: Key findings from the final dataset of published articles (*n* = 25), determined from the Abstracts and Discussions, and conclusions drawn regarding whether or not urban habitats are feasible refugia for red squirrels.

**Abbreviations: AD = alert distance, FCM = faecal cortisol metabolites, FED = fatal exudative dermatitis, FID = flight initiation distance, RTAs = road traffic accidents, SQPV = squirrelpox virus, VED = vertical escape distance*					
Reference	**Geographical Location**	**Key Findings**	**Secondary Findings**	**Tertiary Findings**	**Conclusions: Urban refugia suitable for red squirrels?**
Babińska-Werka and Żółw 2008	Poland	Highest numbers of squirrels observed in city centre parks, compared to woodlands on city outskirts. Significant association between size of urban park and number of squirrels, with larger parks supporting larger populations.	Number of squirrels associated with tree species present in urban parks. Mature and diverse stands of deciduous and coniferous trees provided year-round food source and supported larger squirrel populations.	Higher numbers of squirrels in city parks may also be due to supplemental feeding and fewer predators.	Yes - although size of greenspaces and tree species composition to provide reliable natural food sources are important.
Bertram and Moltu 1986	UK	Squirrels reintroduced to Regent's Park benefitted from specially designed red-only feeding hoppers, which excluded grey squirrels, and became habituated to human disturbance.	Out of ten squirrels released, one was predated by a feral cat and two were killed by road traffic.		Yes - provided grey squirrels are not present. Red squirrels benefitted from supplemental feeding, but potential mortality threats from RTAs* and cat predation.
Blackett et al. 2018	Jersey (UK)	Casualties attributed to RTAs (50.7%), disease (34.4%), predation (7.1%, of which 5% due to cats), other trauma (6.5%), and suspected poisoning (1.2%).	Amyloidosis, often associated with FED* and hepatic capillariasis, highlighted as a cause of concern on Jersey. SQPV* not detected in selected cases.		Potential mortality threats associated with urban areas, including RTAs, cat predation, and disease (exacerbated by high population densities and sharing supplemental feeders).
Chantrey et al. 2014	UK	SQPV has a significant negative effect on red squirrel population densities and population growth rates. Approximately 8% of red squirrels may survive SQPV infection during an outbreak.	Grey squirrels appear to initiate outbreaks, then main driver of red squirrel SQPV cases is infection by conspecifics. Therefore, disease transmission among red squirrels is density-dependent.		Yes - provided contact with grey squirrels is minimised. However supplemental feeding may increase population densities and interactions between squirrels.
Fey et al. 2016	Finland	During routine movements squirrels crossed roads less frequently, particularly those with high traffic volume, and were located further from roads than simulated random walk paths. During dispersal, individuals did not avoid roads regardless of their size.	Roads potentially act as barriers to non-dispersing individuals at the home range-level. However the barrier effect of roads for dispersing individuals is reduced, which can increase potential gene flow at the population-level.	Potential main cause of mortality was predation, most likely by free-ranging cats and foxes.	Partially - roads may act as barriers at the home range-level but not at the population-level. However potential mortality threats from predation, mainly by cats.
Haigh et al. 2017	Republic of Ireland	Squirrels focussed their activities in non-public areas, only moving into public areas when the park closed. There was little difference in behaviours (e.g. foraging and vigilance) between public and non-public areas, which	Levels of FCM* were highest in areas with the most human disturbance, although no correlation with visitor numbers. However FCM levels positively correlated with squirrel		Yes - provided that non-public areas are accessible.

		suggests habituation rather than compensatory strategies.	density, which may be as a result of increased competition for resources.		
Hämäläinen et al. 2018	Finland	Although squirrels preferred areas with more trees available than in the surrounding urban landscape, they were often found using sites with little or no trees.	Movements of squirrels are not restricted to their natural coniferous habitat type, but also seem well-adapted to use structures in urban areas.		Yes - squirrels are well-adapted to use urban areas, although important to maintain greenspaces within urban landscape as preferred habitat.
Hämäläinen et al. 2019	Finland	Barriers to movement may exist in both rural (e.g. open fields) and urban (e.g. buildings and roads) landscapes. Squirrels had very short dispersal distances (on average, 0.5 km) in the urban site, compared with long dispersal distances (up to 16 km) in the rural site.	In the urban landscape, squirrels preferred areas with deciduous or coniferous trees, so movement paths were longer to avoid barriers. However this did not affect how far the squirrels settled from their natal home (i.e. overall straight-line dispersal distance).		Yes - squirrels are well-adapted to use urban areas. Although urban landscape structure may affect movement behaviours, it may not affect other population aspects (e.g. gene flow).
Jokimäki et al. 2017	Finland	Squirrel abundance increased with human population density, number of artificial feeding sites, and spruce cone crop. Squirrel abundance was significantly higher in urban areas compared to woodland habitats.	Goshawk were more abundant in urban areas than woodland habitats, but no effect of presence on squirrel abundance. However, there was a weak negative association between feral cats and squirrel abundance.		Yes – natural food sources and supplemental feeding associated with human presence are important for squirrel abundance. However potential mortality threats from cat predation.
Kopij 2009	Poland	All squirrel home-range core areas were located in broad-leaved or mixed tree stands, with common oaks recorded in most core areas (90.2%).	Dreys tended to be located in the most common tree species, which also provided the largest amount of squirrels' preferred natural food sources.	There was a clear preference for locating dreys in tree-tops, which is most likely an anti-predator adaptation.	Yes – although greenspaces and tree species composition are important for providing natural food sources and drey sites.
Kopij 2014	Poland	Squirrels were most common in largest parks 2-7 km from the city centre, although were not recorded in the small, highly modified, and frequently visited parks in the city centre (0-2 km). Squirrel population density was much higher in city parks, compared to forests.	Squirrel population size affected by size of park, distance and connectivity to surrounding parks, and tree species. Large parks with mature and diverse tree stands supported larger populations than smaller, isolated parks with young, monoculture tree stands.	Squirrel population density was lower in some larger parks where higher densities of nesting hooded crows. Supplemental feeding may have both positive (support higher population density) and negative (spread of parasites/disease) impacts.	Yes – although size, connectivity, and tree species composition of greenspaces are important.
Lurz et al. 2017	Poland and UK	Mercury levels in individuals from three sites (two rural sites in UK and one city site in Poland) were shown to be elevated. Contrary to predictions, mercury levels in female squirrels from one rural UK site were significantly higher than either males or females from other two sites.	Mercury accumulates in urban and terrestrial woodland ecosystems, as well as previously known marine and arctic ecosystems, which may have potential health impacts for endangered species. Monitoring strategy for accumulating mercury levels in the environment currently lacking, with unknown future impacts.		Currently unknown impacts of anthropogenic pollution.
Reher et al. 2016	Germany	Home ranges' always incorporated locations with stable natural food sources, but squirrels responded to seasonal changes in the provision	Stable and abundant food sources result in small home ranges and a lack of seasonal body mass change.		Yes – supplemental and natural food sources are important to ensure high quality habitat, where squirrels will

		of supplemental food by relocating their core areas.			respond to seasonal changes in availability by relocating core areas.
Rézouki et al. 2014	France	Urban parks can maintain self-sustaining and viable long-term red squirrel populations, with relatively high levels of genetic diversity and minimal inbreeding.			Yes – although supplemental feeding may help to ensure the viability of small, isolated populations.
Selonen et al. 2018	Finland	Urban and rural squirrels were found to be part of the same larger population, with gene flow in and out of the city. Increased genetic differentiation found within the urban population, compared to within the rural population or between urban-rural populations.	Increased genetic differentiation was not correlated with proportion of urban landscape between two individuals, but instead may be explained by short dispersal distances of urban squirrels due to abundant food sources.		Yes – although urbanisation may result in increased genetic differentiation, squirrels are still able to move throughout the urban environment and function as a single population with the surrounding rural areas.
Shuttleworth 2001	UK	RTAs accounted for 65% of recorded casualties (26.7% in woodland reserve and 88% in urban area).	RTAs highly seasonal with a clear peak in autumn, but no correlation was found between number of road deaths per month and proportion of time spent foraging on the ground.	Adult males, mostly in breeding condition (78%), were killed more frequently on roads than females during winter months.	Potential mortality threats from RTAs in urban areas.
Stirkė 2019	Lithuania	Most dreys were built in Scots pine (84.8%), which were most widespread tree species and provided stable food source. Most dreys were built in southern orientation (33.9%), potentially to aid thermoregulation and minimise energy loss.	Most dreys were located in the top-height trees (52.9%), which is most likely an anti-predator adaptation. Co-evolved predators only occur occasionally, but feral cats are the most important predator of squirrels in urban areas.	Widespread supplemental feeding has both positive (supports higher population density) and negative (reproductive suppression and spread of parasites/disease) impacts.	Yes – greenspaces and tree species composition are important for providing natural food sources and drey sites. However potential mortality threats from cat predation and mixed impacts from supplemental feeding.
Thomas et al. 2018	Germany	Squirrels had smaller home ranges with a greater overlap in urban site, compared with semi-natural site. Urban squirrels were less active throughout the day and had lower activity rates overall.	Combination of stable and abundant supplemental food sources means less time spent foraging whilst maximising energy intake.	Supplemental feeding can lead to decreased breeding success, increased intraspecific aggression, increased transmission of disease and parasites, and health conditions.	Yes – supplemental food allows squirrels to have smaller home ranges and reduced activity patterns, whilst achieving energy requirements. However, some potential negative impacts regarding breeding success and disease transmission.
Turner et al. 2017	Germany	Constant body mass year-round and minor changes in resting metabolic rate, as supplemental food is stable and abundant.	Stable and abundant food sources allow for modified activity patterns (e.g. smaller home range sizes and reduced activity duration), which maximise energy intake and minimise energy expenditure.		Yes – supplemental food minimises the need for seasonal physiological adaptations.
Uchida et al. 2016	Japan	FID* were significantly shorter for urban squirrels compared to rural squirrels, which	No seasonal differences in FID for urban squirrels, whereas FID were shorter in autumn than in spring for rural squirrels. Reliable		Yes - squirrels demonstrate behavioural flexibility and habituate to human presence.

		suggests that urban squirrels have strongly habituated to human presence.	supplemental feeding in urban areas appears to have buffered seasonality in anti-predator responses.		
Uchida et al. 2017	Japan	VED* was significantly correlated with FID, but not AD*. VED of urban squirrels was significantly shorter than rural squirrels.			Yes - squirrels demonstrate behavioural flexibility and habituate to human presence.
Uchida et al. 2019	Japan	AD was shorter in urban squirrels compared to rural squirrels but did not differ between different approaching objects (humans, model predators, and novel objects). FID was shorter in urban squirrels but differed between the approaching objects (shortest FID towards humans), whereas FID for rural squirrels were similar between the objects.	AD was considered to be related to vigilance, whereas FID related to risk assessment and therefore habituation. Urban squirrels appear to show reduced vigilance but also can assess risk levels.		Yes - squirrels demonstrate the ability to assess risks and appropriately respond to threats.
Verbeylen et al. 2003	Belgium	Modelling found a higher chance of occupancy in woodland patches if larger (>5 hectares), higher quality (based on percentage of seed-bearing trees), and well-connected to nearby source populations.			Yes – although the size, quality, and connectivity of greenspaces are important.
Vieira et al. 2015	Portugal	Reintroductions to urban parks were relatively successful, as squirrels still present 20 years later. However lack of active management and poor-quality habitat has led to population declines and threatens long-term viability of populations.	Recommendations made for three critical stages (pre-project stage, release stage, and post-release stage) to ensure successful reintroductions.		Yes – although important to manage urban parks to provide high quality habitat and to consider the benefits of supplemental feeding.
Wauters et al. 1997	Belgium	Squirrels habituated to human disturbance had higher probability of survival and breeding when reintroduced to an urban park, compared to individuals taken from rural woodlands.	Woodland structure at the removal site did not affect settlement success in the urban park. Reproductive rate was density-dependent, but also positively correlated with size of seed crop.	Road traffic and predation can cause high mortality in populations released into urban habitats.	Yes – although natural food sources are important. However potential mortality threats from RTAs and predation.

APPENDIX III: Research Licences and Permissions

Research Licences

Home Office Licence

Guidance from the Home Office Inspector and NTU's AWERB were sought to determine whether the study required regulation under the Animal (Scientific Procedures) Act 1986. Following consultations, it was decided that no regulated procedures were proposed and therefore a Home Office licence was not required.

Natural England Licence

A Natural England licence (2019-39834-SCI-SCI-7) for disturbing and taking red squirrels for research purposes was obtained, which was renewed annually and amended as required. Following consultations with Natural England and agreement from the National Trust, a permit for conducting research on a SSSI was also obtained.

The Conservation of Habitats and Species Regulations 2017 (as amended) and Wildlife and Countryside Act 1981 (as amended)

NATURAL ENGLAND
Customer Services
Wildlife Licensing
Natural England
Horizon House
Deanery Road
Bristol
BS1 5AH
T: 0208 026 1089
F: 0845 601 3438

LICENCE - Use Prohibited Methods for wild animals: Science, Education & Conservation

This licence authorises acts that would otherwise be offences under the above legislation

Any request for information in this licence will be considered under the Environmental Information Regulations 2004 and the Freedom of Information Act 2000 as appropriate.

Natural England Ref:	**2019-39834-SCI-SCI-7**

Under the The Conservation of Habitats and Species Regulations 2017 (as amended) and Wildlife and Countryside Act 1981 (as amended) Natural England has granted this licence for Prohibited Methods Mammals for the purpose of:

Science or education, under section 55(2)(a) and/or section 16(3)(a)

to:

Name (in full):	Ms Kathryn Fingland
Company Name:	Nottingham Trent University
Address:	School of Animal, Rural and Environmental Sciences Brackenhurst Campus Southwell
County:	Nottinghamshire
Postcode:	NG25 0QF

Between the dates of:

19 July 2019	and	01 September 2020	inclusive

At (locations):

Site/Location Name	County	OS Grid Reference
Formby	Lancashire	SD293074

For the following species:

Species Common Name *(Taxonomic Name)*	Number	Activity	Method	Detailed Location	OS Grid Reference
Red squirrel *(Sciurus vulgaris)*	0	Disturb	Hand	Formby	SD293074
Red squirrel *(Sciurus vulgaris)*	0	Take	Hand	Formby	SD293074
Red squirrel *(Sciurus vulgaris)*	0	Disturb	Cage traps	Formby	SD293074
Red squirrel *(Sciurus vulgaris)*	0	Take	Cage traps	Formby	SD293074

This licence is granted subject to the licensee, including servants and named agents, adhering to the conditions and notes specified below.

Signature: Andrew Laborde **Date:** 18 July 2019

(for and on behalf of Natural England)

WARNING

- **This licence authorises acts that would otherwise be offences under the The Conservation of Habitats and Species Regulations 2017 (as amended) and Wildlife and Countryside Act 1981 (as amended). Any departure from the conditions relating to this licence may be an offence under that legislation;**
- **This licence conveys no authority for actions prohibited by any other legislation;**
- **This licence can be modified or revoked at any time by Natural England, but this will not be done unless there are good reasons for doing so. The licence is likely to be revoked immediately if it is discovered that false information had been provided which resulted in the issue of the licence.**

LICENCE CONDITIONS

1. These conditions apply to the licensee and any additional authorised person. The licensee and any additional authorised person(s) are responsible for ensuring that any licensed operations/ activities comply with all terms and conditions of the licence.

2. The licensee and any additional authorised person(s), shown on the licence, may act under the authority of this licence. The licensee or any additional authorised person(s) may also employ assistants provided they work under the direct personal supervision of the licensee or authorised person.

3. Whilst engaged in activities permitted by this licence, the licensee and/or any additional authorised person(s), must have access to a copy of this licence and produce it to any police officer or any Natural England officer on demand.

LICENCE CONDITIONS

4. The Licensee and any additional authorised person(s) shall permit an officer of Natural England, accompanied by such persons as he/she considers necessary for the purpose, on production of his/her identification on demand, reasonable access to the site for monitoring purposes and to be present during any operations carried out under the authority of this licence for the purpose of ascertaining whether the conditions of this licence are being, or have been, complied with. The Licensee shall give all reasonable assistance to an officer of Natural England and any persons accompanying him/her.

5. This licence does not convey any right of entry upon land, and the landowner's/occupier's prior permission must be obtained, as necessary, before the licence is used.

6. No licensed activity shall be carried out under this licence on a National Nature Reserve or Marine Nature Reserve except with the prior written permission of Natural England.

7. A person authorised by the licensee shall provide him/her with such information as is within his/her knowledge and is necessary for the Report, which the licensee is required to make to Natural England.

8. The 'Report by licensee of action taken under licence' must be completed, even if no licensed action is taken. It must be submitted on line or sent to the Natural England office at the address shown on this licence, to arrive no later than 14 days (two weeks) after the expiry of the licence. Failure to make a report may result in the licence being revoked and/or any future applications being refused.

9. This licence may be modified or revoked at any time by Natural England.

Additional condition(s):

Please see separate Annex.

NOTES

1. Please read the details of your licence carefully to ensure that you comply with it paying particular attention to the number and species licensed as this may differ to what was requested in your application.

2. Under Regulation 60(1) of the Conservation of Habitats and Species Regulations 2017 (as amended), it is an offence to contravene or fail to comply with a licence condition. This includes all persons authorised to act under this licence.

3. An additional authorised person is a suitably trained and experienced person who is able to carry out work under a licence without the personal supervision of the licensee. To carry out licensed activities their name will be on the licence. To comply with the licence conditions, additional licenced persons should have a copy of the licence accessible when acting under the licence.

NOTES

4. An assistant is a person assisting the licensee or the additional authorised person(s). Assistants are only authorised to act under a licence whilst they are under the direct supervision of either the licensee or the additional authorised person(s).

5. Please note the information of the 'Report by licensee of action taken under licence' may have changed from previous years. The data required in your report and the required format can been viewed on the Natural England website. Alternatively you can request a copy from the Natural England address shown on your licence.

Additional note(s):

Please see separate Annex.

Additional Authorised Individuals

The additional authorised individuals listed below are also authorised to act under the terms and conditions of this licence:

Title	First Name	Surname	Address Line 1	Postcode
Mrs	Fiona	Whitfield	Seaforth Nature Reserve	L21 1JD
Miss	Rachel	Miller	Seaforth Nature Reserve	L21 1JD
Mrs	Lesley	Morris	7 Back Lane	WA5 2XQ
Doctor	Samantha	Bremner-Harrison	Nottingham Trent University	NG25 0QF
Doctor	Samantha	Ward	Nottingham Trent University	NG25 0QF

Annex 2: Pro forma "Notice and Consent"

NATURAL ENGLAND

Sefton Coast Site of Special Scientific Interest Merseyside ("the SSSI")

NOTICE OF PROPOSAL TO CARRY OUT AN OPERATION

Section 28E(1)(a) Wildlife and Countryside Act 1981 (as amended and inserted by section 75 and Schedule 9 of the Countryside and Rights of Way Act 2000)

On behalf of the below-named person(s), Natural England has prepared this Notice of Proposal to carry out, cause or permit an operation (or operations) specified in the SSSI's notification. That operation (or operations) will be lawful if carried out, caused or permitted by the below-named person in the manner and on the land specified below once this document is signed by or for the below-named person(s) and for Natural England.

Name and address of owner(s) or occupier(s):

The National Trust

Countryside Office
Blundell Avenue
Freshfield
Formby
Liverpool
L37 1PH

I/we give notice under Section 28(E)(1)(a) of the Wildlife and Countryside Act 1981 of my/our proposal to carry out cause or permit to be carried out the operation(s) specified below on the land specified below:-

Specified operations:

To permit Kat Fingland to carry out research to include trapping of red squirrels.

Contact: School of Animal, Rural and Environmental Sciences

Nottingham Trent University

Nottingham Road

Southwell NG25 0QF

Details of proposed operations:

I think my proposed research will fall under point 10 of the list, as I'm hoping to live-capture trap the red squirrels in order to PIT tag them and attach radio collars to a sub-sample, before releasing them immediately at the capture site. I think I will also need to disinfect the traps between each successful capture (I have Anigene to spray), to help prevent spreading any diseases – I'm not sure whether that falls under point 6? Andrew has also kindly offered to let me store my traps, when they're not being used, which I believe is somewhere near the NT offices – if that falls under point 22?

Live-trap capture of red squirrels in order to tag and attach radio collars, before re-release. Traps will be disinfected using Anigene spray. Research will take place over 3 years, from 2017 to 2020. The research period each year will run from 1st May to 15th August in Year 1 and 1st April to 1st September in Years 2 and 3.

Land on which operations are to be carried out:

The National Trust Estate, Formby

Unit 22 Sefton Coast SSSI ..... *[or see attached map]*

Signed for owner(s) or occupier(s): K Klein

Date: 13/4/17

CONSENT OF NATURAL ENGLAND

Section 28E(3)(a) Wildlife and Countryside Act 1981 (as amended and inserted by section 75 and Schedule 9 of the Countryside and Rights of Way Act 2000)

Natural England gives you consent to carry out, cause or permit to be carried out, the operations specified above on the land specified above.

If you wish to change the proposed operations or their location or to carry out additional operations for which consent has not yet been given, or if a time period given in the notice, above, has expired, you are required to give further written notice to Natural England.

Unauthorised operations may destroy, damage or disturb features of special scientific interest.

It is the responsibility of the grantee of this consent to ensure that no other consents, whether of a public or a private nature, are needed and, if needed, to secure them him/herself. The grantee is also responsible for carrying out the consented operation(s) safely and in all ways according to the law.

Access Permissions

National Trust

Permission to access to the National Trust site was obtained either from the Property Manager or the Head Ranger, which was renewed annually.

National Trust

Justin.Matthews@nationaltrust.org.uk
Direct line: 01704 878591
4th December 2018

Kat Fingland
c/o School of Animal, Rural and Environmental Sciences,
Brackenhurst Campus,
Nottingham Trent University,
Nottingham Road,
Southwell,
Nottinghamshire
NG25 0QF

Dear Kat,

<u>Fieldwork at National Trust Formby</u>

Thank you for your enquiry about carrying out fieldwork at Formby towards your PhD thesis with the working title of "Utilisation of urban environments by the Eurasian red squirrel (*Sciurus vulgaris*): influence on their ecology and implications for their conservation"

Thank you for providing a copy of your method statement for our records.

This field work is due to take place between 13th May and 16th August 2019. You must inform us if you intend to work outside of these dates.

National Trust Formby will permit you to undertake the above on National Trust land providing you follow these requirements:

i) All trapping to be done in areas previously agreed by the National Trust Countryside Manager.
ii) You do not deviate from the methodology proposed in your method statement, for work to be carried out on National Trust land, without prior agreement with National Trust Countryside Manager/Area Ranger
iii) Natural England consent has been provided for this work.

National Trust
Formby Countryside Office
Blundell Avenue
Freshfield
Formby
Liverpool L37 1PH
Tel: +44 (0)1704 878591
Fax: +44 (0)1704 835378
www.nationaltrust.org.uk

Thank you for providing a risk assessment for this work, a copy of the University's public liability insurance and confirmation that you do not require a Home Office licence for this research.

While we make every effort not to alter the conditions set out in this letter, we ask that you will comply with any reasonable requests from National Trust staff, who manage the area, if they think that the public or your own safety maybe compromised.

We would very much like to be kept up to date with developments and findings from your research as it will increase our knowledge of Red squirrels within the Formby area and help us to better manage this population going forward.

Please will you sign where indicated below and return one copy of this letter to me. I would also recommend that you bring a copy of this letter on site whilst you are undertaking your surveys so that you can show it to National Trust staff if requested.

I wish you success in your field work.

Yours sincerely

Justin Matthews
Area Ranger, Formby

I confirm that I accept the guidelines and conditions stated above for conducting my field work at National Trust Formby.

Signed K. Fingland Date 05/12/2018
(Kat Fingland)

Cont/d

Access to Residential Properties

Permission to access residential properties was obtained from the homeowners, who were asked to sign a consent form.

RED SQUIRREL RESEARCH
TRAPPING CONSENT FORM

I consent for live-capture traps to be used at the named property, for the purpose of researching red squirrels as part of a PhD project.

- I give Kat Fingland, with accompanying researchers, permission to access the property for the purposes of placing, baiting, setting, checking, and locking the traps. I understand that, once the trap is set, the researchers have a legal obligation to check the traps on a regular basis and must be able to access the property once the trap is opened.
- I understand and agree that, should any grey squirrels be incidentally trapped, they are an invasive species and legally must be humanely removed by the researchers.
- I understand that I may not damage, move, or set the traps without prior agreement from the researchers.
- I understand that Kat Fingland, Nottingham Trent University, and the Lancashire Wildlife Trust cannot be held responsible for any damage or personal injury that may occur to me as a result of using the trap.
- I understand that I can withdraw my consent and participation in this research at any time – however, if the trap is currently open, I agree that access will be allowed to close and remove the trap.

NAME ________________________________

ADDRESS

TELEPHONE NUMBER ________________________

SIGNED ________________ DATE ____________

Permission to Transport Red Squirrel Carcasses

Permission to transport and necropsy the red squirrel carcasses was granted by the LWT in consultation with the Merseyside Wildlife Crime Officer.

Seaforth Nature Reserve
The Wildlife Trust for Lancashire, Manchester and North Merseyside
Port of Liverpool, Merseyside, L21 1JD

Tel: 0151 9203769
Web: www.lancswt.org.uk

The wildlife TRUSTS
Lancashire, Manchester & N Merseyside

30th March 2017

Lancashire Wildlife Trust have been collecting red squirrel carcasses, reported by the public from around the Formby area. As part of a red squirrel PhD research project, these carcasses will undergo a post-mortem for samples to be collected for disease analysis. Lancashire Wildlife Trust have provided these carcasses to Kat Fingland, and fellow researchers from Nottingham Trent University, for transport back to Nottingham in order to undergo the post-mortems.

Rachel Miller of Lancashire Wildlife Trust spoke to Merseyide Wildlife Crime Officer PC Kreuger 1179 on 30th March to advise her of this.

RMiller

Rachel Miller

Red Squirrel Project Officer

Lancashire Wildlife Trust

Tel: 075907458562

Email: rmiller@lancswt.org.uk

FundRaising Standards Board INVESTORS IN PEOPLE POSITIVE ABOUT DISABLED PEOPLE

The Lancashire Wildlife Trust is a registered charity (Number 229325) and a registered company (Number 731548) registered at
The Barn, Berkeley Drive, Bamber Bridge, Preston, Lancashire, PR5 6BY
VAT No. 604 6629 29.

APPENDIX IV: Fieldwork Protocols

The following fieldwork protocols were developed based on pre-existing protocols provided by the LWT and refined through consultations with the Home Office Inspector and several squirrel experts.

Live-Capture Trapping

1. The traps are to be locked open and pre-baited for at least three days, prior to setting the traps. A trap checklist will be utilised to ensure no traps are accidentally left unchecked.
2. The initial trapping period (Phase 1) will take place from Monday to Friday for up to four weeks, until either no new squirrels are caught for three consecutive days or the four weeks of trapping are complete. The woodlands and urban gardens are to be trapped on alternate weeks, to minimise travelling time and ensure that the traps are checked regularly (i.e. woodland traps open for weeks one and three, while the urban garden traps are locked open, and then vice versa for weeks two and four). Ensure that the traps that are not in use are padlocked open and pre-baited.
3. The traps are to be baited (with sunflower seeds, peanuts, monkey nuts, and apple) and opened from 07:00 each day, with the start time recorded on the 'Trap checklist'. Ensure that the traps are covered with roofing membrane and foliage for shelter. Only bait the trap once per day when opening the traps in the morning unless a squirrel is caught and then the trap can be rebaited if needed.
4. Traps are to be checked at two to three hourly intervals until approximately 4pm, to ensure that the squirrels will only be in the traps for a short period of time. At the start of each new round, record the start time on the 'Trap checklist' and tick off each trap once checked. On the final round, padlock the traps open and record the finish time. At the end of the trapping period, collect all the traps, thoroughly disinfect them in Anigene, and put into secure storage.
5. If the weather is poor (e.g. strong winds and rain), do not set the traps that day. If the weather turns inclement during the day, finish checking the traps but lock them open. On the other hand,

if the weather is very hot for an extended period then trapping can start early (from 05:00 to approximately 12:00) or late (from 13:00 to approximately 20:00) if needed, so that trapping can occur during the cooler times of the day.

6. When re-trapping (Phase 2) to remove the radio collars, follow the same protocols as above but only place the traps where the collared squirrels are located, with two traps in each location, and only for up to three weeks. Trapping should continue until either all collared squirrels are caught and their collars removed, or the three weeks of trapping are complete.

Capture of a Red Squirrel

1. There should always be two people conducting the live-capture trapping: one to handle and process the squirrels, whilst the other records the relevant information on the datasheets.
2. Even if there is not a squirrel present in the trap, record the trap number and time on the trapping checklist.
3. Calmly inspect the squirrel in the trap for any signs of injuries or disease, such as SQPV lesions. If none or only minor typical injuries are observed, then proceed as below. If severe injuries or illness are suspected, then see the separate protocol for 'Capture of a suspected injured or sick red squirrel' below.
4. All equipment (including callipers, Pesola scales, PIT-tagging equipment, radio collar, sharps disposal box, and datasheets) should be easily accessible. Only use one handling sack (or turn inside out, if necessary) and handling cone for each squirrel to minimise the risk of spreading disease. Transfer the squirrel from the trap into the handling sack and then into the handling cone.
5. Measure and record the following:
    a. Sex (male or female)
    b. Breeding condition (breeding or non-breeding)
    c. Estimated age (adult, sub-adult, or juvenile)

d. Weight (in grams) with the cone and then calculate without the cone

e. Length of right shin (in mm), by measuring from the heel to the top of the bent knee

f. Parasite burden

   i. Note both the level (none, low, medium, or high) and type (e.g. fleas, ticks, lice, harvest mites)

   ii. If the parasite burden is particularly high, consider using a rabbit insecticide spray, consult with the vets, or release the individual without further processing

g. If a radio-collared recapture, check for any signs of abrasion from the collar and, if severe or ongoing, remove the radio collar

6. Check the squirrel for a pre-existing PIT tag using the handheld reader (trimmed tail hair may also indicate this). If already PIT tagged, record the ID and record the individual as a recapture. If this is the first recapture in a new round of trapping (e.g. tagged in Phase 1 and caught again in Phase 2, or tagged one year and caught again the following year), then record as above as if a new capture. If recaptured within the same round of trapping (e.g. tagged in Phase 1 and caught again in the same Phase 1, or similarly for Phase 2), the squirrel can be immediately released at the site of capture once the PIT tag has been checked and recorded.

7. If there is no PIT tag, check one with the handheld reader to ensure it works and implant it into the nape of the neck, where the skin is loose and it shouldn't interfere with the radio collar. After implantation, check the PIT tag again with the handheld reader and dispose of the needle in the sharp's disposal box. Trim some tail hair as a marker that it is a tagged individual, in case it is recaptured at a later date. Should the number of PIT tagged squirrels reach the limit designated under the Natural England licence, only recaptures will be recorded, whereas new individuals will not be processed and will immediately be released at the site of capture.

8. Using the Health Check datasheet, make sure the squirrel is suitable to radio collar. If so, place the collar around the squirrel's neck, making sure it is neither too tight nor too loose and

orientated correctly. Again, check the collar with the receiver to ensure it is functioning correctly. If caught during Phase 2, remove the radio collar and check for signs of abrasions.

9. Throughout processing, monitor the squirrel for signs of stress. Should any individuals become particularly stressed (e.g. breath-holding) during processing, release the squirrel immediately at the capture site. Should there be any minor cuts that are bleeding, stem the bleeding with a tissue and consider using a silver nitrate stick.
10. Once processing is complete, immediately release the squirrel at the capture site and clean hands with alcohol gel. Thoroughly clean the handling cone, equipment, and trap with Anigene HLD4V, before either resetting or padlocking the trap open. Place any used handling sacks and handling cones into a bin liner, to be washed thoroughly in Anigene at the end of the day and dried before use the following day.

Capture of a Suspected Injured or Sick Red Squirrel

1. In order to keep contamination of the equipment and local environment to a minimum, transport the squirrel in the trap for veterinary assessment. While one person should immediately contact the LWT Red Squirrel Officer, the other member of the team should retrieve the transportation box from the car and return to the trap location. While wearing nitrile gloves, place the trap containing the squirrel into the box for transportation. If the transportation box is unavailable, cover the traps with handling sacks instead.
2. Ideally, organise for a non-trapping person (e.g. LWT Red Squirrel Officer) to collect and take the squirrel to the local veterinary practice. If this is not possible, one person should continue to check and lock the traps while the other takes the squirrel to receive veterinary attention. However, this should be a last resort as there may potentially be other squirrels in the remaining traps.
3. As a first response, take the squirrel to the local veterinary practice, which should be open and available in case of emergency (08:30 – 18:00) due to the planned days and timings of the

trapping. Following consultation with the vets, if the squirrel may need longer-term rehabilitation then contact the RSPCA inspector to arrange transportation to Stapeley Grange. If the squirrel may need short-term rehabilitation, it may be cared for by the LWT Red Squirrel Officer.

Capture of a Grey Squirrel

Should any grey squirrels be incidentally trapped, they will be humanely dispatched as legally required under Schedule 9 of the Wildlife & Countryside Act 1981.

1. At the start of fieldwork, designate a handling sack and keep this separate from the handling sacks used for processing red squirrels.
2. If a grey squirrel is trapped, transfer it into the designated handling sack for cranial dispatch. This can only be carried out by individuals who have received appropriate training in the dispatch technique and are deemed competent.
3. After dispatch, record the following: date, estimated age, sex, breeding condition, weight (g), and length of right shin (mm).
4. Double-bag the carcass and write the date, location, and species on the outside. This can then be taken to the vets or given to the LWT Red Squirrel Officer for storing in the freezer, until it can be collected for necropsy.
5. Remove the trap and replace with a clean spare, if possible. Otherwise, replace the trap once disinfected. Thoroughly disinfect the trap, handing sack, and any other equipment used in Anigene.
6. Report the grey squirrel to the LWT Red Squirrel Officer and, if caught in the National Trust woodlands, to the National Trust Property Manager and Head Ranger.

APPENDIX V: Supplemental Feeding Survey Forms

Research Information Sheet

NOTTINGHAM TRENT UNIVERSITY

Research Consent Information Sheet

Principal researcher: Kat Fingland

Supervisory team: Dr Samantha Bremner-Harrison, Dr Samantha Ward, Dr Adam Bates

Project title: Utilisation of urban environments by the Eurasian red squirrel (*Sciurus vulgaris*): influence on their ecology and implications for their conservation

What is the purpose of this study?
The red squirrel is the only native British squirrel and was once widespread, but their numbers have severely declined. This is due to a combination of competition and the spread of a fatal disease from grey squirrels, as well as the loss of their woodland habitat. It has been estimated that, without active conservation plans, they could disappear from mainland Britain within 30 years and the continental population is also under threat. As shown in some places already, red squirrels can thrive in urban areas, due to increased food availability and the presence of local conservation volunteers. However, there are risks from road traffic and potential loss of the remaining woodland. This research aims to understand how red squirrels use urban habitats and resources, by surveying the quality of the woodland, mapping the habitat, and monitoring the red squirrels. Using this information, a strategy for managing urban areas for the benefit of this declining native species can be recommended. This could then be used to maintain and improve the suitability of existing long-term strongholds, as well as to develop new urban refuges for red squirrels.

What are we asking you and how would we like to use the information provided?
The questionnaire will be used to determine how many supplemental feeders there are throughout the town of Formby and where they are, as well as what sort of food is being provided to the squirrels, how much, and when throughout the year.

By using this information alongside the data we will collect through monitoring the red squirrels, we can investigate what sort of impact this feeding has on the local red squirrel population. For example, whether their territories overlap with locations of supplemental feeding and how important this additional food is during years with low or high natural food sources. All of this information will contribute to the overall aim of developing a beneficial urban management strategy to aid the conservation of this native species.

We are committed to respecting the ethical code of conduct of the United Kingdom Research Councils. Thus, in accordance with procedures for transparency and scientific verification, the University will conserve all information and data collected during your questionnaire in line with the University Policy, RCUK Common Principles on Data Policy (http://www.rcuk.ac.uk/research/datapolicy/), and the relevant legislative frameworks. The final data will be retained in accordance with the Retention Policy. All data will be anonymised and is available to be re-used in this form where appropriate and under appropriate safeguards.

- You have the right to withdraw your consent and participation at any moment: before, during, or after the questionnaire.

- You have the right to remain anonymous in any write-up (published or not) of the information generated from the questionnaire.

- You have the right to refuse to answer to one, several, or all of the questions you will be asked.

- Your participation does not involve any risks other than what you would encounter in daily life; if you are uncomfortable with any of the questions or topics, you are free not to answer.

- You also have the right to specify the terms and limits of use (i.e. full or partial) of the information generated.

- You have the opportunity to ask questions about this research and ensure these have been answered to your satisfaction.

- Unless required by law, only the research team have the authority to review your records; they are required to maintain confidentiality regarding your identity.

If you want to speak with someone who is not directly involved in this research, or if you have questions about your rights as a research subject, contact Professor Michael White, Chair for the Joint Inter-College Ethics Committee (JICEC) in Art & Design and Built Environment/Arts and Science at Nottingham Trent University. You can call him at 0115 848 2069 or send an e-mail to michael.white@ntu.ac.uk.

Participant Consent Form

Participant Consent Form

Dear Research Participant,

You are being asked to participate in a research study. The purpose of this questionnaire is to investigate the effects of supplementary feeding on an urban red squirrel population (see information sheet for more information). Participation is completely voluntary. You are free to withdraw from this study at any time. If you decide to withdraw, you should notify the researcher immediately. Please read the information below and the information sheet provided, and feel free to ask questions about anything that you do not understand.

Please read the following statements:

- The purpose and nature of the study has been explained to me in writing
- I am participating voluntarily
- I am 18 years old or over
- I understand that I can withdraw from the study, without repercussions, at any time in the next three years
- I understand that I can withdraw permission to use the data, in which case the material will be deleted, at any time in the next three years
- I understand that my responses, which will be made anonymous, may be included in material published from this research
- I understand that anonymized data may be used in other studies in line with the University Research Data Management Policy
- I am willing to take part in this research

Name of participant:____

Signature of participant:______________________________________

Date:____

If you have any questions or comments about this study at any time, please contact Kat Fingland:

- Email address: kathryn.fingland@ntu.ac.uk
- Postal address: Kat Fingland, Nottingham Trent University, Brackenhurst Campus, Southwell, Nottinghamshire, NG25 0QF

Supplemental Feeding Survey

NTU

NOTTINGHAM TRENT UNIVERSITY

Red Squirrel Supplementary Feeding Survey

1. Please provide your address (including house name/number and postcode) – this will allow us to map where the supplemental feeders are, in relation to the red squirrels' territories. This information will not be published, but the general location may be used on a published map to show the locations of any supplementary feeders.

……

……

……

2. (a) On average, how regularly do red squirrels visit or pass through your garden?

Every day ☐ 4–5 days a week ☐ 2–3 days a week ☐ Once a week ☐

Once a fortnight ☐ Once a month ☐ Rarely ☐ Never ☐

(b) On average, how many red squirrels do you see in your garden at the same time?

None ☐ One ☐ Two ☐ Three ☐ Four or more ☐

3. Do you feed the red squirrels in your garden, even if indirectly through feeding the birds?

Yes ☐ **Please continue with the rest of the questionnaire.**

No ☐ **Thank you for your participation in this study! Please return this questionnaire to Kat Fingland at Nottingham Trent University.**

4. What type of feeder(s) do you have and how many of each type (please tick all that apply)?

Squirrel box ☐ …………… Hanging bird feeder ☐ …………… Bird table ☐……………

Other (please describe): ………………………………………………………………………………………

5. What type of food do you provide (please tick all that apply)?

Peanuts in their shell (monkey nuts) ☐ Peanuts ☐ Hazelnuts ☐ Walnuts ☐

Sunflower seeds (in their shell) ☐ Sunflower hearts ☐ Pine nuts ☐ Maize ☐

Dried fruit ☐ Fresh fruit ☐

Other (please describe): ………………………………………………………………………………………

NTU

6. **(a)** Do you provide any additional sources of calcium (e.g. deer antler/cuttlebone) and, if so, what?

Yes ☐ Please describe…………………………………………………………………………………… No ☐

(b) If yes, how often do you provide these additional sources of calcium?

Daily ☐ Weekly ☐ Fortnightly ☐ Monthly ☐

Every 2-3 months ☐ Every 6 months ☐ Yearly ☐

7. When do you generally provide food (please tick all that apply)?

All year ☐

Spring (Mar-May) ☐ Summer (June-Aug) ☐ Autumn (Sept-Nov) ☐ Winter (Dec-Feb) ☐

8. How much food do you estimate that you typically provide per month (based on how often you purchase more food)?

0 – 1kg ☐ 1 – 2kg ☐ 2 – 3kg ☐ 3 – 4kg ☐ 4 – 5kg ☐ 5 – 6kg ☐

6 – 7kg ☐ 7 – 8kg ☐ 8 – 9kg ☐ 9 – 10kg ☐ 10+kg ☐

9. **(a)** How often do you generally clean your feeder?

Daily ☐ Weekly ☐ Fortnightly ☐ Monthly ☐

Every 2-3 months ☐ Every 6 months ☐ Yearly ☐ Never ☐

Other (please describe): ……………………………………………………………………………………

(b) How do you clean it (please tick all that apply)?

Hot water ☐ Cold water ☐ Soap ☐ Disinfectant ☐

Other (please describe): ……………………………………………………………………………………

10. **(a)** Do you provide water in your garden? Yes ☐ No ☐

(b) If yes, how is this water available?

Pond ☐ Bird bath ☐ In dish on ground ☐ In raised dish (e.g. on table) ☐

Other (please describe): ……………………………………………………………………………………

Thank you for your participation in this study! Please return this questionnaire to Kat Fingland at Nottingham Trent University.

APPENDIX VI: Mead D, Fingland K, Cripps R, Migeuz RP, Smith M, Corton C, Oliver K, Skelton J, Betteridge E, Dolucan J, *et al.* (2020) The genome sequence of the Eurasian red squirrel, *Sciurus vulgaris* Linnaeus 1758. *Wellcome Open Research* 5: 18.

Check for updates

DATA NOTE

The genome sequence of the Eurasian red squirrel, *Sciurus vulgaris* Linnaeus 1758 [version 1; peer review: 2 approved]

Daniel Mead[1], Kathryn Fingland[2], Rachel Cripps[3], Roberto Portela Miguez[4], Michelle Smith[1], Craig Corton[1], Karen Oliver[1], Jason Skelton[1], Emma Betteridge[1], Jale Dolucan[5], Olga Dudchenko[5], Arina D. Omer[5], David Weisz[5], Erez Lieberman Aiden[5], Olivier Fedrigo[6], Jacquelyn Mountcastle[6], Erich Jarvis[6,7], Shane A. McCarthy[1,8], Ying Sims[1], James Torrance[1], Alan Tracey[1], Kerstin Howe[1], Richard Challis[1], Richard Durbin[1,8], Mark Blaxter[1]

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Abstract
We present a genome assembly from an individual male *Sciurus vulgaris* (the Eurasian red squirrel; Vertebrata; Mammalia; Eutheria; Rodentia; Sciuridae). The genome sequence is 2.88 gigabases in span. The majority of the assembly is scaffolded into 21 chromosomal-level scaffolds, with both X and Y sex chromosomes assembled.

Keywords
Sciurus vulgaris, red squirrel, genome sequence, chromosomal

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✔ ✔

	1	2
version 1 03 Feb 2020	✔ view	✔ view

1. **Peter H. Sudmant**, University of California, Berkeley, Berkeley, USA
2. **Rob Ogden**, University of Edinburgh, Edinburgh, UK

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Mark Blaxter (mark.blaxter@sanger.ac.uk)

Author roles: Mead D: Conceptualization, Investigation, Writing – Review & Editing; **Fingland K**: Investigation, Resources, Writing – Review & Editing; **Cripps R**: Investigation, Resources, Writing – Review & Editing; **Portela Miguez R**: Investigation, Resources, Writing – Review & Editing; **Smith M**: Investigation, Methodology, Writing – Review & Editing; **Corton C**: Investigation, Methodology, Writing – Review & Editing; **Oliver K**: Methodology, Supervision, Writing – Review & Editing; **Skelton J**: Methodology, Writing – Review & Editing; **Betteridge E**: Methodology, Writing – Review & Editing; **Dolucan J**: Methodology, Software, Writing – Review & Editing; **Dudchenko O**: Investigation, Methodology, Software, Visualization, Writing – Review & Editing; **Omer AD**: Investigation, Methodology, Software, Visualization, Writing – Review & Editing; **Weisz D**: Methodology, Software, Visualization, Writing – Review & Editing; **Lieberman Aiden E**: Funding Acquisition, Supervision, Writing – Review & Editing; **Fedrigo O**: Investigation, Methodology, Writing – Review & Editing; **Mountcastle J**: Investigation, Methodology, Writing – Review & Editing; **Jarvis E**: Funding Acquisition, Supervision, Writing – Review & Editing; **McCarthy SA**: Investigation, Methodology, Software, Supervision, Writing – Review & Editing; **Sims Y**: Investigation, Software, Visualization, Writing – Review & Editing; **Torrance J**: Data Curation, Writing – Review & Editing; **Tracey A**: Data Curation, Writing – Review & Editing; **Howe K**: Data Curation, Validation, Writing – Review & Editing; **Challis R**: Investigation, Visualization, Writing – Review & Editing; **Durbin R**: Conceptualization, Funding Acquisition, Supervision, Writing – Review & Editing; **Blaxter M**: Conceptualization, Data Curation, Funding Acquisition, Project Administration, Resources, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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https://doi.org/10.12688/wellcomeopenres.15679.1

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Species taxonomy
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Sciuromorpha; Sciuridae; Sciurinae; Sciurini; Sciurus; *Sciurus vulgaris* Linnaeus 1758 (NCBI txid 55149).

Background
The Eurasian red squirrel, *Sciurus vulgaris*, is native to northern Eurasia. In the Atlantic Archipelago of Britain and Ireland, *S. vulgaris* is under threat from anthropogenic pressure on its native woodland habitats[1], and from competition from the introduced American grey squirrel, *Sciurus carolinensis*, particularly mediated by squirrelpox virus (Chantrey *et al*., 2014). The current population of *S. vulgaris* in the Atlantic Archipelago is estimated to be 150,000, and there are extensive efforts to conserve this species and expand its range (Hardouin *et al*., 2019). Here we present a chromosomally assembled genome sequence for *S. vulgaris*, based on a male specimen from Britain. This genome sequence will be of utility in population genomic analysis of fragmented *S. vulgaris* populations (Barratt *et al*., 1999), in managing reintroductions and in investigating the biology of susceptibility to squirrelpox virus (Darby *et al*., 2014).

[1] https://www.forestresearch.gov.uk/documents/667/fcpn5.pdf;

Genome sequence report
The genome was sequenced from DNA extracted from a from a naturally deceased male *S. vulgaris* collected as part of a squirrel monitoring project run by the Wildlife Trust for Lancashire, Manchester and North Merseyside. A total of 51-fold coverage in Pacific Biosciences single-molecule long reads (N50 19 kb) and 44-fold coverage in 10X Genomics read clouds (from molecules with an estimated N50 of 69 kb) were generated. Primary assembly contigs were scaffolded with 10X read clouds, chromosome conformation HiC data, and 111-fold coverage of Bionano optical maps. The final assembly has a total length of 2.88 Gb in 638 sequence scaffolds with a scaffold N50 of 153.9 Mb (Table 1). The majority, 92.7%, of the

Table 1. Genome data for *Sciurus vulgaris* mSciVul1.

Project accession data	
Assembly identifier	mSciVul1
Species	*Sciurus vulgaris*
Specimen	NHMUK ZD.2019.213
NCBI taxonomy ID	55149
BioProject	PRJEB35381
Biosample ID	SAMEA994733
Isolate information	Wild isolate; male
Raw data accessions	
PacificBiosciences SEQUEL I	ERR3147845-ERR3147850, ERR3151029, ERR3151031-ERR3151033, ERR3151038-ERR3151041, ERR3151043-ERR3151044, ERR3168377-ERR3168381, ERR3197128, ERR3197129, ERR3218392, ERR3284521, ERR3291651-ERR3291656, ERR3291658, ERR3291671-ERR3291674
10X Genomics Illumina	ERR3316125-ERR3316132
Hi-C Illumina	SRR10119465
BioNano data and assembly	ERZ1283748
Genome assembly	
Assembly accession	GCA_902686455
Accession of alternate haplotype	GCA_902685485
Span (Mb)	2,879
Number of contigs	1,799
Contig N50 length (Mb)	16.3
Number of scaffolds	638
Scaffold N50 length (Mb)	153.9
Longest scaffold (Mb)	213.2
BUSCO* genome score	C:93.8%[S:90.7%,D:3.1%],F:3.0%,M:3.2%,n:4104

* BUSCO scores based on the mammalia_odb9 BUSCO set using v3.0.2. C= complete [S= single copy, D=duplicated], F=fragmented, M=missing, n=number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/mSciVul1_1/dataset/mSciVul1_1/busco.

assembly sequence was assigned to 21 chromosomal pseudo-molecules representing 19 autosomes (numbered by sequence length), and the X and Y sex chromosomes (Figure 1–Figure 4; Table 2). The assembly has a BUSCO (Simão *et al.*, 2015) completeness of 93.8% using the mammalia_odb9 reference set. The primary assembly is a large-scale mosaic of both haplotypes (i.e. is not fully phased) and we have therefore also deposited the contigs corresponding to the alternate haplotype. The genome can be compared to that of the grey squirrel, *Sciurus carolinensis*, which we have also assembled.

Methods

The red squirrel specimen was collected from a garden in Beechwood Drive, Formby, Merseyside, L37 2DQ. Grid ref: SD2829706400 (Lat Long: 53.549316, -3.0836773) by the Wildlife Trust for Lancashire, Manchester and North Merseyside as part of an ongoing programme of recovery of dead squirrels. The spleen was dissected out during autopsy. A full tissue dissection and preservation in 80% ethanol was undertaken and the specimen accessioned by the Natural History Museum, London.

DNA was extracted using an agarose plug extraction from spleen tissue following the Bionano Prep Animal Tissue DNA Isolation Soft Tissue Protocol[2]. Pacific Biosciences CLR long read and 10X Genomics read cloud sequencing libraries were constructed according to the manufacturers' instructions. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL I and Illumina HiSeq X instruments. Hi-C data were generated by the Aiden

[2] https://bionanogenomics.com/wp-content/uploads/2018/02/30077-Bionano-Prep-Animal-Tissue-DNA-Isolation-Soft-Tissue-Protocol.pdf

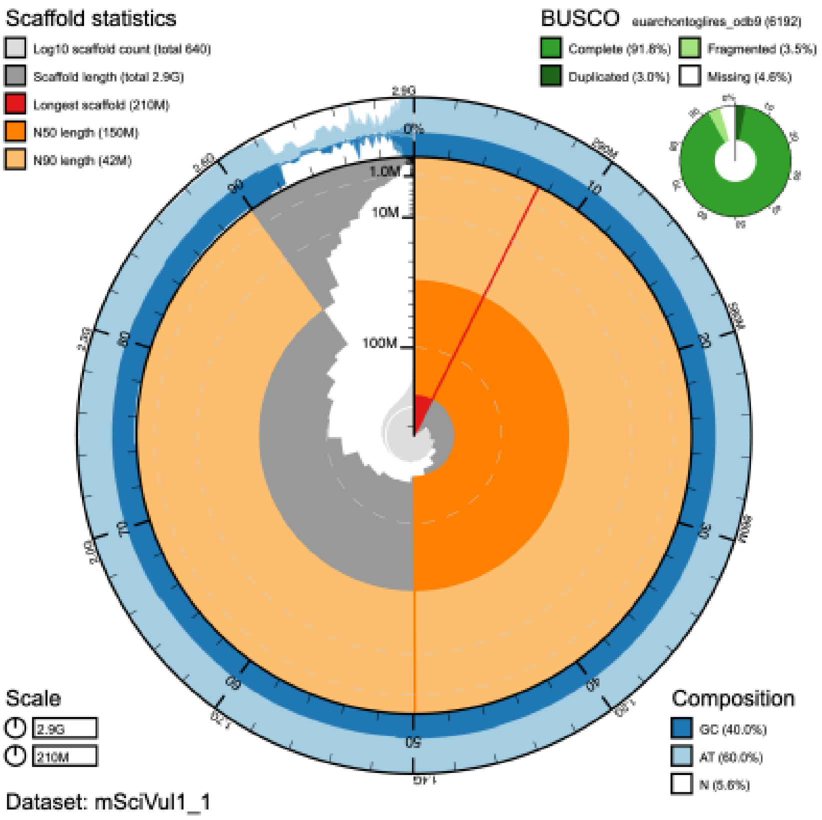

Figure 1. Genome assembly of *Sciurus vulgaris* mSciVul1: Metrics. BlobToolKit Snailplot showing N50 metrics for *S. vulgaris* assembly mSciVul1 and BUSCO scores for the Euarchontoglires set of orthologues. The interactive version of this figure is available here.

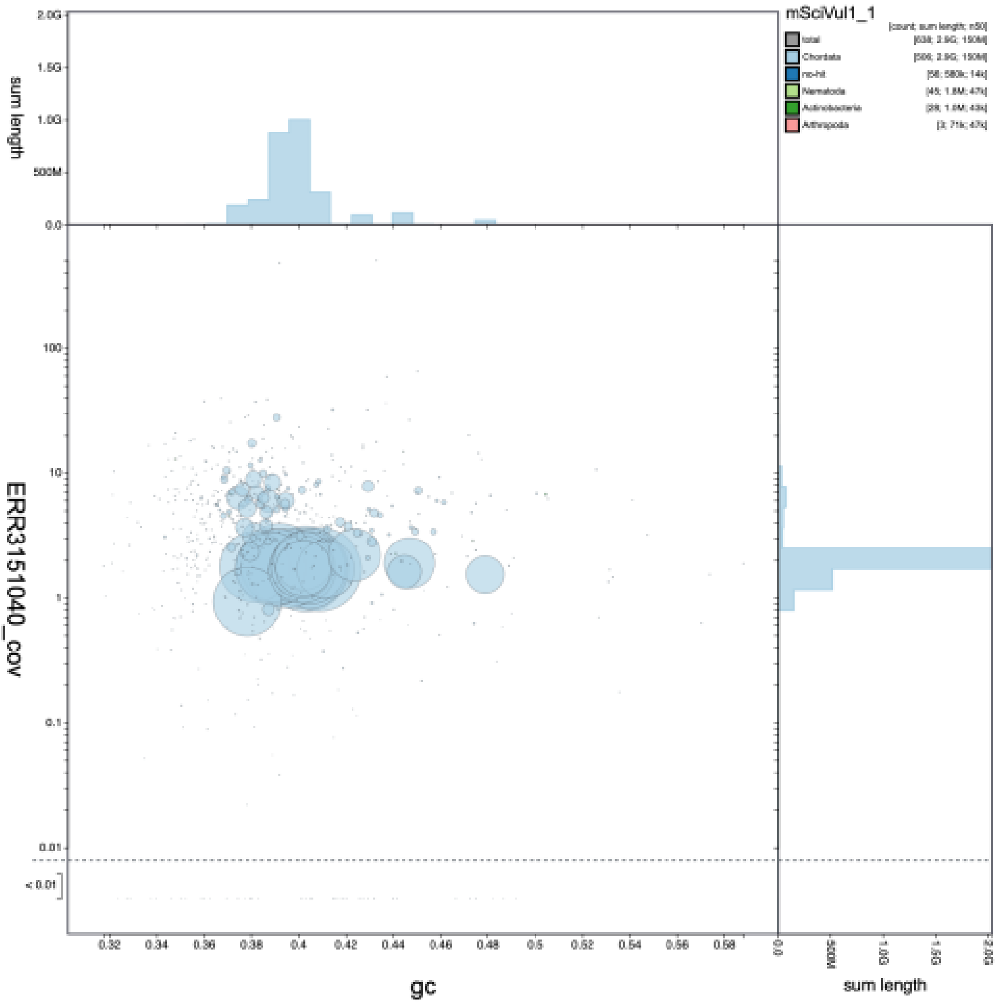

Figure 2. Genome assembly of Sciurus *vulgaris mSciVul1:* GC-coverage plot. BlobToolKit GC-coverage plot of *S. vulgaris* mSciVul1. The interactive version of this figure is available here.

lab using an optimised version of their protocols (Dudchenko *et al.*, 2017). BioNano data were generated in the Rockefeller University Vertebrate Genome laboratory using the Saphyr instrument. Ultra-high molecular weight DNA was extracted using the Bionano Prep Animal Tissue DNA Isolation Soft Tissue Protocol and assessed by pulsed field gel and Qubit 2 fluorimetry. DNA was labeled for Bionano Genomics optical mapping following the Bionano Prep Direct Label and Stain (DLS) Protocol and run on one Saphyr instrument chip flowcell. The total yield of tagged molecules ≥ 150 kb with at least 9

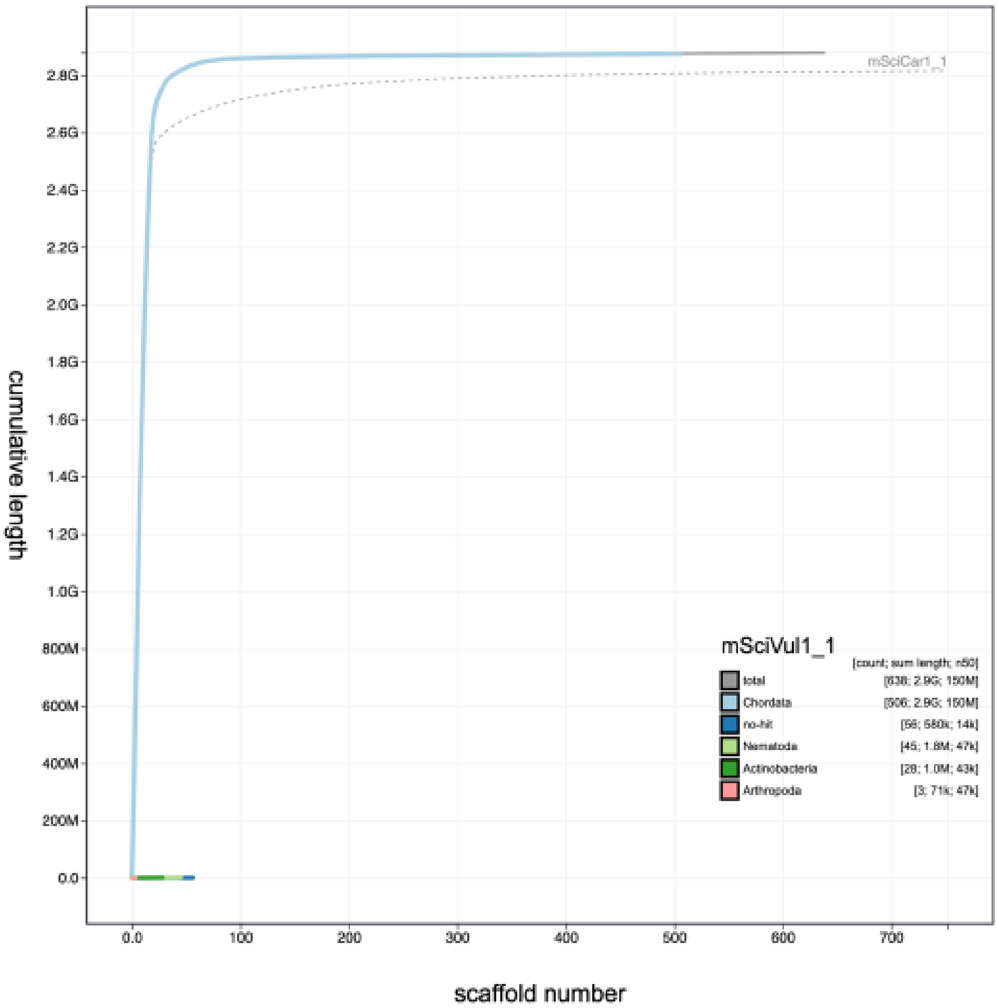

Figure 3. Genome assembly of *Sciurus vulgaris* mSciVul1: Cumulative sequence plot. Dashed line shows the cumulative sequence plot of *S. carolinensis* mSciCar1 for comparison. The interactive version of this figure is available here.

sites was 320.6 Gb (N50 0.25 Mb). A CMAP (Bionano assembly consensus genome map) was *de-novo* assembled using Bionano Solve (see Table 3 for software versions and sources) yielding 574 maps with a total map length of 3.28 Gb and a map N50 of 86.34 Mb.

Assembly followed a modified version of the Vertebrate Genomes Project assembly protocols[3]. In brief, assembly was carried

[3] https://github.com/VGP/vgp-tools

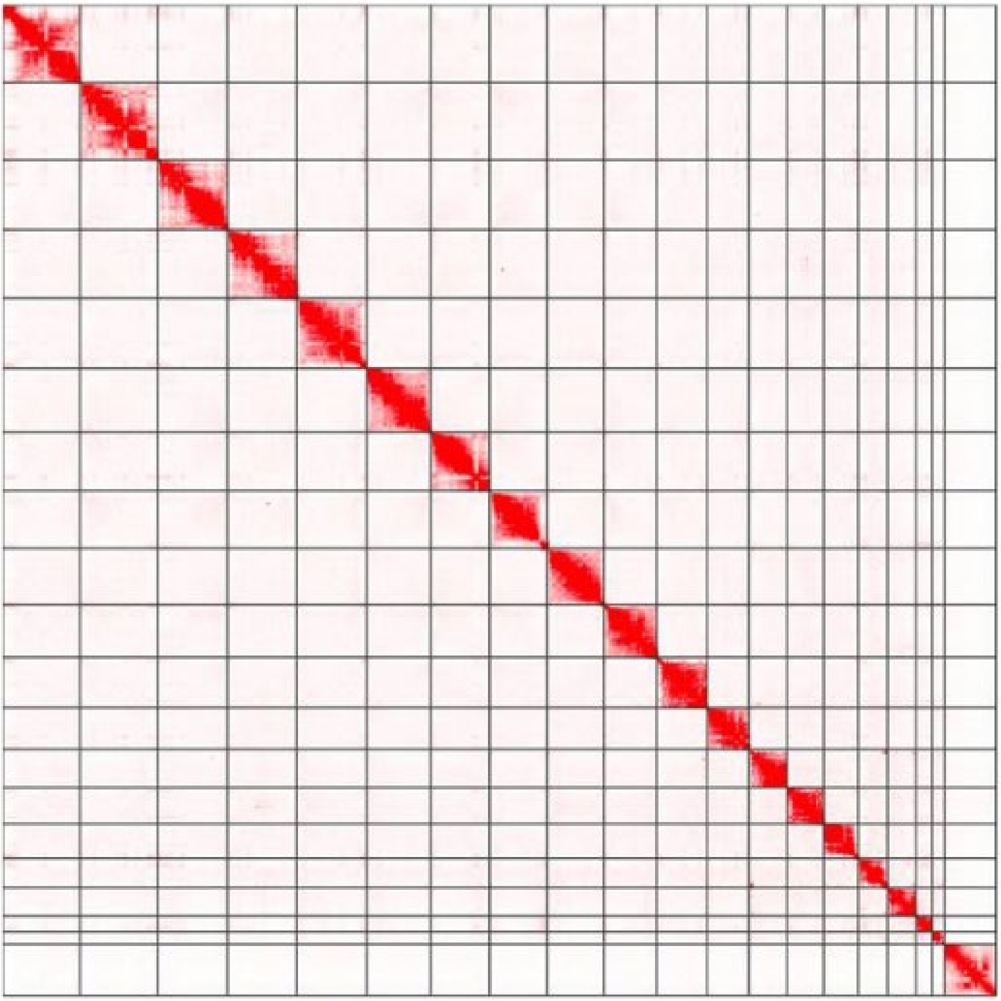

Figure 4. Genome assembly of *Sciurus vulgaris* mSciVul1: Hi-C contact map. Hi-C contact map of the *S. vulgaris* mSciVul1 assembly, visualized in Juicebox. The interactive version of this figure is available here, powered by Juicebox.js (Robinson *et al.*, 2018).

out using Falcon-unzip (Chin *et al.*, 2016), haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2019) and a first round of scaffolding carried out with 10X Genomics read clouds using scaff10x. Hybrid scaffolding was performed using the BioNano DLE-1 data and BioNano Solve.

Scaffolding with Hi-C data (Rao *et al.*, 2014) was carried out with 3D-DNA (Dudchenko *et al.*, 2017), followed by manual curation with Juicebox Assembly Tools (Dudchenko *et al.*, 2018; Durand *et al.*, 2016; Robinson *et al.*, 2018). The Hi-C scaffolded assembly was polished using arrow with the PacBio data, then polished with the 10X Genomics Illumina data by aligning to the assembly with longranger align, calling variants with freebayes (Garrison & Marth, 2012) and applying homozygous non-reference edits using bcftools consensus. Two rounds of the Illumina polishing were applied. The assembly was

Table 2. **Chromosomal pseudomolecules in the genome assembly of *Sciurus vulgaris* mSciVul1.**

ENA accession	Chromosome	Size (Mb)	GC%
LR738611.1	1	213.19	40.3
LR738612.1	2	204.37	40.9
LR738613.1	3	189.66	40.4
LR738614.1	4	187.75	39.4
LR738615.1	5	181.67	39.6
LR738616.1	6	173.03	39
LR738617.1	7	162.82	39.4
LR738618.1	8	153.87	40.6
LR738619.1	9	146.52	38.2
LR738620.1	10	145.29	38.9
LR738622.1	11	132.26	40.2
LR738623.1	12	115.30	40.2
LR738624.1	13	100.60	40.8
LR738625.1	14	99.24	41.3
LR738626.1	15	91.00	40.2
LR738627.1	16	79.70	42.9
LR738628.1	17	75.03	45.1
LR738629.1	18	41.58	47.9
LR738630.1	19	33.12	45.1
LR738621.1	X	138.34	37.9
LR738631.1	Y	4.04	38.9
-	unplaced	210.22	15.6

Table 3. **Software tools used.**

Software tool	Version	Source
BioNano Solve	3.3	http://www.bnxinstall.com/solve/BionanoSolveInstall.html
Falcon-unzip	falcon-kit 1.1.1	Chin *et al.*, 2016
purge_dups	1.0.0	*Guan et al., 2019*
scaff10x	4.2	https://github.com/wtsi-hpag/Scaff10X
3D-DNA	180419	Dudchenko *et al.*, 2017
Juicebox Assembly Tools	1.9.8	Dudchenko *et al.*, 2018
arrow	GenomicConsensus 2.3.3	https://github.com/PacificBiosciences/GenomicConsensus
longranger align	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
freebayes	v1.1.0-3-g961e5f3	Garrison & Marth, 2012
bcftools consensus	1.9	http://samtools.github.io/bcftools/bcftools.html
gEVAL	2016	Chow *et al.*, 2016
BlobToolKit	1	Challis *et al.*, 2019

checked for contamination and further manually assessed and corrected using the gEVAL system (Chow *et al.*, 2016). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2019).

Data availability

Underlying data

European Nucleotide Archive: Sciurus vulgaris (red squirrel) genome assembly, mSciVul1. BioProject accession number PRJEB35381; https://identifiers.org/ena.embl:PRJEB35381.

The genome sequence is released openly for reuse. The *S. vulgaris* genome sequencing initiative is part of the Wellcome Sanger Institute's "25 genomes for 25 years" project[4]. It is also part of the Vertebrate Genomes Project (VGP)[5] ordinal references programme, the DNA Zoo Project[6] and the Darwin Tree of Life (DToL) project[7]. The specimen has been preserved in ethanol and deposited with the Natural History Museum, London under registration number NHMUK ZD 2019.213, where it will remain accessible to the research community for posterity. All raw sequence data and the assembly have been deposited in the ENA. The genome will be annotated and presented through the Ensembl pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in Table 1.

[4] https://www.sanger.ac.uk/science/collaboration/25-genomes-25-years

[5] https://vertebrategenomesproject.org/

[6] https://www.dnazoo.org/

[7] https://www.darwintreeoflife.org/

Author contributions

Collection and identification: KF, RC

DNA extraction and sequencing: DM, MS, CC, KO, JS, EB, JD, ADO, OF, JM, EJ

Genome assembly and curation: SMcC, OD, DW, ELA, KH, RC, YS, JT, AT

Project management: DM, RD, MB

Manuscript: MB, assisted by all authors

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We thank Mike Stratton and Julia Wilson for their continuing support for the 25 genomes for 25 years project. The Wildlife Trust for Lancashire, Manchester and North Merseyside thank many members of the public for support.

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Chow W, Brugger K, Caccamo M, *et al.*: **gEVAL -a web-based browser for evaluating genome assemblies.** *Bioinformatics.* 2016; **32**(16): 2508–2510.
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Dudchenko O, Batra SS, Omer AD, *et al.*: ***De novo* assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds.** *Science.* 2017; **356**(6333): 92–95.
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PubMed Abstract | Publisher Full Text

APPENDIX VII: Mead D, Fingland K, Cripps R, Migeuz RP, Smith M, Corton C, Oliver K, Skelton J, Betteridge E, Dolucan J, *et al.* (2020) The genome sequence of the eastern grey squirrel, *Sciurus carolinensis* Gmelin, 1788. *Wellcome Open Research* 5: 27.

DATA NOTE

The genome sequence of the eastern grey squirrel, *Sciurus carolinensis* Gmelin, 1788 [version 1; peer review: 2 approved]

Dan Mead[1], Kathryn Fingland[2], Rachel Cripps[3], Roberto Portela Miguez[4], Michelle Smith[1], Craig Corton[1], Karen Oliver[1], Jason Skelton[1], Emma Betteridge[1], Jale Doulcan[1], Michael A. Quail[1], Shane A. McCarthy[1], Kerstin Howe[1], Ying Sims[1], James Torrance[1], Alan Tracey[1], Richard Challis[1], Richard Durbin[1], Mark Blaxter[1]

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Latest published: 13 Feb 2020, **5**:27
https://doi.org/10.12688/wellcomeopenres.15721.1

Abstract

We present a genome assembly from an individual male *Sciurus carolinensis* (the eastern grey squirrel; Vertebrata; Mammalia; Eutheria; Rodentia; Sciuridae). The genome sequence is 2.82 gigabases in span. The majority of the assembly (92.3%) is scaffolded into 21 chromosomal-level scaffolds, with both X and Y sex chromosomes assembled.

Keywords

Sciurus carolinensis, grey squirrel, genome sequence, chromosomal

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✔ ✔

	1	2
version 1 13 Feb 2020	✔ view	✔ view

1. **Erik Garrison**, University of California, Santa Cruz, Santa Cruz, USA
2. **Takafumi Katsumura**, Kitasato University School of Medicine, Sagamihara, Japan

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Mead D: Conceptualization, Investigation, Writing – Review & Editing; **Fingland K**: Formal Analysis, Investigation, Resources, Writing – Review & Editing; **Cripps R**: Formal Analysis, Investigation, Resources, Writing – Review & Editing; **Portela Miguez R** : Data Curation, Formal Analysis, Investigation, Resources, Writing – Review & Editing; **Smith M**: Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Corton C**: Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Oliver K**: Formal Analysis, Investigation, Supervision, Writing – Review & Editing; **Skelton J**: Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Betteridge E**: Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Doulcan J**: Writing – Review & Editing; **Quail MA**: Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **McCarthy SA**: Data Curation, Formal Analysis, Investigation, Software, Writing – Review & Editing; **Howe K**: Data Curation, Formal Analysis, Investigation, Resources, Software, Supervision, Writing – Review & Editing; **Sims Y**: Data Curation, Formal Analysis, Investigation, Software, Validation, Writing – Review & Editing; **Torrance J**: Data Curation, Formal Analysis, Investigation, Validation, Writing – Review & Editing; **Tracey A**: Data Curation, Formal Analysis, Investigation, Software, Writing – Review & Editing; **Challis R**: Data Curation, Formal Analysis, Investigation, Software, Validation, Visualization, Writing – Review & Editing; **Durbin R**: Conceptualization, Investigation, Resources, Supervision, Writing – Review & Editing; **Blaxter M**: Conceptualization, Funding Acquisition, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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https://doi.org/10.12688/wellcomeopenres.15721.1

First published: 13 Feb 2020, **5**:27 https://doi.org/10.12688/wellcomeopenres.15721.1

Species taxonomy

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Sciuromorpha; Sciuridae; Sciurinae; Sciurini; Sciurus; *Sciurus carolinensis* Gmelin, 1788 (NCBI txid 30640).

Background

The eastern grey squirrel, *Sciurus carolinensis*, is native to eastern North America, where it plays important roles in forest regeneration through its habit of caching food nuts and seeds (Corbet & Hill, 1991)[1]. In North America, *S. carolinensis* has been introduced outside its native range such that it is now found from the Canadian Pacific northwest to Florida. *S. carolinensis* was introduced to Britain (in 1876), Ireland (in 1911), Italy (in 1948), South Africa (before 1900), Australia (in 1880s, extirpated in 1973) and Pitcairn island (in 1987) (see https://www.cabi.org/isc/datasheet/49075). *S. carolinensis*, which thrives in urban parklands and gardens, is classed as invasive in Europe and on Pitcairn island. In Britain and Ireland the expansion of *S. carolinensis* populations has driven decline in populations of the native red squirrel, *Sciurus vulgaris*, which we have also assembled (Mead *et al.*, 2020). The negative impact of *S. carolinensis* is through interspecific competition, leading to competitive exclusion of *S. vulgaris*, and by their carriage of squirrelpox virus, to which they are resistant but *S. vulgaris* are not (Chantrey *et al.*, 2014) (Darby *et al.*, 2014). The *S. carolinensis* genome will aid analyses of resistance and susceptibility to squirrelpox, as well as to the genomics of invasiveness.

Genome sequence report

The genome was sequenced from DNA extracted from a naturally deceased male *S. carolinensis* collected as part of a squirrel monitoring project run by the Wildlife Trust for Lancashire, Manchester and North Merseyside. A total of 74-fold coverage in Pacific Biosciences single-molecule long reads (N50 28 kb) and 40-fold coverage in 10X Genomics read clouds (from molecules with an estimated N50 of 19 kb) were generated. Primary assembly contigs were scaffolded with chromosome conformation HiC data (42-fold coverage). A contamination check identified a small number of low-coverage contigs that were likely to have derived from an apicomplexan parasite infecting the squirrel (Léveillé *et al.*, 2020); these were removed. Subsequent manual assembly curation corrected 272 missing/misjoins and removed three haplotypic duplications, reducing the scaffold number by 19% and increasing the scaffold N50 by 242% The final assembly has a total length of 2.82 Gb in 752 sequence scaffolds with a scaffold N50 of 148.2 Mb (Table 1). The majority, 92.3%, of the assembly sequence was assigned to 21 chromosomal-level scaffolds representing 19 autosomes (numbered by sequence length), and the X and Y sex chromosomes (Figure 1–Figure 5; Table 2) plus 13 unlocalised scaffolds (assigned to chromosomes but with ambiguous placement). The assembly has a BUSCO (Simão *et al.*, 2015) completeness of 93.7% using the mammalia_odb9 reference set. The primary assembly is a large-scale mosaic of both haplotypes (i.e. is not fully phased) and we have therefore also deposited the contigs corresponding to the alternate haplotype. The *S. carolinensis* mSciCar1 genome sequence is largely collinear with that of *S. vulgaris* mSciVul1 (Figure 4).

[1] See https://animaldiversity.org/accounts/Sciurus_carolinensis/

Methods

The eastern grey squirrel specimen was collected by the Wildlife Trust for Lancashire, Manchester and North Merseyside as part of an ongoing programme of recovery of dead squirrels. A full tissue dissection and preservation in 80% ethanol was undertaken and the specimen accessioned by the Natural History Museum, London.

DNA was extracted using an agarose plug extraction from spleen tissue following the Bionano Prep Animal Tissue

Table 1. Genome data for Sciurus carolinensis mSciCar1.

Project accession data	
Assembly identifier	mSciCar1
Species	*Sciurus carolinensis*
Specimen	NHMUK ZD 2019.214
NCBI taxonomy ID	30640
BioProject	PRJEB35386
Biosample ID	SAMEA994726
Isolate information	Wild isolate; male
Raw data accessions	
PacificBiosciences SEQUEL I	ERR3313242-ERR3313245, ERR3313247-ERR3313255, ERR3313329, ERR3313331, ERR3313332, ERR3313342-ERR3313348
10X Genomics Illumina	ERR3316153-ERR3316156, ERR3316173-ERR3316176
Hi-C Illumina	ERR3312499-ERR3312500, ERR3850937
Genome assembly	
Assembly accession	GCA_902686445.1
Accession of alternate haplotype	GCA_902685475.1
Span (Mb)	2,815,397,268
Number of contigs	2576
Contig N50 length (Mb)	13.98
Number of scaffolds	752
Scaffold N50 length (Mb)	148.23
Longest scaffold (Mb)	208.99
BUSCO* genome score	C:93.7%[S:92.3%,D:1.4%],F:2.8%, M:3.5%,n:4104

* BUSCO scores based on the mammalia_odb9 BUSCO set using v3.0.2. C= complete [S= single copy, D=duplicated], F=fragmented, M=missing, n=number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/mSciCar1_1/dataset/mSciCar1_1/busco

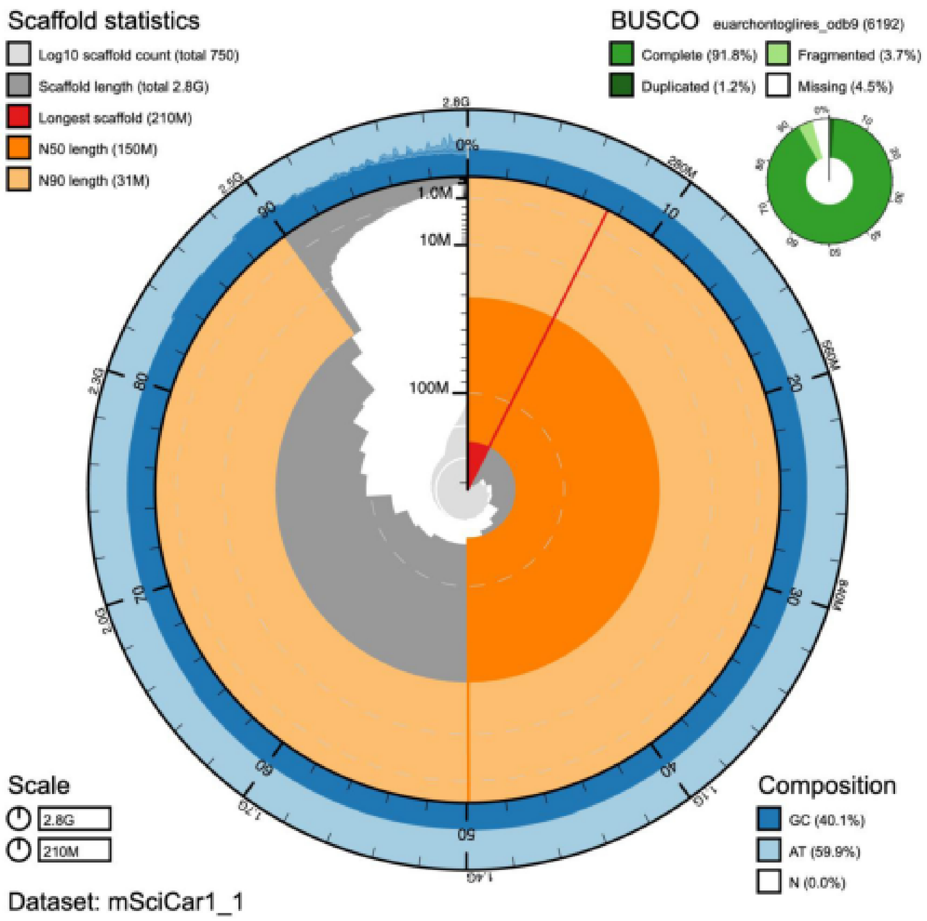

Figure 1. Genome assembly of *Sciurus carolinensis* mSciCar1: Metrics. BlobToolKit Snailplot showing N50 metrics for *S. carolinensis* assembly mSciCar1 and BUSCO scores for the Euarchontoglires set of orthologues. The interactive version is available here.

DNA Isolation Soft Tissue Protocol[2]. Pacific Biosciences CLR long read and 10X Genomics read cloud sequencing libraries were constructed according to the manufacturers' instructions. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL I (single molecule long read) and Illumina HiSeq X (10X Genomics Chromium). HiC data were generated using the Dovetail v1.0 kit and sequenced on HiSeq X.

See Table 3 for software versions and sources. Assembly was carried out using Falcon-unzip (Chin *et al.*, 2016), haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020) and a first round of scaffolding carried out with 10X Genomics read clouds using scaff10x. Scaffolding with Hi-C data was carried out using SALSA2. The Hi-C scaffolded assembly was polished with arrow using the PacBio data, then polished with the 10X Genomics Illumina data by aligning to the assembly with longranger align, calling variants with

[2] https://bionanogenomics.com/wp-content/uploads/2018/02/30077-Bionano-Prep-Animal-Tissue-DNA-Isolation-Soft-Tissue-Protocol.pdf

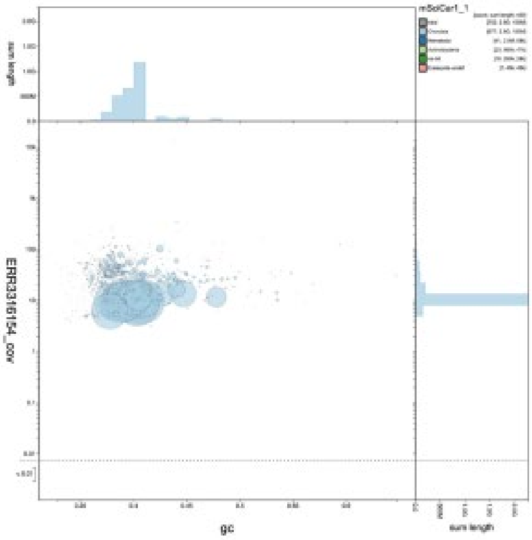

Figure 2. Genome assembly of *Sciurus carolinensis* mSciCar1: GC-coverage plot. BlobToolKit GC-coverage plot of *S. carolinensis* mSciCar1 from long read data submission ERR3316154. The interactive version is available here.

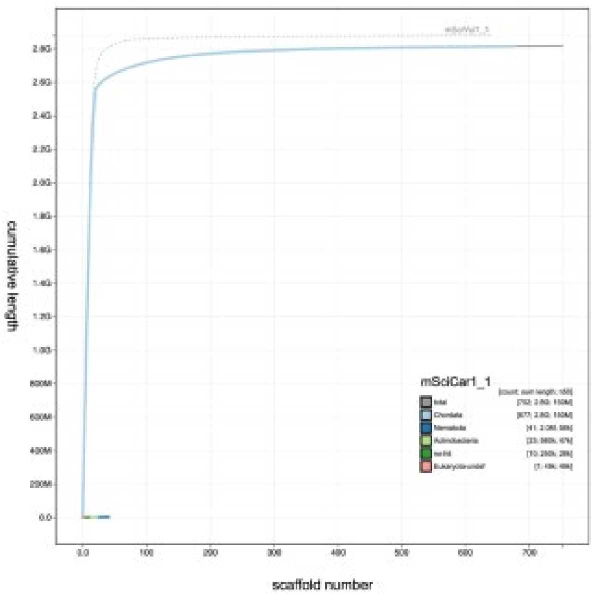

Figure 3. Genome assembly of *Sciurus carolinensis* mSciCar1: Cumulative sequence plot. The blue line in the main plot shows the cumulative sequence plot for mSciCar. The sashed line shows the cumulative sequence plot of *S. vulgaris* mSciVul1 for comparison. The interactive version is available here.

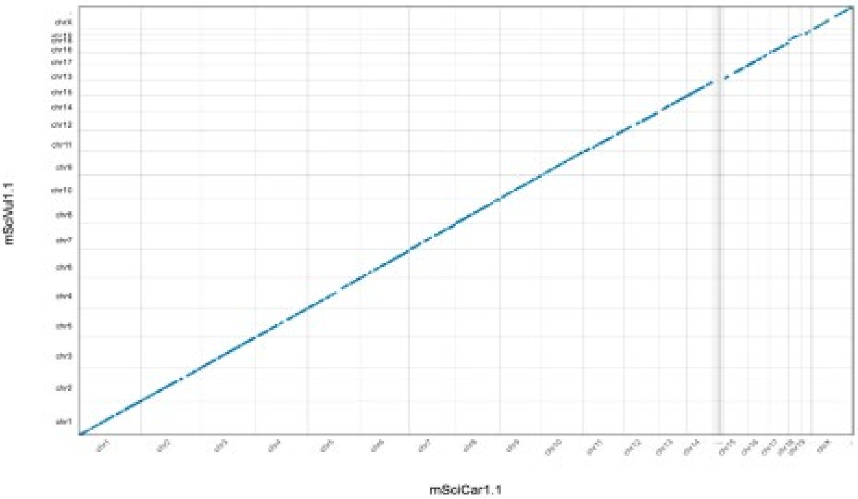

Figure 4. Genome assembly of *Sciurus carolinensis* mSciCar1: Whole genome alignment with *Sciurus vulgaris* mSciVul1. A nucmer (Kurtz *et al.*, 2004) pairwise alignment of mSciCar1 (x-axis) with mSciVul1 (Y axis).

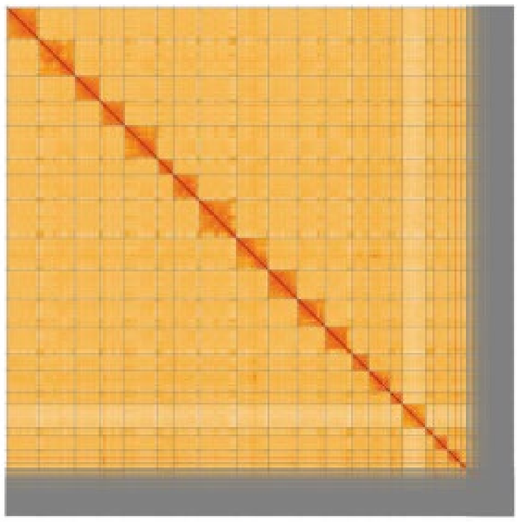

Figure 5. Genome assembly of *Sciurus carolinensis* mSciCar1: Hi-C contact map. Hi-C scaffolding of the *S. carolinensis* mSciCar1 assembly visualised in HiGlass (Kerpedjiev *et al.*, 2018).

Table 2. **Chromosomal pseudomolecules in the genome assembly of *Sciurus carolinensis* mSciCar1.**

ENA accession	Chromosome	Size (Mb)	GC%
LR738590.1	1	208.99	40.3
LR738591.1	2	199.83	40.8
LR738592.1	3	183.55	40.3
LR738593.1	4	177.11	39.5
LR738594.1	5	175.91	39.1
LR738595.1	6	162.27	38.7
LR738596.1	7	154.99	39.1
LR738597.1	8	148.23	40.5
LR738598.1	9	141.42	38.8
LR738599.1	10	140.98	38.1
LR738600.1	11	135.23	40.1
LR738602.1	12	118.65	40.1
LR738603.1	13	94.68	41.1
LR738604.1	14	88.65	40.2
LR738605.1	15	83.14	40.5
LR738606.1	16	68.57	44.7
LR738607.1	17	66.05	42.7
LR738608.1	18	41.56	47.8
LR738609.1	19	30.99	44
LR738601.1	X	131.72	37.8
LR738610.1	Y	4.81	38.3
-	Unplaced	258.08	40

freebayes (Garrison & Marth, 2012) and applying homozygous non-reference edits using bcftools consensus. Two rounds of the Illumina polishing were applied. The assembly was checked for contamination and corrected using the gEVAL system (Chow *et al.*, 2016). Since Hi-C data were sparse, curation was aided by synteny with the assembly for *Sciurus vulgaris* simultaneously being curated by the Wellcome Sanger Institute. The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2019).

Table 3. **Software tools used.**

Software tool	Version	Source
Falcon-unzip	falcon-kit 1.2.2	(Chin *et al.*, 2016)
purge_dups	1.0.0	(Guan *et al.*, 2020)
SALSA2	2.2	(Ghurye *et al.*, 2018)
scaff10x	4.2	https://github.com/wtsi-hpag/Scaff10X
arrow	GenomicConsensus 2.3.3	https://github.com/PacificBiosciences/GenomicConsensus
longranger align	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
freebayes	v1.1.0-3-g961e5f3	(Garrison & Marth, 2012)
bcftools consensus	1.9	http://samtools.github.io/bcftools/bcftools.html
gEVAL	2016	(Chow *et al.*, 2016)
BlobToolKit	1	(Challis *et al.*, 2019)
nucmer from MUMmer 3	3.0	(Kurtz *et al.*, 2004)

Data availability

Underlying data

European Nucleotide Archive: *Sciurus carolinensis* (grey squirrel) genome assembly, mSciCar1. BioProject accession number PRJEB35386; https://identifiers.org/ena.embl:PRJEB35386.

The genome sequence is released openly for reuse. The *S. carolinensis* genome sequencing initiative is part of the Wellcome Sanger Institute's "25 genomes for 25 years" project[3]. It is also part of the Vertebrate Genome Project (VGP)[4] and the Darwin Tree of Life (DToL) project[5]. The specimen has been preserved in ethanol and deposited with the Natural History Museum, London under registration number NHMUK ZD 2019.214, where it will remain accessible to the research community for posterity. All raw data and the assembly have been deposited in the ENA. The genome will be annotated and presented through the Ensembl pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in Table 1.

Acknowledgements

We thank Mike Stratton and Julia Wilson for their continuing support for the 25 genomes for 25 years project. The Wildlife Trust for Lancashire, Manchester and North Merseyside thank many members of the public for support.

[3] https://www.sanger.ac.uk/science/collaboration/25-genomes-25-years

[4] https://vertebrategenomesproject.org/

[5] https://www.darwintreeoflife.org/

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Ghurye J, Rhie A, Walenz BP, *et al.*: **Integrating Hi-C links with assembly graphs for chromosome-scale assembly.** *PLoS Comput Biol.* 2019; **15**(8): e1007273.
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Guan D, McCarthy SA, Wood J, *et al.*: **Identifying and removing haplotypic duplication in primary genome assemblies.** *Bioinformatics.* 2020; pii: btaa025.
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Mead D, Fingland K, Cripps R, *et al.*: **The genome sequence of the Eurasian red squirrel, *Sciurus vulgaris* Linnaeus 1758 [version 1; peer review: awaiting peer review].** *Wellcome Open Res.* 2020; **5**: 18.
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APPENDIX VIII: Power Analysis for Home Range Analysis (Chapter Four)

A power analysis (Table A8.1), to determine an approximate sample size to radio collar each research season, was conducted in Minitab (Minitab 2023) using the 'sample size for estimation' tool. Sample sizes (n) and standard deviations (SD) were obtained from published research to calculate the standard error, a confidence interval of 95% was selected to obtain the Z value (Z = 1.96), and then the margin of error was calculated using the following equation:

$$\textit{Margin of error} = Z \times \left(\frac{SD}{\sqrt{n}}\right)$$

Table A8.1. Summary of the power analysis conducted using mean home range sizes (100% MCP) ± SD and sample sizes from published research and the margin of error (calculated using Z = 1.96) to estimate a sample size for this study.

Reference	Home Range Size (ha) ($\bar{x}$ ± SD)	Sample Size (n)	Margin of Error	Estimated Sample Size (n)
Lurz et al. 2000	M: 17.75 ± 6.20	6	4.961	9
	F: 6.29 ± 0.64	8	0.443	12
Wauters et al. 2001b	1997			
	M: 7.57 ± 3.42	7	2.534	10
	F: 2.70 ± 0.80	7	0.593	10
	1998			
	M: 7.26 ± 3.41	8	2.363	11
	F: 4.31 ± 2.13	8	1.476	11
Pierro et al. 2007	Site 1: 16.54 ± 2.73	23	1.116	26
	Site 2: 9.97 ± 1.65	20	0.723	23
	Site 3: 8.80 ± 1.51	23	0.617	26
Thomas et al. 2018	Site 1: 3.50 ± 2.70	4	2.646	7
	Site 2: 6.70 ± 1.70	5	1.490	8