Developing and implementing novel methods for managing grey squirrels

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Declaration

The material contained within this thesis has not previously been submitted for a degree at Durham University or any other university. The research reported within this thesis has been conducted by the author unless indicated otherwise.

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Abstract

Improving levels of biodiversity and preventing further decline is a global issue. Alien invasive species have been identified as one of the main causes of global biodiversity loss. In total 1 million species of plants and animals are assessed as at risk from extinction due to the impacts of invasive species and in 2019, global annual costs of biological invasions were estimated to exceed US $423 billion. Rodents are the most widely introduced invasive species around the world, mainly due to their colonisation of many islands and countries facilitated by human global expansion. Rodents cause a large number of negative economic and environmental impacts, including losses to the food industry, damage to property and the transmission of diseases. Conservative analyses estimate the cost of rodent invasions as US$ 3.6 billion between 1930 and 2022.

It is important to have practical and effective tools to manage wildlife populations and mitigate their impacts. Culling, traditionally used to reduce wildlife population numbers and human-wildlife conflicts, can be ineffective, inhumane, expensive or environmentally hazardous. Fertility control is increasingly used as a wildlife management tool for a range of mammal species, offering a number of potential benefits, particularly when compared with culling. Fertility control is more publicly acceptable than other methods, which is likely to increase landowner and practitioner support and facilitate deployment. Fertility control has been proposed as a method for controlling a range of overabundant rodent species, but most of the currently used fertility control agents are injectable single-dose immunocontraceptive vaccines and rodent populations are often too numerous for these agents to be delivered cost-effectively.

Oral contraceptives are currently being developed to manage the invasive non-native grey squirrel *Sciurus carolinensis* in the UK. Grey squirrels were introduced into England in the 19th century and it is estimated that their numbers now exceed 2.5 million. As a result, the red squirrel *Sciurus vulgaris*, the only squirrel native to the UK, has declined to less than 300,000 individuals and is now considered to be 'Endangered' in Great Britain. The grey squirrel is responsible for the decline of the native red squirrel *Sciurus vulgaris*, through the transmission of the squirrel pox virus and competition for resources. It is also estimated that the grey squirrel causes £37 million in tree damage per annum.

Oral contraceptives require a delivery system that is effective, practical, targeted and economically viable, so it is equally important that a suitable bait delivery system is developed to ensure that a bait is delivered to a sufficient proportion of the target population with minimal impact on nontarget animals. The development and testing of this bait system is the focus of this thesis.

In Chapter 2 I designed and tested a novel bait hopper, equipped with a PIT-tag reader and bait weighing device, that could record the frequency of feeding visits and amount of bait consumed per visit by free-living grey squirrels. In captive trials, the hopper proved highly effective at measuring patterns and quantities of bait uptake by individual grey squirrels. I also found that the bait marker rhodamine B (RB) was effective at marking grey squirrels when low amounts were consumed with no apparent adverse effects, making it suitable for measuring population level bait uptake. The adaptability of the hopper design means that it has wider applications for wildlife management; in particular, efficacy studies for bait-delivered drugs in the context of wildlife disease control and/or population reduction

Understanding grey squirrel feeding behaviour with regards to feeding devices is important so that delivery methods can be designed to ensure that a sufficient number of target individuals receive an effective contraceptive dose at a time of year that will guarantee their infertility throughout peak times of breeding. In Chapter 3, using the purpose-designed bait hoppers baited with RB bait for four days, I demonstrated that it was possible to deliver baits to the majority of grey squirrels in 10 woodland environments. Season, hopper density and squirrel density were all important factors determining bait uptake, with better bait uptake found in summer and with a higher density of hoppers.

In Chapter 4, I assessed individual level bait uptake for six woodland populations of squirrels in three seasons, with four days of rhodamine B bait deployment via purpose-designed squirrel-specific bait hoppers with integrated PIT-tag readers, developed and tested in Chapter 2. I demonstrated that it was possible to deliver multiple doses on most days to most male and female grey squirrels, with bait deployment more likely to be effective in spring, immediately before the second annual peak in squirrel breeding. Winter was also found to be a suitable month to deploy contraceptives.

Effective wildlife population management requires an understanding of the abundance of the target species. Camera traps are increasingly used to estimate animal abundance, and methods have been developed that do not require the identification of individual animals. In Chapter 5 of this study, I developed a practical and cost-effective method, based on a camera trap index, that could be used by practitioners to estimate densities of grey squirrels in woodlands, to provide guidance on the numbers of traps or contraceptive feeders required for grey squirrel control. Camera traps were deployed in ten independent woods of between 6 and 28 ha in size. An index, calculated from the number of grey squirrel photographs recorded per camera per day had a strong linear relationship ($R^2 = 0.90$) with the densities of squirrels removed in trap and dispatch operations. This method can easily be adapted to other rodent or small mammal species, making it widely applicable to other wildlife management interventions.

It is important that species-specific bait delivery systems are designed and tested to ensure that oral contraceptives can be delivered effectively and in a targeted way without impacting other wildlife. In Chapter 6 I discuss two studies that investigated two different species-specific bait systems, that could be used to ensure oral contraceptive are targeted towards grey squirrels and the potential impact on nontarget animals minimised. From the trials conducted in this study, I found that a feeder with a weighted bait door was successful in excluding most other species of UK wildlife, in areas where there were grey squirrels, but no red squirrels present. I also found that body weight could be used to develop a feeder that allows access by most grey squirrels whilst excluding red squirrels. This was confirmed from data collected from a prototype selective feeder where both red and grey squirrels were present.

The methods designed and tested in this study proved highly effective at measuring patterns and quantities of bait uptake by individual grey squirrels. Oral contraceptives could offer suitable control methods to manage invasive non-native rodents and small mammals in the future. In addition to the grey squirrel, key species on the International Union for Conservation of Nature 100 world's worst invasive alien species list include; the ship rat *Rattus rattus*, house mouse *Mus muscola*, small Indian mongoose *Herpestes javanicus* and stoat *Mustela ermina*. The methods and devices developed in this study could be adapted to optimise contraceptive delivery methods for the management of these species in the future.

Contents

Chapter 1. General Introduction

1.1 Biodiversity and the impact of invasive non-native species

Between 1970 and 2016, it is estimated that globally there has been a 68% decrease in the global population of mammals, birds, amphibians, reptiles and fish (Almond et al., 2020). Improving biodiversity and preventing further decline is a worldwide issue. In 2022, at the fifteenth meeting of the Conference of the Parties (COP 15), leaders from 196 countries adopted the Kunming-Montreal Global Biodiversity Framework; a UN agreement calling for the protection of 30% of the planet's land, ocean, and waters, with the aim of restoring and protecting ecosystems and endangered species (Convention on Biological Diversity, 2024). One of the main Framework targets set for 2030, was to 'Reduce the introduction of invasive alien species by 50% and minimise their impact'. This included the prevention of the introduction and establishment of new invasive species and the control of invasive species already established.

Alien invasive species have been identified as one of the main causes of global biodiversity loss. In total 1 million species of plants and animals are assessed as at risk from extinction due to the impacts of invasive species and in 2019, global annual costs of biological invasions were estimated to exceed US $423 billion, with over 66% related to reduced human food supply (IPBES, 2023). Rodents are the most widely introduced invasive species around the world, mainly due to their colonisation of many islands and countries facilitated by human global expansion (Drake & Hunt, 2009). Rodents have many characteristics that make them successful colonisers: high reproductive rates, adaptability to most environments, high mobility and the ability to cache food to overcome periods of low food availability (Blanco & Fernandes, 2012).

Rodents cause a large number of negative economic and environmental impacts worldwide, including losses to the food industry, damage to property and the transmission of diseases (Stenseth et al., 2003; Witmer & Raymond-Whish, 2021). Conservative analyses have found that the costs associated with rodent invasions have significantly increased over time, and between 1930 and 2022 totalled US$ 3.6 billion, with the highest costs attributed to muskrat *Ondatra zibethicus*, *Rattus* spp. and American beaver *Castor canadensis* (Diagne et al., 2023). In total, 87% of costs were damage-related, principally impacting agriculture, and were predominantly in Asia, Europe and North America.

1.2 Fertility control and the management of wildlife populations

It is important to have practical and effective tools to manage wildlife populations and mitigate their impacts. Culling, traditionally used to reduce wildlife population numbers and mitigate human-wildlife conflicts, can be inhumane and environmentally hazardous. For example, in recent years, anticoagulant rodenticides have been banned for use in the UK, mainly due to their wider impact on nontarget species and the environment (British Pest Control Association, 2024). The introduction of animal welfare and environmental protection legislation such as the EU Biocides Regulation 528/2012(EU BPR) (Health and Safety Executive, 2018) and the UK Wildlife and Countryside Act 1981 (UK Government, 2018), together with growing public opposition towards lethal methods (Dunn et al., 2018; van Eeden et al., 2017), has meant that there are fewer available and acceptable methods for wildlife control. The continuous effort required to remove animals and suppress population recovery means that culling can be expensive; between 1998 and 2015, grey squirrel eradication from the 714 km^2 island of Anglesey in the UK and the introduction of a small number of red squirrels, cost approximately £1 million (Derbridge et al. 2016).

Fertility control is increasingly used as a wildlife management tool for a range of mammal species (Massei & Cowan, 2014), offering potential benefits, particularly when compared with culling (Massei, 2023). Fertility control is more publicly acceptable than other methods (Dunn et al. 2018; Fagerstone et al., 2002), which is likely to increase landowner and practitioner support and facilitate deployment. Unlike lethal control methods, individuals treated with fertility control agents are likely to remain in the population, potentially decreasing recruitment, and immigration rates, therefore mitigating population recovery through density dependent feedback (Knipling & McGuire, 1972). More effective wildlife control can therefore be achieved by initially removing individuals in a population through culling, then maintaining populations at a low level through the introduction of fertility control (e.g. Hobbs et al., 2000; Croft et al. 2020 and 2021). In addition, disease transmission and prevalence is likely to be reduced, particularly when fertility control is used in conjunction with a disease control vaccine (Carroll et al., 2010) due to a reduction in the likelihood of social perturbation (Tuyttens &

MacDonald, 1998) and a decline in the number of disease-susceptible individuals (i.e., juveniles) present in the population (Smith and Cheeseman, 2002; Miller et al., 2004).

Fertility control has been proposed as a method for controlling a range of overabundant rodent and small mammal species, but most of the currently used fertility control agents are injectable single-dose immunocontraceptive vaccines and rodent populations are often too numerous for these agents to be delivered cost-effectively (Fagerstone et al. 2008; Kirkpatrick et al., 2011; Massei, 2023). To be effective, a critical level of contraceptive coverage is required, and a sufficient proportion of the target population must be treated (Cowan & Massei, 2008; Croft et al., 2021). In most instances, injectable contraceptives require the capture or restraint of animals, which limits their practical application to relatively small and localised populations (Asa et al. 2024; Massei, 2023; Wimpenny et al., 2021).

Oral baits facilitate the deployment of pharmaceuticals, on a landscape scale, using methods that are generally more practical and cost-effective. This has led to their successful utilisation in the control of wildlife disease, such as the rabies vaccines for wild carnivores (Cross et al., 2015; EFSA, 2015; Slate et al, 2005). There are, however currently only two oral contraceptives available for mammalian wildlife management: EP-1 and ContraPest® both developed for rodent control (Jacoblinnert, 2022; Massei, 2023).

EP-1 consists of the synthetic hormones levonorgestrel and quinestrol, which reduce female conception rate through the enlargement of the uterus and inhibit testicular function in males. EP-1 has been used as a once-a-month human contraceptive pill in China since the 1960s (Kejuan et al., 2007) and is registered in Tanzania as a control agent for multimammate mice *Mastomys natalensis* (Jacoblinnert et al., 2022). EP-1 has been shown to reduce the fertility of a range of rodent species, including plateau pikas *Ochotona curzoniae* (Liu et al., 2012), black rats *Rattus rattus* (Selemani et al, 2022) and Brandt's voles *Lasiopodomys brandtii* (Zhao et al. 2008). There are, however, several concerns surrounding the use of EP-1 as a wildlife control agent, mainly regarding the potential impact hormones could have on the environment and on nontarget species. For example, the administration of EP-1 has been shown to affect egg production in chickens (He et al., 2021). As a preventative measure, EP-1 is often deployed inside tubes or bamboo cuttings (Imakando et al. 2022). Though these devices do not fully contain the contraceptive and may allow access by other small animals. This means that nontarget access is only reduced rather than completely prevented.

The second oral contraceptive available is ContraPest®. ContraPest® is a liquid contraceptive containing 4-vinylcyclohexene diepoxide and triptolide, that has been shown to cause ovarian follicular depletion in female rats and impair spermatogenesis in male rats, leading to reduced fertility in both sexes (Mayer et al., 2002; Witmer et al., 2017). ContraPest® is registered for use in the United States for Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) and can be deployed via a range of purpose-designed bait boxes, aimed at reducing nontarget access in different environments and monitoring bait uptake (Senestech, 2024). The use of ContraPest® is restricted to in and around buildings and very little is known about its effect on other species.

1.3 Oral contraceptives for grey squirrel management in the UK

Oral contraceptives are currently being developed to manage the invasive non-native grey squirrel *Sciurus carolinensis* in the UK. The International Union for Conservation of Nature has included the grey squirrel in the 100 world's worst invasive alien species list (Lowe et al., 2024). Grey squirrels were introduced into England in the 19th century and their numbers now exceed 2.5 million. In comparison, the native red squirrel *Sciurus vulgaris* has declined to less than 300,000 individuals and is now considered to be 'Endangered' in Great Britain (Figure 1.1; Croft et al., 2017; Mammal Society, 2024; Mathews et al., 2018).

The grey squirrel is largely responsible for the decline of the native red squirrel, through the transmission of the squirrel pox virus (that the grey squirrel can carry asymptomatically) and competition for resources (Everest et al., 2023; Gurnell & Pepper, 2016; Rushton et al, 2006; Wauters et al., 2002). There are several aspects of the grey squirrel biology, that make it a very successful coloniser and that give it a competitive advantage over the red squirrel. The grey squirrel occupies a wide range of habitats in the UK, including broadleaf and conifer forests and urban parks and gardens. The grey squirrel's adaptability to different environments can largely be attributed to its broad omnivorous diet, which predominantly includes a large range of tree seeds, but also flowers, buds, leaves, fruits, fungi as well as invertebrates, birds eggs, nestlings and adult birds (Moller, 1983).

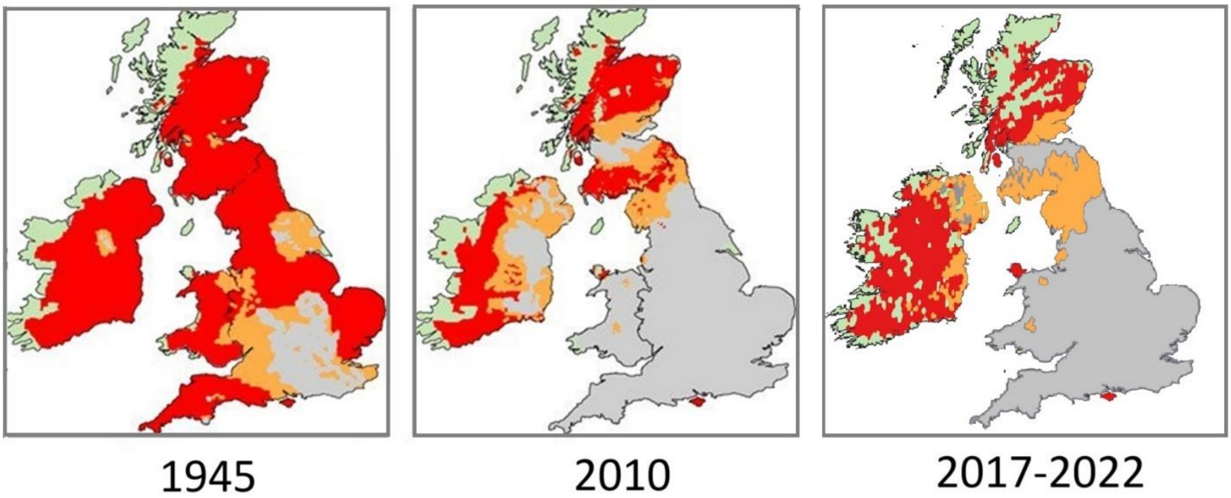

Figure 1.1. Estimated distributions of grey squirrels (grey), red squirrels (red) and both species together (orange) in the British Isles for 1945, 2010 (Copyright Craig Shuttleworth/RSST) and 2017-2022 (UK Squirrel Accord, 2024: Data provided by CEDaR, Clocaenog Red Squirrels Trust, Colin Lawton, Mammal Society, Mid-Wales Red Squirrels Partnership, National Biodiversity Data Centre, National Parks and Wildlife Service, Red Squirrels Northern England, Saving Scotland's Red Squirrels, Trees for Life, Ulster Wildlife, University of Galway and Vincent Wildlife Trust).

Grey squirrels are also known to obtain nutrients from trees through bark stripping (Nichols et al., 2016). As a result of this process, it is estimated that grey squirrels cause £37 million in tree damage per annum and it is predicted that, over the next 40 years, this will cost the forestry sector in England and Wales at least £1.1 billion ($1.4 billion USD) in damaged timber, lost carbon capture, and tree replacements (Royal Forestry Society, 2021).

The grey squirrel is particularly known for scatter-hoarding; widely dispersing the seeds of hardwood trees such as oak *Quercus spp.* in caches buried in soil or hidden in leaf litter (Steele & Wauters, 2016). This reduces resource competition from other animals, including red squirrels, and maintains seed availability throughout winter, when natural food availability is low. It has also been found that in oak dominated woodlands, grey squirrels can digest acorn polyphenols more effectively than red squirrels, allowing them to extract more energy from acorns and consume them earlier in the season (Kenward & Holm, 1993).

If there is direct competition for food between co-existing grey and red squirrels, the grey squirrel has a size advantage. The body weights of adult grey squirrels (440-650 g) are often double that of adult red squirrels (up to 350 g; Mammal Society, 2024). These competitive advantages may benefit the delivery of oral contraceptives to grey squirrels, as they are likely to out-compete other animals including red squirrels for access to a bait, thus reducing nontarget impact. By incorporating a weight threshold, body weight could be used as a selective tool, ensuring that grey squirrels can access a contraceptive and smaller animals such as red squirrels cannot.

Red squirrels typically inhabit conifer forests, being more suited to the conifer environment and food resources. This has allowed red squirrels to persist and even co-exist with grey squirrels in some forest areas in Scotland, England and Wales (Bryce et al., 2002). Conifer tree seeds are, however, nutritionally relatively poor and conifer forests have higher levels of squirrel predation levels compared with broadleaf forests (Kenward et al., 1998). This and the competitive advantages mentioned above, might explain why red squirrels are found at much lower densities than grey squirrels. Densities of grey squirrels across different environments can vary considerably, from <1 to >13 squirrels ha^{-1}, with densities in urban areas (8 squirrels ha^{-1}) on average double that of rural areas (4 squirrels ha^{-1}; Merrick et al., 2016). In comparison, rural red squirrel densities are typically <2 squirrels ha^{-1} (Bryce et al., 2002; Lurz et al., 1995)

Female grey and red squirrels can live up to 5-6 years in the wild and can breed from 10-12 months old (Mammal Society, 2024). Both grey and red squirrels exhibit similar breeding patterns, having two peaks of breeding a year, one in winter and one in spring/early summer (Hayssen, 2016). Both species have on average between 2-3 kits per litter and give birth in a nest or drey (Mammal Society, 2024). For grey squirrel females, producing two litters per year is usual, providing there are sufficient food resources. Red squirrel breeding is more sensitive to food resources, with females more likely to produce one litter per year (Wauters & Dhondt, 1995). Two peaks of breeding may allow for a more cost-effective delivery strategy for oral contraceptives to grey squirrels, as the contraceptives could deployed for a short time immediately before each breeding peak, rather than having to be deployed all year round.

The UK Squirrel Accord (2024) is a UK charity with 45 signatories, which include red squirrel conservation, forestry and land management organisations, Defra and other Government organisations. The charity has funded research on oral contraceptive for grey squirrels since 2017. The aims of this research, led by the Animal and Plant Health Agency (APHA), is to develop an oral immunocontraceptive vaccine for grey squirrels and to design a method to deliver the contraceptive to the target species. The development of an oral contraceptive for grey squirrels is also an objective in the current UK Government management plan for the species (Forestry Commission, 2014).

The contraceptive in development is based on an immunogenic protein attached to gonadotropin-releasing hormone (GnRH), which in one trial induced infertility in 6 out of 10 laboratory rats treated with 6 doses of an oral formulation (Massei et al. 2020). This contraceptive is mammal specific and should induce infertility in both males and females. It is estimated that the development of the oral contraceptive and its registration for general use in the UK will take a minimum of six years (UK Squirrel Accord, 2024).

Oral contraceptives require a delivery system that is effective, practical, targeted and economically viable, so it is equally important that a suitable bait delivery system (a feeder together with bait deployment methodology) is developed to ensure that a contraceptive is delivered to a sufficient proportion of the target population with minimal impact on nontarget animals. The development and testing of this bait delivery system is the focus of this thesis.

1.4 Chapter 2. Developing methods to measure bait uptake in grey squirrels

In Chapter 2, I develop and test two methods to measure bait uptake by free-living grey squirrels. Rhodamine B (RB), commonly utilised as a bait marker, has been used to measure bait uptake by wildlife including the proportion of a population that will consume a bait (Fisher 1999). Following ingestion of RB-treated baits, RB is incorporated in hairs and whiskers of mammals and can be observed as a fluorescent orange band under UV light. It is, however, important to determine the useable concentration of RB in baits for each wildlife species, as RB may decrease the palatability of a bait (Fernandez & Rocke 2011). For Chapter 2, I conducted trials on captive grey squirrels, to determine useable concentrations of RB in bait, by assessing palatability, detectability in the hair and changes in detectability with bait age.

The effectiveness of bait-delivered contraceptives depends on the number of visits to bait stations by individual animals over time and on the quantity of bait they consume at each visit. Consequently, understanding how bait uptake differs between individuals in a population is important when optimising the delivery of contraceptives, vaccines and biocides. Passive

Integrated Transponder (PIT) tags are increasingly used to mark and monitor animals, as they have minimal welfare impacts, are relatively cheap and easy to apply and generally have a lower tag loss rate than external tags (Smyth & Nebel, 2013). This technology has been used to collect remote data on wildlife in the field, including recording the behaviour of wood ducks *Aix sponsa*a at nest boxes (Bridge et al., 2019), monitoring the home ranges of wood mice *Apodemus sylvaticus* using bait stations (Godsall et al., 2014) and recording den use by edible dormice *Glis glis* (Kukalová et al., 2013).

In Chapter 2 I design and test a novel bait hopper, equipped with a PIT-tag reader and bait weighing device, that could record the frequency of feeding visits and amount of bait consumed per visit by free-living grey squirrels. I co-developed the bait hopper with NatureCounters, Kent, UK, who specialise in PIT-tag based wildlife technology. The bait hopper design, aimed to minimise nontarget access in several ways. The bait compartment was contained within a metal tube, which was accessed by a door. The positioning of the door was based on a previous hopper design, developed by the Forestry Commission to deliver the anticoagulant rodenticide Warfarin to grey squirrels (Mayle et al., 2007), before the license to use it was revoked by the EU in 2014 due to welfare concerns. The door had a 70 g weight attached and pivoted on a top hinge, angled to encourage a squirrel to push it open with its head to access the bait.

The hopper had an integrated PIT-tag reader in front of the door to record the date and time it was accessed by a PIT-tagged squirrel, while a strain gauge underneath the bait tray recorded how much bait was taken. The hopper was tested in a trial using captive grey squirrels, to ensure PIT-tag reads could be accurately assigned to feeding visits and a laboratory trial was conducted to ensure it could read accurate weights under different temperature ranges.

1.5 Chapter 3. Factors affecting population level bait uptake by the grey squirrel

In Chapter 3, I used the bait hopper and bait marker methods I developed in Chapter 2, to monitor bait uptake by grey squirrel populations in ten woods. The trials were conducted in summer and winter and at different densities of hoppers, to measure the potential efficacy of delivering a contraceptive bait via feeders and the main factors affecting bait uptake. Season is an important factor when considering contraceptive delivery. In UK woodlands, there are two main peaks in grey squirrel mating: December to January and April to May, with some females producing two litters within the same year (Hayssen, 2016). A contraceptive should be delivered so that it is effective over these months. There are also seasonal variations in bait uptake by grey squirrels. Due to the relatively low availability of natural food resources, squirrels are more likely to feed on cached food and baits in winter (Steele & Wauters, 2016). Conversely, squirrels are more active for longer during the day in summer than in winter (Thompson, 1977) and it is generally more practicable to deliver bait in summer due to more favourable weather conditions and longer daylight hours.

1.6 Chapter 4. An assessment of individual bait uptake by the grey squirrel

The effectiveness of a contraceptive is determined by the number of individuals it is delivered to, together with the proportion of individuals rendered infertile. To maximise effectiveness, oral contraceptive delivery should predominantly target the females in a population and the majority of the females should be rendered infertile for the peak times in breeding (Massei & Cowan, 2014; Massei et al. 2024). Understanding grey squirrel feeding behaviour related to feeding devices is important so that delivery methods can be designed to ensure a sufficient number of individuals receive an effective contraceptive dose.

In group-living rodents, such as the grey squirrel, high reproductive rates and high population densities may result in intraspecific competition for resources, particularly between individuals of different sex and breeding status and this may determine how much contraceptive an individual would consume. For instance, in California ground squirrels *Spermophilus beechey,* Whisson and Salmon (2009) found that a greater proportion of males visited bait stations than females and that males consumed more bait and made more visits to bait stations than females. Jacob et al. (2003) found higher bait uptake in breeding than non-breeding female house mice *Mus domesticus*, while Inglis et al. (1996) found that female Norway rats *Rattus norvegicus* made more feeding visits to bait than males. Within grey squirrel populations a hierarchical system has been reported, with males usually dominant over females and adults over sub-adults (Pack et al., 1967).

The aim of Chapter 4 was, using the bait hopper developed in Chapter 2, assess how sex, body weight, season, squirrel density and bait point density influenced the probability of a grey squirrel in a woodland visiting a feeder and the amount of bait it consumed from feeders.

1.7 Chapter 5. A camera trap method for estimating densities of grey squirrels

Effective wildlife population management requires an understanding of the abundance of the target species. This knowledge is important to plan how much effort, in terms of equipment and hours, is required to achieve a set population reduction and to assess the effect of fertility control on population reduction. Camera traps are increasingly used to estimate mammal population sizes (Jayasekara & Mahaulpatha, 2021; Massei et al., 2018; Mason et al., 2022; Noss et al., 2012) and, in the last few decades, methods have been developed that estimate animal abundance based on the detection rates of animals by camera traps that, unlike traditional capture-mark recapture applications, do not require the identification of individual animals (Gilbert et al, 2021; Howe et al., 2017; Loonam et al., 2021; Moeller, 2017; Palencia et al., 2021). Most of these methods have been focussed on medium to large mammal species with large range sizes and may be unsuitable for measuring local abundances of smaller mammals that have variable detection rates and hard to measure movement behaviour.

Developments in camera trap technology, which include longer battery life, faster trigger speeds, higher sensitivity and greater memory capacity, have widened their application for monitoring the activity of rodents and other small, fast-moving mammals and indices calculated from the number of camera trap photos per unit effort have been found to be closely correlated with other measures of population size, such as tracking plates and capture mark-recapture from trapping records, for Norway rats *Rattus norvegicus* (Lambert et al., 2018), red-backed voles *Myodes rutilus* and deer mice *Peromyscus maniculatus* (Vilette et al, 2016) and snowshoe hares *Lepus americanus* and red squirrels *Tamiasciurus hudsonicus* (Vilette et al. 2017).

The aim of Chapter 5 was to develop and test a practical and cost-effective camera trap method that could be used by practitioners to estimate densities of grey squirrels in woodlands. I deployed camera traps in ten woodlands, baited for 3-6 days, immediately prior to the bait uptake trials described in Chapter 3. Indices were calculated for each wood based on the number of squirrel photographs recorded per camera per day and these were compared with the total number of squirrels removed through trap and dispatch, undertaken at the end of the bait uptake studies.

1.8 Chapter 6. Developing species-specifc feeders for oral contraceptive delivery

Oral contraceptives for wildlife population management offer some significant benefits over their injectable counterparts, which often require the capture of individuals for administration. Oral contraceptives delivery methods are likely to be more practical, cost-effective and provide better animal welfare outcomes, as it is likely that they could be deployed remotely, on a larger scale and to more individuals (Massei, 2023). However, as oral contraceptives are not administered directly to individuals, there is a greater risk that they could be accessed by nontarget animals. It is therefore important that species-specific bait delivery systems are designed and tested to ensure that fertility control can be delivered effectively and in a targeted way without impacting other wildlife.

The GnRH based contraceptives in development for grey squirrels in the UK are mammal specific, targeting reproductive systems that are similar in structure and function across different families of mammals (Massei, 2023; Pinkham et al., 2022; UK Squirrel Accord, 2024). In the UK, there are concerns that oral contraceptives delivered via feeders to grey squirrel control, could be accessed by vulnerable species protected under Schedule 5 of the Wildlife and Countryside Act 1981. Red squirrels are, of course, an important nontarget consideration, but another Schedule 5 species that could be affected is the pine marten *Martes martes*, which are gradually recovering in the UK, both in terms of numbers and geographical range, after suffering significant declines in the 19th and 20th centuries due to persecution and habitat loss (Macpherson & Wright, 2021). As well as negatively impacting species of conservation concern, the ability of any nontarget animals to remove baits would likely reduce the efficacy of oral contraceptives, by limiting the bait available to the target species, therefore increasing the amount of bait required along with the associated levels of effort and cost necessary to deploy it.

In Chapter 6 I discuss two studies that investigate two different species-specific bait systems, that could be used to ensure oral contraceptive are targeted towards grey squirrels and the potential impact on nontarget animals minimised. The first study was conducted simultaneously with the bait trials in Chapters 3 and 4, where the only squirrel species present was grey squirrels. In these trials I tested the species-selectivity of the bait hopper described in Chapter 1, which was designed to exclude nontarget animals using a weighted door that had to be pushed open to access the bait. The bait was contained within a metal tube, which was fixed approximately 1 meter above the ground on a wooden stand. Nontarget access to bait was assessed by matching the date and times the bait door was opened (recorded via a magnetic switch) to photos and videos recorded by a remote camera.

The second study was designed to assess whether squirrel body weight could be used to select between grey and red squirrels, in areas where both species were present, so that a bait hopper could be developed that would allow most adult greys access to a bait, while excluding all red squirrels. Red squirrel juvenile bodyweight is on average 100-150g, with adults up to 350g, while the weight range for adult grey squirrels is reported to be 440-650g (Mammal Society, 2024).

For the study, I commissioned the design and construction of squirrel feeders with integrated automatic weighing platforms. These devices were used to gather data on red squirrel weights from 8 red squirrel habitats in Cumbria, Northumberland, North Yorkshire and North Wales at different times of year. The maximum red squirrel weights recorded were compared to the weights of grey squirrels collected at the same time of year, as part the bait uptake trials described in Chapters 3 and 4. From the data collected, a prototype feeder was developed and tested in a small pilot trial, in a woodland in Cumbria where both red and grey squirrels were present.

In Chapter 7 I discuss the collective findings from Chapters 2-6, including how these could be more widely applied to other wildlife management applications and species.

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Chapter 2. Developing methods for measuring bait uptake in grey squirrels

The findings of this chapter are published in:

Beatham, S. E., Goodwin, D., Coats, J., Stephens, P. A., & Massei, G. (2021). A PIT-tag–based method for measuring individual bait uptake in small mammals. *Ecological Solutions and Evidence,* 2(2). https://doi.org/10.1002/2688-8319.12081

Beatham, S. E., Coats, J., Stephens, P. A., & Massei, G. (2023). Factors affecting bait uptake by the grey squirrel (*Sciurus carolinensis*) and the future delivery of oral contraceptives. *Wildlife Research*. https://doi.org/10.1071/WR22159

The data from the captive PIT-tag trials are published in:

Beatham, S (2021) Trials testing a bait hopper equipped with a PIT-tag reader and bait weighing device, to record bait uptake by individual grey squirrels. *Dryad Digital Repository*. doi:10.5061/dryad.jq2bvq888

2.1 Abstract

Baits are used to deliver biocides and contraceptives to reduce overabundant wildlife populations and as vehicles for vaccines to control disease outbreaks. Bait-delivered contraceptives are also being developed to manage grey squirrel *Sciurus carolinensis* populations in the UK. The effectiveness of bait-delivered drugs on wildlife populations depends on the amount of bait consumed by individuals over time and the proportion of a population that consumes a bait. It is important that suitable methods are available to measure individual and population level bait uptake in free-living wildlife populations.

Passive Integrated Transponder (PIT) tags are increasingly used to mark wildlife and monitor individual behaviour, as they are cost-effective, have minimal negative welfare impacts and have a lower tag loss rate than external tags, particularly in small animals. There are, however, no suitable PIT-tag systems available for measuring bait uptake by grey squirrels. The bait marker rhodamine B (RB) has been used to measure bait uptake by small animals including the proportion of a population that will consume a bait. Following ingestion of RB-treated baits, RB is incorporated in hairs and whiskers and can be observed as a fluorescent orange band

under UV light. It is, however, important to determine the useable concentration of RB in baits for each wildlife species, as RB concentration can effect bait palatability.

The main aims of Chapter 2 were to, in trials on captive grey squirrels: 1. design and test a novel bait hopper equipped with a PIT-tag reader and bait weighing device, to record bait uptake by individual grey squirrels; and 2. determine useable concentrations of RB in bait to measure bait uptake, by assessing palatability, detectability and changes in detectability with bait age.

The bait hopper developed proved to be highly effective at recording feeding visits by squirrels, as 95% of the visits could be attributed to a PIT-tag record. The bait removed per feeding visit was measured to an accuracy of 0.1 g and 97% of the bait taken from six hoppers attributed to a PIT-tag ID. It was found that a concentration of 0.18% RB mixed in hazelnut butter was palatable to grey squirrels and that individuals needed to consume only small amounts (< 5 g) for it to be detected in the flank hair using an ultraviolet microscope.

The methods developed in this chapter have wider applications for wildlife management; in particular, efficacy studies for bait-delivered substances in the context of wildlife disease control and/or population reduction.

2.2 Introduction

Baits are used to deliver biocides and contraceptives to reduce wildlife populations (Towns & Broome, 2003; Pyzyna et al., 2016) and as vehicles for vaccines to control disease outbreaks in wildlife (Slate et al., 2005). Bait-delivered oral contraceptives are being developed to manage the invasive non-native grey squirrel in the UK (UK Squirrel Accord, 2024). Understanding factors that affect bait uptake is important for the development of effective contraceptive delivery methods and this requires accurate measurements of bait uptake both at the population and individual level. Oral contraceptives require delivery devices, that are robust enough for the environments they will be used in, easy to maintain, cost-effective to manufacture and that can deliver a bait to the target species with minimal effects on nontarget species.

The effectiveness of rodenticides, oral vaccines and bait-delivered contraceptives depends on the number of visits to bait stations by individual animals over time and the quantity of bait they consume at each visit. Consequently, understanding how bait uptake differs between the individuals in a population is important when optimising the delivery of contraceptives, vaccines and biocides.

Passive Integrated Transponder (PIT) tags are increasingly used to mark and monitor animals, as they have minimal welfare impacts, are relatively cheap and easy to apply and generally have a lower tag loss rate than external tags (Smyth & Nebel, 2013). Once a number of individuals have been PIT-tagged, Radio Frequency Identification (RFID) technology can be used to create remote sensing stations that record and monitor the presence of individuals via their PIT-tags. PIT-tags do not rely on an internal power source, so can feasibly be used over the lifetime of an animal, providing an important improvement in animal welfare by reducing the amount of trapping and/or handling required. Examples of how this technology has been used to collect remote data on wildlife in the field include recording the behaviour of wood ducks *Aix spons*a at nest boxes (Bridge et al., 2019), monitoring the home ranges of wood mice *Apodemus sylvaticus* using bait stations (Godsall et al., 2014) and recording den use by edible dormice *Glis glis* (Kukalová et al., 2013).

Rhodamine B (RB), commonly utilised as a bait marker, has been used to measure bait uptake by wildlife including the proportion of a population that will consume a bait (Fisher 1999). Following ingestion of RB-treated baits, RB is incorporated in hairs and whiskers and can be observed as a fluorescent orange band under UV light. It is, however, important to determine the useable concentration of RB in baits for each wildlife species, as RB may decrease the palatability of a bait (Fernandez & Rocke 2011).

The main aims of Chapter 2 were to, in trials on captive grey squirrels: 1. design and test a novel bait hopper equipped with a PIT-tag reader and bait weighing device, to record bait uptake by individual grey squirrels; and 2. determine useable concentrations of RB in bait to measure bait uptake, by assessing palatability, detectability and changes in detectability with bait age. The hopper was designed to overcome a number of limitations typically encountered when using RFID systems to gather long-term field data. I discuss how this hopper represents a significant improvement over other systems, in terms of data quality and quantity, battery management and practicality; in particular. I also discuss how the methods developed could be applied to studies with other small mammal species.

2.3 Materials and methods

Ethical statement

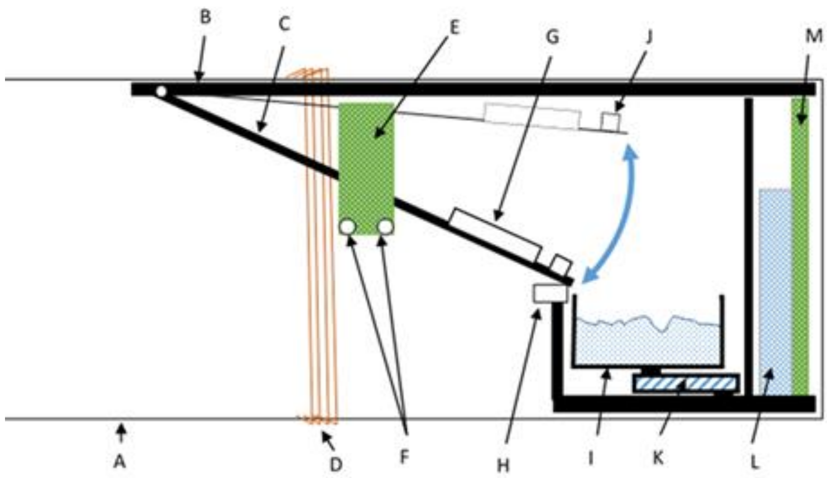
This study was conducted by trained Home Office Personal Licence holders under the advice of the Named Veterinary Surgeon and under a UK Home Office licence, in accordance with the Animals (Scientific Procedures) Act 1986. The study was approved by the joint Animal and Plant Health Agency (APHA) and Fera Science Ltd Animal Welfare and Ethical Review Body (AWERB).

Bait hopper technical design

The grey squirrel bait hopper was designed to record the identity of any PIT-tagged squirrel that entered it and to restrict access to bait by non-target animals through a weighted door and metal exterior (Figure 2.1). I based the hopper structure and dimensions on a previous hopper design, developed by the Forestry Commission to deliver the rodenticide Warfarin to grey squirrels (Mayle et al., 2007). To reduce nontarget access, a door fixed with a 70 g weight, pivots on a top hinge, angled to encourage a squirrel to push it open with its head in order to access the bait.

From the dimensions and requirements I provided, NatureCounters, Kent, UK, designed and produced the hopper, incorporating an RFID system for the detection of PIT-tags. To detect a PIT-tag, an RFID system must be active, which utilises a significant amount of battery power. The system in this study conserves battery power through the use of infrared light beams. Each visit to the hopper by an animal is designated as an “event”. An event is triggered when both infrared light beams are broken, and finishes when both light beams are clear again. When an event is triggered, the RFID reader is activated, creating a frequency field in the antenna coil.

a)



b)

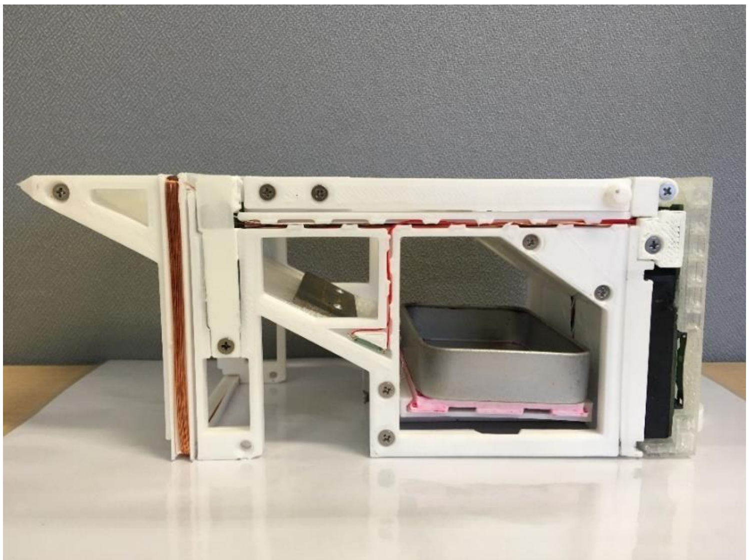


Figure 2.1. Design of a grey squirrel bait hopper with PIT-tag reader (a) and photograph of the 3D-printed plastic bait compartment (b). The hopper design shows: A. Body of hopper; a 10 x 10 x 55 cm length of aluminium tubing, entrance left. Includes a plastic section to avoid disruption of the RFID signal; B. 3D-printed plastic insert which latches into the aluminium tube; C. Pivoting plastic door; D. RFID antenna coil set to a frequency of 134 kHz; E. Light beam driver/detector circuit boards; F. Infrared light beam sensors including LED emitters on one side of the entrance and photodiode receptors on the other side, forming a dual light beam across the entrance; G. Steel weight (70 g) attached to the door flap; H. Magnet sensitive reed switch to detect when the door has been pushed open; I. Metal bait tray which can be inserted into the hopper from the side. Includes plastic runners on the base to allow secure attachment; J. Magnet attached to the door; operates the door reed switch; K. Plastic grooves to attach the bait tray; L. Five AA batteries to power the data-logger; M. Main data-logger circuit board incorporating a microcontroller with non-volatile memory and clock functionality, RFID reader, LCD display, removable SD card, analogue-to-digital converter (ADC), reed switch to flush data and reed switches to set date/time (NatureCounters, Kent, UK).

The field remains active until the event had finished, a PIT-tag has been successfully read, or a timeout of approximately 3 seconds is reached. Using this system, the hopper requires only five AA batteries and can be used in the field for at least four weeks before the batteries have to be replaced. The RFID coil is positioned immediately before the bait compartment. This meant that it is more likely that PIT-tag individual identities (IDs) were recorded for feeding visits only, as opposed to visits where an animal enters the hopper and does not feed. If a PIT-tag is detected but the identification is not established, a '0' is recorded. This offers an advantage over other RFID systems, which typically only record positive identities from PIT-tagged individuals.

A strain gauge, upon which the bait tray is attached, weighs the amount of bait consumed by an individual, identified by its PIT-tag record. The hopper records, via a magnetic door switch, every time the bait compartment is accessed. When the door magnet is disengaged, the hopper records the weight of the bait tray at least 5 times per second and calculates an average weight from these readings. The PIT-tag IDs, door switch and weight data associated with each event are recorded to files on the SD card, along with the date and time.

Captive trial 1: Test of bait hopper capacity to record feeding visits by squirrels

For all captive trials, animal husbandry was provided by Fera Science Ltd. The trial was conducted using two outdoor pens (width = 2.7 metres, length = 9.7 metres, height = 2.4 metres), each containing one male and two female grey squirrels previously fitted with a PIT-tag (Identichip®, York, UK) subcutaneously in the scruff of the neck. Throughout the trial, the squirrels were provided with a varied diet including maize, peanuts, bird seed and fruit, along with environmental enrichment including wool bedding, foliage, tubes, ropes, nest boxes, branches and sticks. Two hoppers were placed in each pen and each hopper was positioned on top of a wooden stand (approximately 90 cm high) so that they were visible by closed circuit television (CCTV) cameras. During the trial, one of the hoppers became obscured by a branch so was not included in the analysis.

For four consecutive days per week, for two weeks, 10 g of fresh 100% hazelnut paste was provided daily in each hopper. Feeding visits to the hoppers by individual squirrels were recorded using CCTV at peak times of feeding activity (4:00 to 8:00 and 16:00 to 20:00 GMT) in the second week of the trial. For the CCTV analysis, when a squirrel entered a hopper, the visit was assigned to one of the following categories: 'feeding' (when it entered the hopper and was subsequently observed masticating), 'full' (only the tail was visible and no evidence of feeding) or 'partial' (part of the hind quarters were still visible). Only visits that could be definitively assigned to one of these categories were used in the analysis.

The accuracy with which the hoppers recorded feeding visits was determined by checking whether a PIT-tag was detected for each feeding, full or partial visit recorded on CCTV and whether the ID of the squirrel was established. A Fisher's exact test was used to determine whether PIT-tag IDs were more likely to be recorded for feeding visits over non-feeding visits.

Laboratory trial and captive trial 2: Testing hopper capacity to measure bait uptake by individual squirrels

A trial was designed to test the accuracy of the strain gauge in each hopper, using manually weighed baits. To measure the accuracy of bait weight taken per visit, the first part of the trial was conducted in a laboratory with nine hoppers. Approximately 70 g of 100% hazelnut paste was weighed in a bait tray and placed in each hopper. To simulate different field conditions, five hoppers were placed in a refrigerator and left for one hour to acclimatise to between 6^{o}c and 8^{o}c; the remaining four hoppers were left at room temperature, between 20^{o}c and 21^{o}c. A small amount (0.1 to 2.2 g) of paste was then removed from each hopper using a pre-weighed metal spoon, and the spoon and paste weighed (to the nearest 0.1 g). After at least 10 minutes, a larger amount of paste (4.9 to 18.5 g) was removed from each hopper and weighed (to the nearest 0.1 g). This was repeated until there were are at least 5 weights for small amounts of bait and at least 5 weights for larger amounts of bait for each hopper. To ensure the strain gauge was stable, the amount of bait taken from each hopper was calculated from the weight recorded by each hopper 2 minutes prior to the bait removal minus the weight taken 2 minutes after the removal. These were compared with weights obtained manually using a standard balance.

To test whether it was possible for the hoppers to weigh bait consumed by individual squirrels, a second trial was conducted using captive PIT-tagged grey squirrels and six of the nine hoppers used in the laboratory trial. Two hoppers per pen were deployed in three outdoor pens containing 2-3 squirrels per pen, 7 squirrels in total. Hoppers were placed on the floor along each side of a pen, weighed down by bricks, to ensure the squirrels could not move or overturn them. In week 1, the hoppers were baited on Monday, Wednesday and Friday with approximately 40 g 100% hazelnut paste to encourage the squirrels to feed from them. On the Tuesday and Wednesday of week 2, 20 ± 0.5 g of hazelnut paste was weighed into bait trays and installed in each hopper at 7:15 am, immediately before the squirrel peak feeding time. After 6 hours, the bait trays were removed and the bait weighed (to the nearest 0.1 g).

The manual weights taken for each trial period were compared to those recorded by the hoppers. The hopper weight of bait taken for each event was calculated by taking the minimum weight from the most stable values recorded; those where the raw figures for the first and fifth repeat strain gauge readings (before they were converted to a weight) were within 5. The weight decrease at each event was then matched with a PIT-tag record, if available, to calculate the amount of bait taken per visit by individual squirrels.

Captive trial 3: Determining the best concentration of rhodamine B for bait

Two concentrations of RB (CAS number 81-88-9, Sigma-Aldrich), 0.12% and 0.18% weight/weight (w/w) mixed into 100% hazelnut butter, were tested for their comparative palatability to captive grey squirrels and detectability in squirrel hair. The 0.12% concentration was selected based on the minimum amount of RB per body weight required for detection, 10 mg/kg, found by a study on prairie dogs *Cynomys ludovicianus* (Fernandez & Rocke 2011). At 0.12%, it was calculated that a grey squirrel with a body weight of 522 g (the average weight recorded on a sample of 23 captive squirrels housed) would have to eat at least 4 g of bait to ingest a sufficient dose of RB to mark its hair. This was deemed to be a reasonable minimum value, based on the amount of bait consumed by individual squirrels in Captive trial 1. A previous trial conducted at APHA (unpublished) found that 100% hazelnut butter was readily consumed by captive grey squirrels and 0.25% weight/weight (w/w) RB in hazelnut butter was detectable in the hair of squirrels that ingested it but was less palatable to them than hazelnut butter alone, therefore 0.18% was selected as a midway concentration between 0.12% and 0.25%.

Two grey squirrel-specific bait hoppers with integrated PIT-tag readers were placed in nine outdoor purpose-built pens (width = 2.7 m, length = 9.7 m, height = 2.4 m), each housing one male and two female grey squirrels. The hoppers were placed opposite each other, on either side of the pen on 90 cm high wooden stands. Each squirrel had previously been fitted with an

Identichip® PIT-tag subcutaneously in the scruff of the neck. Every time a squirrel entered a hopper, the date, time and ID of the squirrel was recorded by the hopper and saved onto an SD card.

Throughout the trial the squirrels were provided with a diet of maize, peanuts and mixed bird seed, along with environmental enrichment including wool bedding, foliage, tubes, ropes, nest boxes and branches. The hoppers in each pen were baited with 7 g +/- 0.5 g of 100% hazelnut butter once a day for one week. The six pens that had the most bait consumed were split into three groups, two pens per group. Each hopper was provided with 7 g +/- 0.5 g of bait at the same time of day on four consecutive days and the bait weighed after 24 hours. Group 1 were given 100% hazelnut butter, group 2 were given 0.12% RB w/w in 100 % hazelnut butter and group 3 were given 0.18% RB w/w in 100% hazelnut butter. The RB baits were mixed fresh each day. Each time the bait was removed from the hoppers, the area around the hopper was checked for bait spillage (the bait was bright pink, so easy to identify) and the spillage weight recorded, to the nearest gram.

Three weeks after RB bait deployment, the squirrels were caught, restrained and 10-20 hairs plucked from the flank and the tail and placed in a transparent plastic sample bag. Each hair sample was analysed in its bag using a Leica DMLB ultra-violet (UV) microscope (Leica Microsystems UK Ltd.) at 4 x magnification and the presence or absence of RB fluorescence was recorded. As squirrel hair exhibits natural fluorescence, a control squirrel hair sample was used as a reference. The detectability of each RB dose was assessed by comparing the numbers of individuals in each treatment group that exhibited RB bands in the flank and tail hair with the relative amount of bait consumed by each individual squirrel. The latter was estimated from the comparative number of PIT-tag records for each squirrel over the four trial days, as a PIT-tag is more likely to be recorded for a visit where bait is consumed than one where it is not (see Captive Trial 1 results).

The relative palatability of the different baits was assessed from the total amount of bait consumed per pen per day for each group. During the bait trial, 7 +/- 0.5 g of hazelnut butter was left in a hopper in an area with similar environmental characteristics (e.g. temperature and humidity) to the RB bait in the pens. This was weighed every day to ensure that there was no effect of moisture absorption or other environmental factors on the weight of the RB baits.

Captive trial 4: Testing the viability of 14-day old rhodamine B bait

A concentration of 0.18% RB bait was mixed with 100% hazelnut butter and stored in a sealed plastic container, 14 days prior to use. This was deemed a practicable length of time in which bait could be mixed and transported to external practitioners, if required, ready for deployment in the field. Eight pens, containing one male and two female captive grey squirrels were installed with two hoppers per pen, as described in the first captive trial. Each hopper was pre-baited with approximately 7 g of 100% hazelnut butter per day for four days. The following week, the four pens that had the most bait consumed were provided with 7 +/- 0.5 g RB bait per day and the amount of bait consumed weighed for each hopper each day. Each time the bait was removed from the hoppers, the area around the hopper was checked for signs of bait spillage and any recorded along with the spillage weight, to the nearest gram. PIT-tags are more likely to be recorded for visits where a squirrel feeds, compared to those where they do not (see Captive trial 1 results), therefore the relative proportion of bait taken by each squirrel in each pen was estimated based on the relative number of PIT-tag records.

Two weeks following the final treatment day, male squirrels from each of the four treatment pens were caught as part of routine husbandry procedures. Hair from the flank and tail were taken and the samples analysed RB fluorescence, as described above.

2.4 Results

Captive trial 1: Test of bait hopper capacity to record feeding visits by squirrels

In total, 97 feeding visits, 47 full visits and 102 partial visits to the three hoppers by grey squirrels were recorded on CCTV during the trial. Feeding visits were recorded for five of the six squirrels and full and partial visits for all six squirrels. An average of 27 (range = 9 to 48) PIT-tag records were obtained for each squirrel. A PIT-tag was detected for 100% of feeding visits, 96% of full visits and 64% of partial visits; a PIT-tag ID was established for 95% of feeding visits, 77% of full visits and 25% of partial visits. The percentage of PIT-tag IDs recorded was significantly higher for visits where bait was taken (95%), compared with visits where no bait was taken (41%; Fisher's exact test, $p<0.001$). The ratio of feeding visits recorded by CCTV to PIT-tag IDs was 1:1.6, as the hoppers would sometimes record multiple

IDs for animal that spent more time inside them. On 28 occasions, it was observed that the squirrel visiting a hopper was displaced by another squirrel.

Laboratory trial and captive trial 2: Testing hopper capacity to measure bait uptake by individual squirrels

Over 107 occasions of bait removal, the average difference between the manually weighed bait removed and the weight of bait removed recorded by the nine hoppers was 0.3 g (range = 0.0 to 5.6) and there was no significant difference between the two sets of weights (Paired two sample T test; $t_{106} = 0.30$, $p = 0.76$). The seven highest differences (all greater than 1 g) were recorded by one hopper. When the data from this hopper were removed from the analysis, the average weight difference was 0.1 g (range = 0.0 to 0.9). This hopper was subsequently excluded from the captive squirrel trial 2.

The average amount of bait taken by squirrels during captive trial 2 was 11.5 g (range = 0.0 to 23.5) per hopper per day. The average difference between the manually weighed bait weights and the weights recorded by six hoppers on both trial days was 0.5 g (range = 0.1 to 1.2) and there was no significant different between the two sets of weights (Paired two sample T test; $t_{12} = 0.50$, $p = 0.63$). All seven PIT-tagged squirrels were recorded feeding from the hoppers. In total, on the two trial days, 138 g of bait was removed from the six hoppers. All of the bait consumed could be attributed to an unconfirmed PIT-tag, while 122 g could be attributed to a positive PIT-tag ID. On trial day 2, 12 g were removed from one hopper that later stopped recording PIT-tag IDs, with the fault likely caused by wet weather conditions. The average amount of bait taken by a squirrel on each visit was 1.2 g (range = -0.4 to 6.9). On one occasion there was a misread PIT-tag but the error concerned the last two digits of the thirteen digit ID only and was easily corrected.

Captive trial 3: Determining the best concentration of rhodamine B for bait

There was no evidence that the higher concentration of RB bait was less palatable than the lower concentration or the control bait (Table 2.1). The two control pens consumed 103 g of hazelnut butter over the 4-day treatment period, compared with 101 g for the 0.12% RB treatment group and 108 g from the 0.18% RB treatment group. The blank hopper in pre-bait week gained 1.6 g, while in treatment week it did not differ in weight by more than 0.1 g on any day. It was likely that water absorption was the cause for the increase. Bait spillage was recorded on five of the 48 checks made (in each case <1 g) in the treatment pens and was typically recorded as pink staining or smears of bait found at the hopper entrance.

RB was detected in both the tail and flank hair of 2 out of 6 squirrels in the 0.12% treatment group and four out of six squirrels in the 0.18% treatment group (Table 2.1; Figure 2.1). The only PIT-tags recorded by the hoppers were from squirrels that tested positive for RB. In pen 4, one squirrel tested positive for RB despite having a low number of visits to the hopper (four PIT-tag records). From the total amount of bait taken and the representative number of PIT-tag records, it is estimated that this squirrel took less than 5 g. A high level of natural fluorescence was found in two tail hair samples from pens where no bait was taken.

Table 2.1. The amount of bait containing 0.12% or 0.18% w/w of RB consumed from hoppers over 4 days by captive grey squirrels housed as one male and two females per pen and the presence of RB in flank and tail hair samples. The number of PIT-tags recorded by the hoppers for each squirrel was used as measure of the relative amount of the bait was eaten by an animal.

Treatment Group	Pen	Bait consumed pre-bait period (g)	Bait consumed treatment period (g)	Squirrel	Pit-tag records	RB fluorescence in hair Y = Yes, N = No	
						Flank	Tail
0.12%	1	50.8	51.8	M1	0	N	N
				F1	112	Y	Y
				F2	0	N	N
	2	49.8	49.1	M2	29	Y	Y
				F3	0	N	N
				F4	0	N	N
0.18%	3	47.6	52.7	M3	36	Y	Y
				F5	13	Y	Y
				F6	0	N	N
	4	51.3	54.8	M4	4	Y	Y
				F7	92	Y	Y
				F8	0	N	N
Control	1	54.7	50.4				
	2	53.6	52.8				

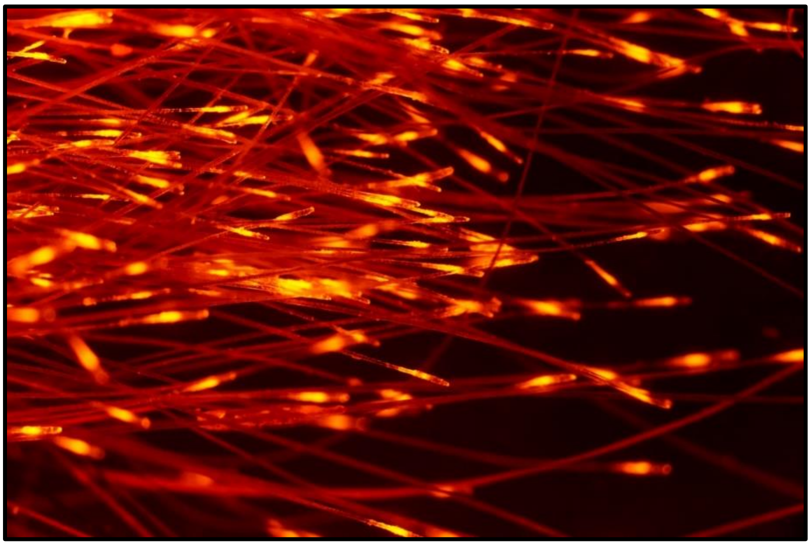


Figure 2.1. Fluorescence within the bulbs of the flank hair from a grey squirrel that had consumed rhodamine B bait. Each hair sample was analysed in a clear sample bag using a Leica DMLB ultra-violet (UV) microscope (Leica Microsystems UK Ltd.) at 4 x magnification.

Captive trial 4: Testing the viability of 14-day old rhodamine B bait

Between 45.5 and 52.7 g of RB bait was consumed from each of the four pens over the four trial days (Table 2.2), and in total over 80% of the bait provided via the hoppers in each pen. All four males sampled exhibited RB fluorescence in either the bulb or shaft of the flank hair. Only one male did not exhibit RB fluorescence in the tail hair; that squirrel had the lowest number of PIT-tag records and was estimated to have eaten less than 5 g of bait. No bait spillage was recorded around any of the hoppers during baiting.

Table 2.2. The amount of fourteen-day old bait, containing 0.18% w/w of RB, consumed over four days by four captive male grey squirrels and the presence of RB in flank and tail hair samples. The number of PIT-tag records for each squirrel was used estimate the amount of the bait was eaten by an animal.

Pen	Bait consumed over four days (g)	Squirrel ID	N Pit-tag records	RB fluorescence in hair? Y = Yes, N = No	
				Flank	Tail
5	45.4	M5	13	Y	N
6	52.7	M6	79	Y	Y
7	49.6	M7	16	Y	Y
8	49.2	M8	44	Y	Y

2.5 Discussion

The squirrel hopper with integrated PIT-tag reader proved effective for measuring patterns and quantities of bait uptake by individual grey squirrels and the results in this study represent a significant advancement when measuring the dose rates of rodenticides, oral vaccines and contraceptives and other bait-delivered drugs. A PIT-tag was detected for every feeding visit made to a hopper by a grey squirrel in captivity, a PIT-tag ID was recorded for 95% of individuals that fed from a hopper and 97% of the bait taken from functioning hoppers could be attributed to a PIT-tag ID. A PIT-tag ID was more likely to be recorded for visits where bait was taken than those where it was not. In other bait station designs, the RFID reader has been positioned at the entrance of the feeder, rather than immediately before the bait compartment (Kenward et al., 2005). This will increase the likelihood that PIT-tags will be recorded for squirrels who visit or pass the feeder but do not take any bait.

A novel component of the hopper design was the addition of a strain gauge to weigh the bait taken on each visit. When combined with the PIT-tag data using numbered events, the amount of bait taken on each visit by individual squirrels was measured to an accuracy of 0.1 g. Remote devices with strain gauges have been used to measure the body weights of animals (Bosch et al., 2015) but none have demonstrated the accuracy of weighing necessary to calculate the dose rates of contraceptives or other drugs.

Research requiring marked individuals has traditionally relied on external tags, which have the potential to be lost, damaged or misread, and often require some kind of human intervention, through recapture or tracking, to gather data (Gibbons & Andrews, 2004). This study further demonstrated that the detection of PIT-tags using hoppers can be achieved remotely, with minimal human interference and a high identification accuracy. This method therefore represents an improvement in welfare standards and behavioural data quality for animal research and provides a practical and accurate method to determine individual bait uptake in field conditions where otherwise multiple captures of animals would be required.

The battery management system meant there were no issues with battery life during the trials, with each hopper functioning for several weeks on the same set of five AA batteries. Other studies have not been able to demonstrate the same degree of longevity, despite using larger batteries such as car batteries to power bait stations (Kenward et al., 2005). Larger batteries reduce the portability and practicality of devices for use in the field and the regular monitoring and changing of batteries can be labour-intensive and could potentially disrupt the focal species' natural behaviour. Alternatively, to reduce power consumption, RFID systems can be programmed to activate at intervals when PIT-tagged individuals are more likely to be present (Bridge et al., 2019), but this will often result in some loss of data and is unsuitable if 24-hour monitoring is required.

The 3D-printed insert upon which the electronics are fixed allows the hoppers to be easily modified; therefore, the hoppers can be adapted to record other types of data from different animal species. Adaptations of the RFID system described in this study are currently being used in studies on other small mammals (NatureCounters, Kent, UK) including the monitoring of burrow use by European hamsters and a weighing device for measuring the body weights of a captive colony of fruit bats.

This study found that RB is a suitable bait marker to measure bait uptake in populations of grey squirrels. The results of the captive squirrel trials showed that, based on amount of bait consumed, a concentration of 0.18% RB mixed in hazelnut butter was as palatable to squirrels as hazelnut butter alone and that an individual needed to consume only small amounts of bait (<5 g) for it to be detected in the flank hair. This is comparable to a study by Fernandez & Rocke (2011), who found that concentrations of 0.05% to 0.30% of RB in bait were readily accepted by prairie dogs and that RB concentrations of 12 mg/kg bodyweight were sufficient for detection in whiskers under UV light. The grey squirrels in the captive studies were not individually housed, so the exact amount of bait consumed by each squirrel is not known. However, based on average captive squirrel body weights, the total amount of bait consumed in each pen and the relative number of PIT-tag records recorded for each squirrel by the hoppers, it is estimated that RB detection was achieved at less than 17 mg/kg body weight.

RB appeared to be as effective at marking squirrel hair, 14 days after it was mixed with a bait, making it more practical for field applications, where large quantities of bait may have to be mixed in advance. Squirrel flank hair appeared to be more reliable than tail hair for RB detection, as tail hair was found to have more natural fluorescence, that made lower levels of RB fluorescence harder to identify. In previous studies, hair samples for RB analysis have been washed and mounted on slides in preparation for analysis (Weerakoon, Price, & Banks 2013).

In this study, hair samples were analysed effectively whilst inside the transparent plastic sample bags that they were placed in when taken in the field; thus, the process was much more time efficient and less susceptible to labelling errors or sample contamination.

2.6 Conclusion

The novel design of hopper with integrated PIT-tag reader and bait weighing capability proved highly effective at measuring patterns and quantities of bait uptake by individual grey squirrels. The unique modifications to the hopper design in this study resulted in improved data collection and battery life in the field when compared to other similar devices. The findings of this study suggest that the bait marker rhodamine B would be effective at measuring population level bait uptake in grey squirrels. The bait marker proved to be effective at marking grey squirrels, even when only low amounts were consumed, with no apparent adverse effects. The adaptability of the hopper design means that it has wider applications for wildlife management; in particular, efficacy studies for bait-delivered drugs in the context of wildlife disease control and/or population reduction.

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Chapter 3. Factors affecting population level bait uptake by the grey squirrel

The main findings of this chapter are published in:

Beatham, S. E., Coats, J., Stephens, P. A., & Massei, G. (2023). Factors affecting bait uptake by the grey squirrel (*Sciurus carolinensis*) and the future delivery of oral contraceptives. *Wildlife Research*. https://doi.org/10.1071/WR22159

3.1 Abstract

Oral contraceptives have the potential to reduce numbers of grey squirrels in the UK, however, to be effective a sufficient proportion of a population must consume a bait containing contraceptives. The aim of this study was to estimate the efficacy of delivering baits via feeders to grey squirrels and to determine the factors most important to bait uptake.

Bait uptake was measured using the bait marker rhodamine B mixed with 100% hazelnut butter, using the concentration (0.18%) and analysis methods developed in Chapter 2. The bait was delivered to grey squirrels via the purpose-designed bait hoppers developed in Chapter 2. Bait uptake field trials were conducted in ten UK woodlands in summer and winter and at different densities of hoppers.

It was possible to deliver bait to between 55% and 88% of the grey squirrels in six out of ten woods within four days. Season, hopper density and squirrel density were important factors affecting bait uptake, with more squirrels consuming bait in summer than in winter and from three hoppers per hectare rather than one per hectare. This study demonstrated that, using the delivery system trialled, oral contraceptives could be delivered to grey squirrels across the UK.

3.2 Introduction

Oral contraceptives are currently being developed for grey squirrel management (Massei 2018; UK Squirrel Accord, 2025) and oral contraceptives delivered in baits have the potential to reduce population sizes of grey squirrels, reduce the rate of population recovery after culling and eradicate local populations (Croft et al. 2021). Fertility control offers a number of potential advantages to manage wildlife, particularly when compared with traditional methods, such as culling. These advantages include: 1. infertile animals remain in the population and, thus, potentially contribute to density-dependent feedback that constrains recruitment and survival, slowing population recovery (White, Lewis, & Harris 1997; Merrill, Cooch, & Curtis, 2003); 2. fertility control could be particularly effective at maintaining populations at relatively low density after initial culling by reducing breeding and population recovery (Prentice et al., 2019; Merrill, Cooch, & Curtis, 2003); 3. fertility control would not be expected to disrupt social groups in the same way as the removal of individuals, thus causing less social perturbation than culling and decreasing risk of disease transmission (Woodroffe et al., 2006) and 4. population control via contraceptives is more publicly acceptable than lethal methods, meaning it could be deployed on a greater scale than other methods (Dunn et al., 2018; Jacoblinnert et al., 2022).

For fertility control to be effective, a bait should be easily administered to a sufficient proportion of the population, with predominantly the females targeted (Massei & Cowan 2014; Jacoblinnert et al., 2022). Bait uptake by wildlife can be affected by many factors such as sex (Whisson & Salmon, 2009), the time of year at which bait is deployed (Robinson, Cushman, & Lucid, 2017), bait density and distribution, the availability of alternative food sources (McRae et al., 2020), and the density of the target species (Haley, Berentsen, & Engeman, 2019). Understanding the relative importance of these factors for a target species is integral to the design of effective bait delivery strategies.

Season is an important factor when considering contraceptive delivery. I hypothesised that an oral contraceptive may need to be deployed twice per year, to maintain infertility year-round. Winter was trialled as it coincides with the main breeding peak in grey squirrels (Gurnell, 1996) and bait uptake should be greater in winter, as natural food resources are depleted and grey squirrels are more reliant on cached food (Steele & Wauters, 2016). Summer was trialled as a more practical season to deploy a bait, in terms of climate and daylight hours and grey squirrel activity should be greater due to the longer daylight hours (Thompson, 1977). Autumn was not trialled as this is when tree seeds are at their most abundant, so supplementary feeding is unlikely to be successful (Gurnell, 1996).

Bait uptake was measured using the bait marker rhodamine B (RB) mixed with 100% hazelnut butter, using the concentration (0.18%) and methods developed in Chapter 2. The bait was delivered to grey squirrels via purpose-designed bait hoppers, developed in Chapter 2. Bait uptake field trials were conducted in ten UK woodlands in summer and winter and at different densities of hoppers. The aims of this study were to: 1. estimate the efficacy of delivering baits via hoppers to woodland populations of grey squirrels; and 2. analyse the effect on bait uptake by male and female squirrels of season, hopper density, ratio of squirrels to hoppers, and squirrel density. The study findings will ultimately be used to optimise delivery methods for an oral contraceptive for grey squirrels.

3.3 Materials and methods

Ethical statement

This study was conducted by trained Home Office Personal Licence holders under the advice of the Named Veterinary Surgeon. The study was approved by the joint Animal and Plant Health Agency (APHA) and Fera Science Ltd Animal Welfare and Ethical Review Body (AWERB).

Field trials

Field trials were conducted between December 2017 and September 2020. I led the study design and analysis. I conducted most of the field work and sample analysis, with assistance from Animal and Plant Health Agency (APHA) colleagues that I had trained in the methodology or that were already experienced in some of the techniques. For wood 9, the field work was largely conducted by volunteers, whom I trained in the RB bait deployment and data collection and had been previously trained in grey squirrel trap and dispatch.

The ten woods trialled were between 6 and 18 ha in area. The area of each wood was measured from a satellite base map using a measure tool (Google My Maps 2017 to 2020). All woods were mature in age. Woods 1, 8, 9 and 10 contained broadleaf tree species only, woods 2 to 6 were broadleaf/conifer mixed and wood 7 was conifer only. Woods 1-8 and 10 were located in Yorkshire, England; within 25 km of York, England (53.96° N, −1.09° E). Wood 9 was located in Denbighshire, Wales, within 10 km of Denbigh (53º N, −3º W). All woods had relatively low connectivity to other woodland areas, sharing <10% of their boundary with other woodland areas, though woods 9 and 10 were connected to adjacent woods of equal or greater size. Wood selection was dependent on landowner permission and restricted to those that had a good level of access for field operatives but very low levels of public access

The RB bait methods successfully tested in Chapter 2 were used for this study. A concentration of 0.18% RB (CAS number 81-88-9, Sigma-Aldrich) mixed in 100% hazelnut butter (Bulk^{TM}) was deployed in each wood via the same design of bait hoppers developed and tested in Chapter 2. Hoppers were either deployed in summer (July/August) or winter (December/January) and were distributed approximately evenly across each wood, guided by accessibility to operators.

In winter 2017, hoppers were deployed at a density of 3/ha in two woods. In 2018, a balanced design was used to compare hopper densities of 1/ha and 3/ha for four woods in summer and two woods in winter. Based on the 2018 results, the hopper density was increased to 3/ha for two woods trialled in summer 2019 and summer 2020. To reduce access to bait by non-target animals, each hopper was mounted on a stand, approximately 1 metre in height (Figure 3.1). Hoppers were pre-baited with 40 g 100% hazelnut butter three times in one week. The following week, the hoppers were baited with 40 g of fresh RB bait per day on four consecutive days and the amount of bait consumed weighed after 24 hours. The hoppers were removed on the fifth day.

Figure 3.1. Grey squirrel using a metal bait hopper during a bait uptake study, recorded by a camera trap. Hoppers were fixed to 1-metre-high stands to help prevent access by nontarget animals.

Within three weeks of the hopper removal, squirrel live-capture cage-traps were deployed, attached to 1-metre-high wooden stands evenly distributed throughout each wood at a density of 3 traps/ha. The traps in each wood were wired open and pre-baited for three to 11 days, dependent on the availability of time and resources. Traps were then set and checked at least once every 24 hours. For animal welfare and health and safety reasons, traps were not set if heavy rain, snow, high winds or temperatures below 2°C were forecast. This meant that for winter surveys, traps were often set early morning and checked mid-late afternoon. Trapping was typically conducted over consecutive days, Monday to Friday until squirrel capture rates were reduced to an average of less than one per day over three consecutive days.

Squirrels were trapped and humanely dispatched using a Home Office approved (schedule 1) method by a trained and competent person. Each squirrel was given a unique ID, weighed, sexed, aged and health checked, and at least 20 hairs were sampled from the flank. The hair was stored at room temperature in transparent, sealed plastic bags. The total number of squirrels trapped was used to estimate the squirrel population size and density in each wood. The flank hair and whiskers from each squirrel were analysed for RB fluorescence using a UV microscope, as described in Chapter 2. The percentage of squirrels sampled from each wood that tested positive for RB were calculated and the most important factors affecting bait uptake for male and female squirrels, and female squirrels alone, as the target sex. I conducted the data management and analysis using Microsoft Excel and built Generalised linear models (GLM) using the MuMIn in the software R (R Core Team, 2019). GLMs were based on the global model:

```
glm(formula = cbind(RB.positive, RB.negative) ~ season + wood size + hopper density + squirrel density, family = “binomial”, data = dat, na.action = “na.fail”)
```

For each GLM, diagnostic checks of residual plots were used to confirm that they were approximately normally distributed and that model assumptions were met. For binomial models, overdispersion was measured using the parameter, theta. For male and female squirrels and for female squirrels alone, 16 candidate models were assessed and the most important factors affecting bait uptake were selected based on Akaike’s information criterion (corrected for small sample sizes; AICc) using the method recommended by Richards et al. (2008). Models were selected that had an AICc within six units of the model with the lowest AICc; however, those that had simpler, nested models with a lower AICc were excluded. A second global model, that replaced hopper density and squirrel density with squirrels per hopper, was also assessed to see if it described the data more parsimoniously.

3.4 Results

A total of 613 squirrels (320 females, 293 males) were trapped and sampled from ten woods. Between 55% and 88% of the squirrels trapped and sampled in six out of ten woods tested positive for RB (Table 3.1). In five of these woods, over half of the females tested positive. The best results were achieved in summer with the higher hopper density, with over 70% of the squirrels sampled in three woods consuming the bait.

In total, between 20% and 64% of the total amount of bait deployed in each wood was removed from the hoppers. Data from camera traps monitoring the hoppers (see Chapter 6) suggested this was achieved entirely by grey squirrels. Bait spillage was recorded on 14% of the total checks made to hoppers and in all cases the amount was ≤1 g. For 9 of 10 woods, 3 weeks of trapping and removing squirrels proved sufficient to reduce numbers caught to less than one squirrel per day over three consecutive days. For Wood 10, after 13 trap-days, limited human resources meant that the trapping was stopped at 1.3 squirrels per day for the last 3 days.

Table 3.1. The percentage of female and male and female grey squirrels that consumed bait containing rhodamine B from hoppers, placed in woodlands at a density of 1 or 3/ha in winter and summer, from the numbers trapped and removed until the rate of captures per day was less than one over three consecutive days.

Wood ID	Year	Season	Hopp. density (n/ha)	Size (ha)	N trap days	N squirrels trapped		Squirrels / hopper	Squirr. density (n/ha)	% RB positive	
						F	Total			F	Total
1	2017	Wint.	3	8	8	15	31	1.29	3.88	53.3	54.8
2				8	7	20	38	1.58	4.75	60.0	65.8
8	2018			10	6	22	39	1.39	3.90	45.5	43.6
7			1	14	15	105	207	14.79	14.79	8.6	8.7
5	2018	Summ.	3	6	7	12	22	1.22	3.67	75.0	77.3
6				10	9	8	17	0.57	1.70	62.5	88.2
9	2019			14	10	34	62	1.48	4.43	67.7	71.0
10	2020			15	13	53	105	2.33	7.00	22.6	19.1
3	2018		1	18	15	43	75	4.17	4.17	27.9	34.7
4				9	6	8	17	1.89	1.89	37.5	58.8

There was evidence from the trial at Wood 10 that minor modifications made to the hoppers, to incorporate the strain gauge device described in Chapter 2, may have reduced the accessibility of the bait to the squirrels, so that the majority of squirrels did not enter the hoppers during prebait and even fewer during the deployment of RB bait (88 squirrels in this study were microchipped prior to hopper deployment and their hopper access was monitored). In addition, 24 of the 45 hoppers deployed showed an increase in RB bait weights between 1 and 4 g on at least one trial day, which was not observed to the same extent in other trials. The most likely cause was the absorption of water due to high environmental humidity, which may reduce bait palatability by causing the RB to become more concentrated in parts of the bait.

In several respects, including the higher final trap rate and lower hopper acceptance by squirrels, wood 10 was an outlier. For that reason, the analysis of factors affecting uptake was conducted twice: once with wood 10 included and once with wood 10 excluded. For both males and females combined, and females alone, a model including just hopper density and season had the lowest AICc value for explaining bait uptake in nine populations of grey squirrels (Table 3.2; Figure 3.2).

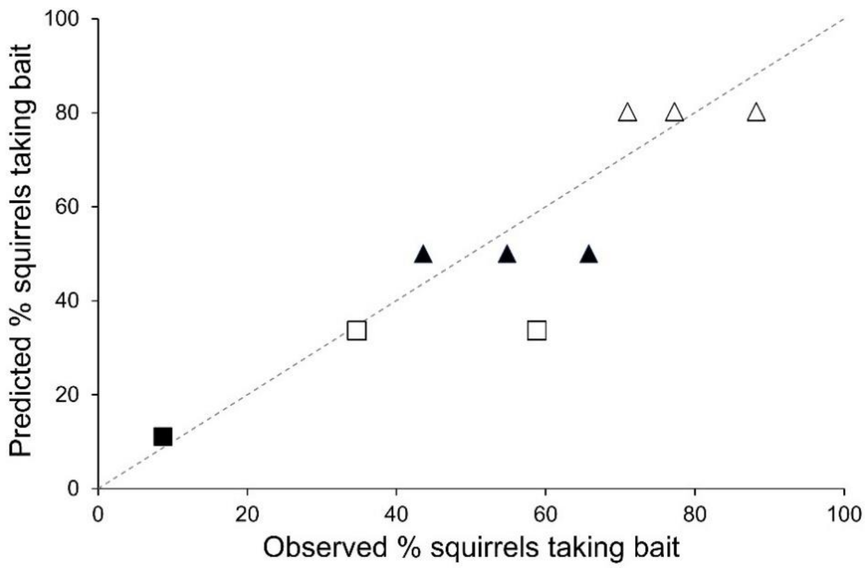

Figure 3.2 The percentage bait uptake recorded in nine woods for male and female grey squirrels in summer (white) and winter (black) and for three hoppers per hectare (triangle) and 1 hopper per hectare (square). Observed values are plotted against values predicted from the most parsimonious model (hopper density and season).

A model including hopper density and squirrel density performed similarly well (ΔAICc ≤ 1.26), suggesting that all three factors (squirrel density, hopper density and season) may play some role in determining bait uptake. The results were qualitatively similar when wood 10 was included for the male and female analysis, although season was not selected as an important predictor. Despite its strong similarity and lower parameter burden, a model including squirrels per hopper did not explain variation in squirrel bait uptake as well as a model containing both hopper density and squirrel density (Table 3.).

Table 3.2. GLM results from 16 candidate models based on global model 1 (season + wood size + hopper density + squirrel density) and 8 candidate models based on global model 2 (season + wood size + squirrels/hopper) testing factors affecting bait uptake in nine woodland populations of grey squirrels. Models with an AICc within 6 units of the model with the lowest AICc were selected for male and female squirrels and for female squirrels alone. Models that had simpler nested models with a lower AICc were excluded.

Subject	Global model	Terms	d.f.	AICc	ΔAICc	Weight	R^2
Males and females	1	Hopper density + Season	3	60.1	0	0.451	0.802
	2	Squirrels/hopper	2	60.9	0.8	0.302	0.724
	1	Hopper density + Squirrel density	3	61.3	1.2	0.247	0.764
Females	1	Hopper density + Season	3	44.0	0.0	0.646	0.898
		Hopper density + Squirrel density	3	45.2	1.2	0.354	0.838

3.5 Discussion

The aim of this study was to estimate the efficacy of delivering baits via feeders to woodland populations of grey squirrels and to determine the most important factors affecting population level bait uptake. This information can be used to develop an effective strategy to deliver an oral contraceptive to reduce numbers of grey squirrels in the UK.

Baits deployed for wildlife management purposes have the potential to affect nontarget species (Shore & Coeurdassier, 2018) and an oral contraceptive is unlikely to be species specific, therefore it is important that specificity is achieved via the bait delivery method (Massei & Cowan, 2014). The feeders (bait hoppers) used in this study have an outer metal case, have a weighted door on the entrance and were fixed to 1-metre-high stands, to reduce the number of non-target species that can access the bait. The hazelnut paste used as the bait was chosen deliberately for its attractiveness to squirrels and its thick, liquid consistency; making it difficult to remove from the hoppers, so that it will be consumed *in situ*. The minimal spillage recorded during the field trials suggests that this was probably the case and squirrels that did not enter hoppers are unlikely to have been able to access the RB bait. For contraceptive delivery, baits of similar properties deployed via hoppers of a similar design should only be available to animals that can access the hopper bait compartment. The species-specificty of the hoppers is more fully investigated in Chapter 6.

The hoppers proved very effective at delivering bait to grey squirrel populations, with the majority of squirrels in six out of ten woods consuming bait within four days. This included one wood where the bait delivery was conducted by trained volunteers and practitioners. To be effective for grey squirrel control, oral contraceptives will rely on the efforts of volunteer groups and practitioners to deliver them on a landscape scale. Working with volunteers on this study was therefore important, to demonstrate the practicality and robustness of the methods developed and to engage practitioners and volunteer groups in the wider oral contraceptive project.

In three woods surveyed in summer using three hoppers per hectare, over 70% of squirrels consumed bait from the hoppers. In woods where the squirrel to hopper ratio was <2, the average bait uptake was 66% (57% for female squirrels) and the three woods with the lowest bait uptake (8.7% to 34.7%) were the only woods where the squirrel to hopper ratio was higher than 2. It was clear from the RB model results that both hopper density and squirrel density are likely to play some role in determining bait uptake. It is possible that when there are too few hoppers per squirrel, some individuals monopolise the hoppers, as the grey squirrel is known to have a hierarchy of dominance, particularly at bait stations (Pack et al., 1967). The results from this study suggested that natural food availability was not a significant factor affecting bait uptake from hoppers by grey squirrels in summer or winter; therefore, if a vaccine required multiple doses within a year to be effective, provided that the numbers of squirrels per hopper was low enough, it should be possible to deliver bait to the majority of male and female squirrels in most woods within these seasons.

It is particularly important that females are targeted by contraceptive control as, like many other mammal species, the grey squirrel exhibits polyandry and fertility control models for other mammal species have demonstrated that a high proportion of females in a population need to be rendered infertile for fertility control to be effective (Massei & Cowan 2014). For a better understanding on how effective oral contraceptives could be for grey squirrel control, further research is required to estimate the frequency of feeding visits and the amount of bait consumed by individual squirrels, per visit to a hopper, the contraceptive dose rate required to render an individual squirrel infertile and the percentage of squirrels in a population that would need to be rendered infertile to achieve eradication within a reasonable timescale.

Landscape based grey squirrel models have suggested that an oral contraceptive would have to render approximately 75% of grey squirrels to provide a tool for effective control (Croft et al., 2021; Croft & Massei, 2023). The RB results suggest this threshold should be achievable, with further optimisation of delivery methods, however, the overall effectiveness of a contraceptive will also be determined by the proportion of individuals rendered infertile. What constitutes effective control is also subject to interpretation and is highly dependent on management goals. For example, there is some evidence that reducing grey squirrel densities to under 4 squirrels per ha, may significantly reduce tree damage (Beatham et al., unpublished data; Mayle & Broome, 2013). The data collected in this chapter could be used to further refine model parameters and improve model predictions regarding the infertility threshold required.

Season, hopper density and squirrel density were important factors affecting bait uptake, with a larger proportion of squirrels consuming bait in summer than in winter and with three hoppers rather than one hopper per hectare. Fitted models based on these factors explained large proportions of the variance in the proportions of grey squirrels that consumed bait from hoppers deployed in woods of between 6 ha and 18 ha in size and with varied tree composition and degrees of connectivity to other woodland. These preliminary results suggest that the relationship between bait uptake and the density of squirrels relative to the density of hoppers should remain constant across woodlands of different size and tree composition.

This study provided further evidence that RB can be used as an effective bait marker to measure bait uptake in mammals (Fisher, 1999). There was evidence from the captive and field trials that the RB bait had the potential to absorb water under some environmental conditions. The conditions during the bait trial in wood 10 were very humid and this may have caused the RB to become concentrated in parts of the bait, decreasing its palatability. RB bait has been found to decrease in palatability with increasing concentration in other studies (Fernandez & Rock, 2011). Any oral contraceptive will have to be designed to remain palatable under a range of environmental conditions.

In addition to possible bait palatability issues, the estimates of bait uptake recorded in this study are conservative, as bait was deployed for four days only and the number of squirrels removed might have included some animals that had moved into the woods after baiting ceased. The population size of each wood was measured from the number of squirrels trapped and removed until less than one per day were caught over three consecutive days. There will be some error in this estimate, as squirrels will begin moving into an area soon after removal begins. For example, in two 12 ha woods where squirrels had been completely removed, squirrel numbers returned to pre-cull levels in less than 10 weeks (Lawton & Rochford 2007).

During the trapping and removal of squirrels, a population estimate must ideally be taken before the last few, more difficult to catch squirrels (Croft et al., 2021), are outnumbered by the squirrels moving into the area. This is more likely to be achieved in woods that are reasonably isolated, as was the case with eight out of 10 woods studied. The movement of squirrels into the study woods was likely to be minimal due to the short time period (less than three weeks) between the deployment of rhodamine B-treated baits and the start of squirrel removal.

Future work should explore bait uptake in larger woods, with different natural food availability and methodological refinements, such as different distributions and densities of hoppers and the timing, frequency and longevity of bait deployment. This information could then be used to maximise bait uptake by squirrels and the cost effectiveness of population control via contraception.

3.6 Conclusion

This study demonstrated that, using purpose-designed bait hoppers, it is possible to deliver baits to the majority of grey squirrels in woodland environments and the same methods could be used to employ oral contraceptives to reduce grey squirrel numbers in the future. Season, hopper density and squirrel density were all important factors determining bait uptake. To understand how effective oral contraceptives could be for grey squirrel control, further research is required to estimate the frequency of feeding visits and the amount of bait consumed by individual squirrels per visit to a hopper, the contraceptive dose rate required to render an individual squirrel infertile and the percentage of squirrels in a population that would need to be rendered infertile to achieve eradication within a reasonable timescale.

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Chapter 4. An assessment of individual bait uptake by the grey squirrel

The main findings of this chapter are published in:

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4.1 Abstract

Understanding grey squirrel feeding behaviour is important when designing delivery methods, to ensure that a sufficient number of target individuals receive an effective contraceptive dose at a time of year that will guarantee their infertility throughout peak times of breeding. The main aims of this study were to assess how sex, season, squirrel density and bait point density influenced; 1. the probability of a squirrel visiting a feeder and 2. the amount of bait consumed from feeders. This study further investigated the findings from Chapter 3. Field trials were conducted on six woodland populations of squirrels in three seasons, with four days of rhodamine B bait deployment via purpose-designed squirrel-specific bait hoppers with integrated PIT-tag readers, developed and tested in Chapter 2.

In total, 58% of PIT-tagged females and 67% of PIT-tagged males were recorded visiting hoppers in six woods. Male and female squirrels visited hoppers multiple times and on most days of the four-day bait deployment, demonstrating that if oral contraceptives were deployed in baits, individuals that visited hoppers are likely to receive multiple doses within a short space of time. It was predicted that bait deployment would be more effective in spring, immediately before the second annual peak in squirrel breeding, followed by winter. The results from this study could be used to design methods for delivering oral contraceptive baits to grey squirrels in the future and the methods used could be applied to other small mammal species and other bait delivery systems.

4.2 Introduction

To maximise effectiveness, oral contraceptive delivery should predominantly target the females in a population and the majority of the females should be rendered infertile for the peak times in breeding (Massei, 2023; Massei & Cowan, 2014). Understanding grey squirrel feeding behaviour around bait devices is important so that delivery methods can be designed to ensure that a sufficient number of individuals receive an effective contraceptive dose. What constitutes an effective dose, and the longevity of its effect will be determined in laboratory trials. It is estimated that the development of an oral contraceptive and its registration for use in the UK will take a minimum of six years (UK Squirrel Accord, 2024). Within this time frame, it is important that a suitable bait delivery system is developed for trialling contraceptives in the field to ensure that they are effective and have minimal impact on nontarget animals.

As discussed in Chapter 3, bait uptake can be affected by several factors including sex, body weight, reproductive status, season, population density and bait point density. In Chapter 3, I found that a greater proportion of squirrels consumed bait when population sizes were lower and when three hoppers per hectare rather than one hopper per hectare were deployed. Grey squirrels can live at high densities. In UK woodlands, the average size of a grey squirrel home range is <5 ha (Wauters et al., 2002; Lawton & Rochford, 2007) and average grey squirrel density is reported to be between 4 and 5 squirrels/ha, ranging from less than 1 to over 13 squirrels/ha (Merrick et al., 2016; see Chapter 5).

In group-living rodents, such as the grey squirrel, high reproductive rates and high population densities may result in intraspecific competition for resources, particularly between individuals of different sex and breeding status. For instance, in California ground squirrels *Spermophilus beechey,* Whisson and Salmon (2009) found that a greater proportion of males visited bait stations than females and that males consumed more bait and made more visits to bait stations than females. Jacob et al. (2003) found higher bait uptake in breeding female house mice *Mus dometicus* versus other females, whereas Inglis et al. (1996) found that female Norway rats *Rattus norvegicus* made more feeding visits to bait than males.

The choice of season in which to deliver fertility control is particularly important if the duration of the effect of a contraceptive is relatively short, which would make it necessary to treat females immediately prior to mating. In grey squirrels, there are two main peaks in mating: December to January and April to May, with some females producing two litters within the same year (Hayssen, 2016). Therefore, it may be necessary to deploy a contraceptive in different seasons and it is important that levels of bait uptake are sufficient across these seasons, to ensure fertility control is effective.

The bait hoppers and bait marker methods developed in Chapter 2 were trialled in two grey squirrel populations in winter 2017/2018, two populations in summer 2022 and in two populations in spring 2023. Over four days of bait deployment, using hazelnut paste mixed with 0.18% rhodamine B (RB), the duration of visits to hoppers and the amount of bait taken per visit were measured for individual male and female squirrels in different seasons and at different squirrel and hopper densities.

The main aims of this study were to assess how sex, body weight, season, squirrel density and bait point density influenced: 1) the probability of a squirrel visiting a feeder: and 2) the amount of bait consumed from feeders. The findings from this study will inform systems for delivering oral contraceptive baits to grey squirrels in the future and the methods used could be applied to other small mammal species and other bait delivery systems.

4.3 Materials and methods

Ethical statement

This study was conducted by trained Home Office Personal Licence holders under the advice of the Named Veterinary Surgeon and under a UK Home Office licence, in accordance with the Animals (Scientific Procedures) Act 1986. The study was approved by the joint Animal and Plant Health Agency (APHA) and Fera Science Ltd Animal Welfare and Ethical Review Body (AWERB).

Field trial assessing factors affecting feeding visits and bait uptake by grey squirrels

For the trials in this chapter, I led the study design and analysis and I conducted most of the field work and sample analysis, with assistance from Animal and Plant Health Agency (APHA) colleagues that I had trained in the methodology or that were already experienced in some of the techniques. Trials were conducted on six grey squirrel populations in five independent woods (one wood was trialled twice) that were located at least 3.5 km apart and within 25 km of York, England (53.96ºN, -1.09ºE; Figure 4.1). Populations of grey squirrels were sampled from woods HW and SC in winter 2017/2018, woods SC and PW in summer 2022 and woods GE and BW in spring 2023. Winter and spring were selected as suitable seasons to deploy a contraceptive, as they coincide with the peaks in grey squirrel breeding (Hayssen, 2016); summer was selected as a season when good levels of bait delivery via feeders can be achieved, based on the findings of Chapter 3. Autumn was not trialled due to reduced squirrel breeding activity and high natural food availability, which could deter squirrels from using feeders (Gurnell, 1996)

To provide two suitable replicates for each season, woods were selected that were of a similar size (between 7 and 8 ha) and structure (mature broadleaf or mature broadleaf conifer mixed tree species), that had high levels of grey squirrel activity reported by landowners and established squirrel populations, with minimal squirrel control conducted within the previous 12 months. At the end of each trial, the squirrel population was trapped and removed from each wood as part of other related studies and for wildlife management purposes. Therefore, woods could not be re-used for trials that were within 12 months of each other. The squirrel population at wood SC was removed in January 2018, and the wood recolonised by squirrels. SC was reused in Summer 2022, as another suitable wood could not be found in the timeframe necessary. Thus, it was considered that six independent populations of squirrels were assessed.

All woods had relatively low connectivity to other woodland areas, sharing between 5 and 39% of their boundary with other woodland areas (Figure 4.1). Wood selection was dependent on landowner permission and restricted to those that had a good level of access for field operatives but very low levels of public access. Some of the methods employed by this study had to be undertaken by Home Office licensed personnel and required specialist equipment and significant time and resources. Due to these limitations, it was not possible to trial more than two woods for each season.

Single-catch squirrel cage traps (density = 3/ha, n = 21-24) were deployed across the whole area of each wood on 1-metre-high wooden stands and pre-baited with a combination of maize, peanuts, a small number of whole hazelnuts and approximately 2 grams of 100% hazelnut paste

(Bulk™, UK) every 2 to 4 days for between 3 and 13 days, dictated by human resource availability.

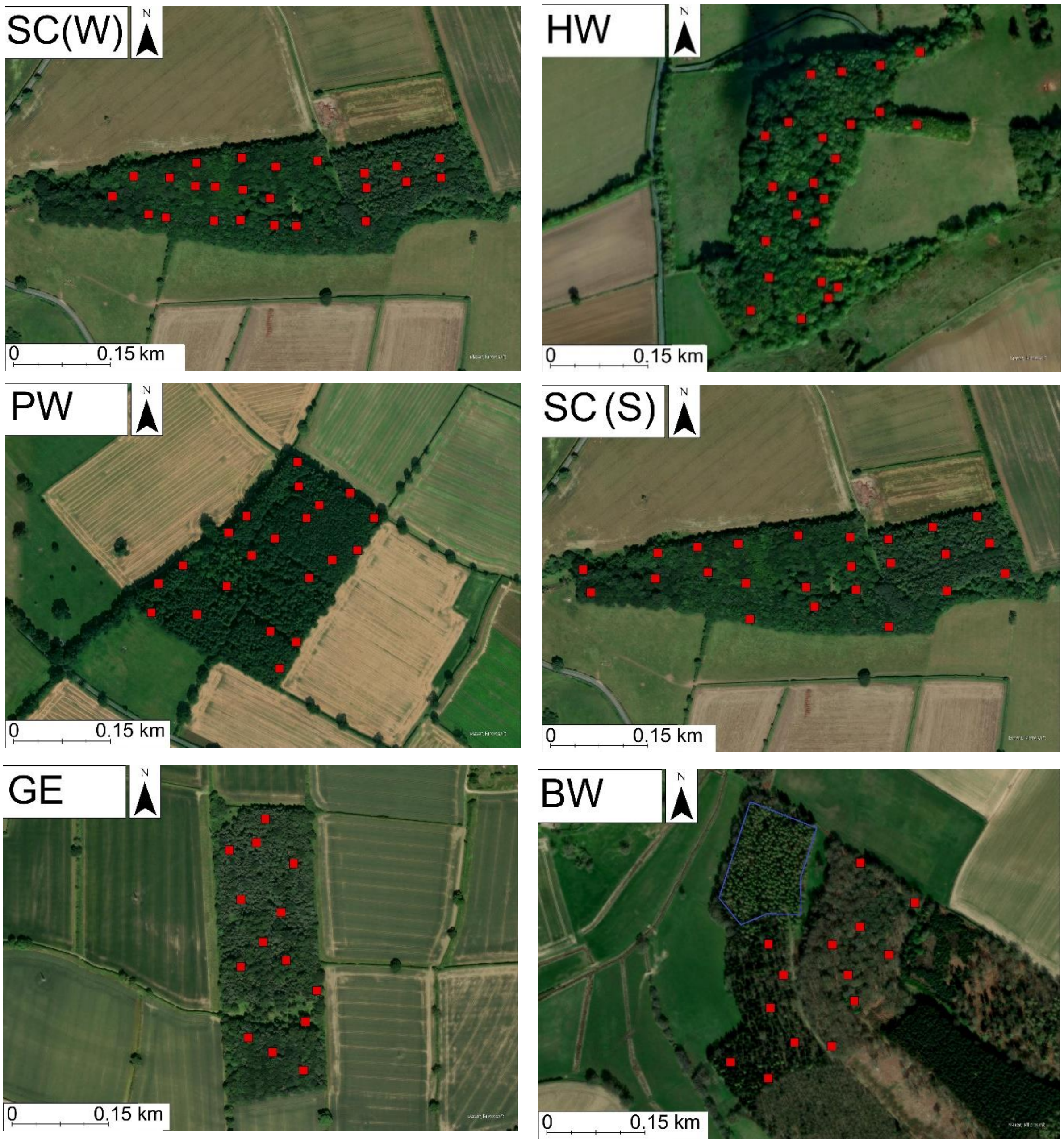

Figure 4.1. Woods where field trials were conducted to assess individual bait uptake by grey squirrels. HW and SC(W) were assessed in winter 2017/2018, PW and SC(S) in summer 2022 and GE and BW in spring 2023. Red squares show the locations of bait hoppers deployed for four days to monitor the feeding behaviour of squirrels via integrated PIT-tag readers. Woods HW, SC, PW and GE shared less than 5% of their boundary with other woodland areas, while for BW this was 39%. The wooded area to the north of BW (blue outline) had been deforested over a year before the trial commenced.

For animal welfare and health and safety reasons, traps were not set if heavy rain, high winds (>30 mph) or high (>30^{o}c) or low (<2^{o}c) temperatures were forecast. Traps were partly covered with a waterproof sheet to provide animals with shelter. Traps were set early morning and checked in the afternoon or set late afternoon and checked the following morning.

Trapped squirrels were anaesthetised on site using isoflurane via a mask and a PIT-tag (Identichip®, York, UK) implanted subcutaneously in the scruff of the neck. Squirrels were then sexed, weighed (to the nearest gram) and recorded as adult or sub-adult (<450 g, slim, soft pelage, body lacking muscle, head large in proportion to body and female nipples or male testes not visible). Females were assessed for evidence of recent breeding (extended or lactating nipples and palpable foetuses). Hair was clipped from the tail for visual identification. Once recovered from anaesthesia, squirrels were released under a Natural England licence in the location at which they had been trapped. Trapping was conducted for 3 to 4 days, completed within 18 days; the traps were then removed.

In spring and summer, within two days of PIT-tagging, hoppers were deployed at each wood, while in winter, hoppers were deployed within four weeks of PIT-tagging (Figure 4.1). Hoppers were deployed evenly across the whole area of each wood, with locations guided by a 1 ha grid generated in ArcGIS (version 10.7.1) overlayed onto a satellite map using the ArcGIS Collector mobile phone application. Locations were, however, adjusted according to accessibility; for example, steep slopes or thick vegetation were avoided. Each hopper was fixed to a 1-metre-high wooden stand to reduce nontarget access. In winter and summer, hoppers were deployed at a density of 3/ha (n = 21-24). Squirrel hopper use data from these studies suggested that lowering the hopper density to 2/ha could be more cost-effective for bait delivery in terms of field hours and bait quantity required. This was tested in spring 2023, using a Before-After-Control-Impact (BACI) study design (Fenn et al., 2020). At woods GE and BW, hoppers were deployed at a density of 2/ha (N = 14) and baited for four days, then increased to 3/ha (N = 21) at BW and maintained at 2/ha at GE for an additional four days each.

For all woods, to simulate the deployment of a contraceptive, each hopper was pre-baited with approximately 40 g of hazelnut paste every 2-3 days for 6-7 days, with the bait doors propped open, to encourage the squirrels to feed from them. Each hopper was then baited daily with 40 g of hazelnut paste mixed with the bait marker dye RB **(**Merck Life Sciences UK Limited, Dorset, UK) at a concentration of 0.18% for four consecutive trial days in winter and summer and eight consecutive trials days in spring. The bait was manually weighed in and out of the hoppers each day, and the hopper entrance and stand checked for evidence of bait spillage, easily identified due to the pink colouration of the bait. Any spillage found was weighed.

In the summer and spring trials, hoppers were also fitted with a strain gauge, which recorded the weight of the bait taken for each squirrel visit (see Chapter 2). Hoppers were monitored with Reconyx™ HC500 or HS2X remote cameras, and the footage analysed at times when the bait doors were opened, to identify animals feeding from them, including any nontarget animals (see Chapter 6).

Within 4 days of hopper deployment in spring and summer, and within 21 days of hopper deployment in winter, squirrel live-capture cage-traps were installed in each wood at a density of 2-3 traps/ha. Traps were secured to the same 1-metre-high wooden stands used in the hopper trials. Trapping was conducted using the same protocol as the trapping for the PIT-tagging though, for this session, traps were set and checked at least once every 24 hours where possible. If weather conditions did not permit trapping overnight, traps were set early morning and checked late afternoon the same day. Trapping was conducted within a four-week period, typically Monday to Friday, for a minimum of 5 days and for a maximum of 14 days, or until squirrel capture rates were reduced to an average of less than one per day over three consecutive days.

The short trapping period was designed to minimise the movement of grey squirrels into the study woods from other areas once removal began. Trapping effort was high, short-term and conducted across relatively discrete squirrel habitats and across an area larger than the average squirrel home range (less than 5 ha; Wauters et al., 2002). A high level of demographic population closure should therefore have been achieved. Lawton & Rochford (2007) confirmed through capture mark-recapture that most, if not all, grey squirrels in a population could be trapped within five days using a similar trapping regime. The number of squirrels trapped was therefore used as a proxy for the total number of squirrels in each wood that were available to visit hoppers, as per Chapter 6. As a comparison, the number of squirrels was also calculated using the closed-population capture mark-recapture (CMR) Schnabel method, from the capture records taken from the 3-4 days of PIT-tagging conducted at each wood (Mares et al., 1981).

Squirrels that were trapped were humanely dispatched using a UK Home Office approved (Schedule 1) method, by a trained and competent person, and the PIT-tag ID, sex and tail clip (if present) were recorded and at least 20 hairs were taken from the flank, placed in a plastic sample bag, to be later analysed using a Leica DMLB ultra-violet microscope (Leica Microsystems UK Ltd) at x 4 magnification for the presence or absence of RB fluorescence.

Data analysis

Data management and analysis was conducted using Microsoft Excel® and R (R Core team, 2023). All Generalised linear models (GLM) used were assessed using the MuMIn package (Bartoń., 2024) and for each model, the relative significance of the fixed effects linked through interactions was assessed through pairwise comparisons of estimated marginal means using the Emmeans package (Lenth, 2024). Diagnostic checks of residual plots were used to confirm that were approximately normally distributed and that model assumptions were met. For binomial models, overdispersion was measured using the parameter, theta.

From the PIT-tag data collected by hoppers, for each individual squirrel a dataset was created containing site, season, sex, body weight (g), date, time, PIT-tag ID and hopper ID. Consecutive PIT-tags recorded for the same individual at the same hopper were categorised into visits. The duration of a visit was defined as the time between the first PIT-tag read and the last PIT-tag read for that individual visit. A visit was deemed complete once the PIT-tag of a new individual was recorded, or there was more than a 2-minute gap until the next PIT-tag record from the same individual. The 2-minute time frame was used to standardise the data, as the hoppers used in different years had different levels of PIT-tag detection sensitivity and squirrels would often enter hoppers multiple times in quick succession during the same visit. When only one PIT-tag read was recorded for an individual, a minimum visit duration of 1 second was applied.

The effect of hopper density on hopper use and bait uptake by grey squirrels in spring

To assess whether reducing the hopper density to 2/ha in spring 2023 affected hopper use and bait uptake by individual squirrels at woods GE and BW, the PIT-tag records from squirrels recorded by hoppers deployed at both woods were analysed using a BACI framework, as described in Fenn et al. (2020). The variable 'Site' represented treatment, with GE assigned as the control site and BW as the treatment site. The variable 'Period' was defined as 1 (before treatment; trial days 1-4, hopper density set at 2/ha at GE and BW) and 2 (after treatment; trial days 5-8, hopper density maintained at 2/ha at GE and increased to 3/ha at BW). Binomial GLMs were used to test how the fixed effects wood, period, sex and number of squirrels per hopper influenced the probability that a PIT-tagged squirrel was recorded visiting a hopper (yes/no) in each time period. Squirrels per hopper was calculated from the total number of squirrels (PIT-tagged and untagged) trapped and removed in each wood divided by the number of hoppers deployed for that time period. It was included in the model as it was hypothesised that there may be increased competition between individual squirrels in higher density populations, potentially resulting in a reduction in hopper use by some squirrels. The inclusion of squirrel ID as a random factor was considered, using a generalised linear mixed model structure, however, it was found that the datasets were too small to support this. The GLM model structure was, thus:

```
glm(Visited ~ Period*Site + Sex + squirrels_hopper, family="binomial"(link = cloglog), data = dat_spring, na.action=na.fail)
```

For PIT-tagged squirrels that were recorded visiting at least one hopper in each of the study woods, a gaussian GLM with a log link was used to assess the influence of the fixed effects period, sex and site on the quantity of bait uptake by each squirrel, inferred from the total time in minutes (duration) that each squirrel was recorded visiting hoppers. Collinearity between squirrels per hopper and the period/site interaction meant that squirrels per hopper was not included in this model. ANOVAs were used to test whether variation in hopper use or bait uptake was better explained by models that included an interaction term between period and site (indicating a hopper density effect) than those that did not.

Factors affecting hopper use and bait uptake by grey squirrels in different seasons

Binomial GLMs were used to test the fixed effects of season, squirrel body weight, sex and the number of squirrels per hopper on the probability of a PIT-tagged squirrel entering a hopper (yes or no) during trial days 1-4 in each wood. Body weight was included, as it was hypothesised that larger individuals may outcompete smaller ones when accessing bait. The model structure was:

```
glm(Visited ~  Bodyweight_g*Sex + Bodyweight_g*Season + Sex*Season + squirrels_hopper, family="binomial"(link = cloglog), data = dat_spring, na.action=na.fail)
```

Interactions were included for body weight and sex, and body weight and season, as it was hypothesised that body weight may have been influenced by these factors. An interaction also was included between season and sex, based on the evidence that female squirrels may visit hoppers less during periods of breeding when they are less active and are spending more time in dreys with dependent young. Age and female breeding status were not included in the model, due to seasonal variation in the ability to accurately assess these factors from appearance alone, which might have caused underestimates in the number of breeding females and sub-adults. The percentage of trapped squirrels that, through the analysis of their hair samples, tested positive for the bait marker RB in each wood was used as a comparative measure of hopper use, as per Chapter 3.

For PIT-tagged squirrels that were recorded visiting at least one hopper, a gaussian GLM with a log link was used to test the effect of season, sex and number of squirrels per hopper on the total amount of time individual squirrels spent visiting hoppers during the four trial days. The data did not support the inclusion of body weight as a fixed effect (the model including body weight produced much larger standard errors for both the sex and season estimates; Supplementary Material) however, this was investigated separately. Time spent visiting hoppers was used as a proxy for the amount of bait consumed by squirrels, as bait weight data were only collected in summer and spring.

Time spent in hoppers vs weight of bait taken for individual squirrels

To assess whether time spent visiting hoppers by individual squirrels was directly related to amount of bait taken, the weight of bait taken was recorded for each hopper visit. This was calculated from the combined weight of the bait and bait tray recorded at the time of the first PIT-tag read minus the weight recorded at the time of the final PIT-tag read. The weights taken at the precise time of the reads were often unstable, due to spikes in weight readings caused by squirrels exerting force on the bait trays with their bodies as they fed. Thus, for each visit, the stable weight (at least three repeat values) closest to the time of the first PIT-tag read, or immediately prior to the first associated spike in weight, was selected as the start weight and the stable weight closest to the time of the final PIT-tag read or immediately after the associated spike in weight, was selected as the final weight. If a stable weight was not recorded within 20 seconds of a PIT-tag read, a null entry was recorded.

The total weight of bait taken during the four trial days by each individual squirrel from the four woods surveyed in summer and spring (PW, SC, GE and BW) was compared with the time each individual spent visiting feeders (the sum of time of last PIT-tag read minus time of first PIT-tag; read across all visits). Data were only included from squirrels if they did not have significant amounts (>10% of values) of weight data missing, due to weighing errors or hopper failures. As data were not normally distributed and contained multiple tied observations, a Kendall's Tau correlation analysis was used to assess whether there was a significant relationship between weight of bait taken and time spent visiting hoppers.

4.4 Results

The effect of hopper density on hopper use and bait uptake by grey squirrels in spring

In total, 66 squirrels (25 male, 41 female) were PIT-tagged in woods GE and BW in spring 2023 (Table 4.1). The hoppers across both woods recorded visits by 10 males and 26 females during trial days 1-4 and 14 males and 26 females during trial days 5-8. Two new males recorded in period 2 came from GE and two came from BW. There was a high resight rate with 94% of the individuals recorded in time period 1 also recorded in time period 2. During trial days 1-4, male grey squirrels at GE and BW spent a median of 39 min (IQR =33 min) and 50 min (IQR = 45 min) visiting hoppers respectively, compared with 38 min (IQR = 19 min) and 35 min (IQR = 36 minutes) during trial days 5-8. Female grey squirrels at GE and BW spent a median of 57 min (IQR = 117 min) and 29 min (IQR = 59 min) visiting hoppers during trials days 1-4, compared with 62 minutes (IQR = 117 minutes) and 53 minutes (IQR = 37 minutes) during trial days 5-8.

There was no evidence that hopper density (2/ha versus 3/ha) influenced the probability that an individual squirrel would visit a hopper or the time they spent visiting hoppers, with no significant difference between GLMs that included an interaction between site and time period and those that did not; $\chi^2_{2,\,125} = -0.974$, $p = 0.615$ and $\chi^2_{2,72} = -176.25$, $p = 0.747$ respectively (Supplementary Material). Site was the only variable that significantly affected hopper use, with tagged squirrels at GE significantly more likely to enter hoppers than tagged squirrels at

BW (Supplementary Material; $z_{4,127} = 3.66, p = < 0.001$). This was supported by the RB analysis that showed that 56% of the 41 squirrels trapped at GE and 38% of the 91 squirrels trapped at BW had fed from hoppers (Table 4.1).

Table 4.1. The number of grey squirrels PIT-tagged in five woods in winter, summer and spring, including the number of tagged males (M) and females (F), the percentage that entered bait hoppers within a four day bait deployment, the number of squirrels that tested positive for the bait marker RB, the number that were trapped and removed, and number of squirrels per hopper; CMR Schnabel estimate (Mares et al., 1981) or number trapped divided by number of hoppers deployed.

Season/ Year	Wood ID	No. PIT-tagged			No. visited hoppers				No. trap-ped	% +ve RB	% tagged trapped	No./ hopper	
		M	F	Total	M	F	Total	%				Est.	Trap
Winter 2017-2018	HW	9	12	21	8	10	18	86	31	55	90	0.9	1.3
	SC	15	14	29	14	12	26	90	38	66	86	1.4	1.6
Summ. 2022	PW	19	23	42	13	11	24	57	35	57	60	2.4	1.7
	SC	20	24	44	14	7	21	48	32	63	45	2.0	1.3
Spring* 2023	GE	9	15	24	6	13	19	79	41	56	100	2.6	2.9
	BW	16	26	42	4	13	17	40	91	38	90	5.8	6.5
All woods		88	114	202	59	66	125	62	271	56	79	2.4	2.2

*In Spring 2023 PIT-tag records were monitored for 4 days, RB bait was deployed for 8 days

The binomial and gaussian GLMs performed significantly better than the respective null models (Supplementary Material; $X^2_4 = 21.91$, $P < 0.0001$ and $X^2_4 = 21.91$, $P < 0.0001$). Residual plots indicated a good model fit for the binomial GLM, while the gaussian GLM data were right skewed. When the 8 highest data points were removed (4 from each time period) and the analysis repeated, the residuals were normalised and the initial model results confirmed (Supplementary Material).

Factors affecting hopper use and bait uptake by grey squirrels in different seasons

In total, 202 squirrels (88 male, 114 female) were PIT-tagged in six woods (Table 4.1). Out of the 202 individuals, 7 were assessed as sub-adult (spring = 5, summer = 1, winter = 1) and 23 females exhibited evidence of recent breeding (spring = 16, summer = 4, winter = 3). In winter and spring, at least 86% of the squirrels PIT-tagged were recaptured during the removal exercise, compared with 38-52% in summer. After between 7 and 14 days of trap and remove, the squirrel capture rate at woods SC (both seasons), HW, GE and BW were reduced to less than 1 per day over 3 consecutive days and 80-98% of the squirrels trapped were caught within the first 5 days. After 14 days of trapping at PW, the capture rate was 1 per day, with 51% caught within the first 5 days. Trapping at PW and SC during summer 2022 had to be postponed due to a heat wave and temperatures were higher than average UK summer temperatures throughout the trapping period. For each wood, the number of squirrels per hopper calculated from the trap records were within 0.7 squirrels per hopper of those calculated from the CMR method (Table 4.1). Out of the 271 squirrels trapped, 56% tested positive for the bait marker RB, between 38% and 66% per wood (Table 4.1).

Out of 121 hoppers deployed for the six field trials, during the four trial days, 111 hoppers recorded 1,676 visits by PIT-tagged squirrels. Of the 10 hoppers that did not record any visits, 6 hoppers that were deployed in summer did not record any PIT-tags during the trial days, due to electronic faults. Bait spillage was found on 16% of the 484 checks made to hoppers, with most quantities recorded at <1 g spilled and no more than 2 g recorded at any one check. A total of 114 hoppers were analysed for nontarget access to the bait.

Overall, 73% of PIT-tagged squirrels visited hoppers during the pre-bait period, compared with 62% (58% of PIT-tagged females and 67% of PIT-tagged males) during the trial period. The proportion of female squirrels that visited hoppers was much lower in the two woods surveyed in summer (29% and 48%) than those surveyed in winter (86% and 90%) and spring (50% and 87%). A total of 27 individuals visited hoppers during pre-bait only and 6 individuals visited during the trial period only. Most of the sub-adult squirrels (6 out of 7) and breeding females (15 out of 23) visited hoppers during the trial period. During the four trial days for all woods, 59 PIT-tagged male squirrels recorded by hoppers made a median of 14 visits (IQR = 12) to 5 different hoppers (IQR = 3) for a total of 42 minutes (IQR = 31 minutes). In comparison, 66 PIT-tagged females recorded by hoppers made a median of 11 visits (IQR = 14) to 3 different hoppers (IQR = 3) for a total of 31 minutes (IQR = 47 minutes). In total 93% of male squirrels and 71% of female squirrels that visited hoppers did so on at least three separate dates during four trial days.

PIT-tagged male and female squirrels within the same season that did and did not visit hoppers were not significantly different in body weight (Figure 4.2). The median body weights for male and female squirrels that entered hoppers during the trial period was 533 g (IQR = 125) and 564 g (IQR = 72) respectively, and 524 g (IQR = 89) and 568 g (IQR =83) for males and females that did not. Binomial GLM analysis found that body weight was not a significant factor determining whether a squirrel visited a hopper (Supplementary Material; $z_{10, 191} = 0.540$, $p = 0.589$).

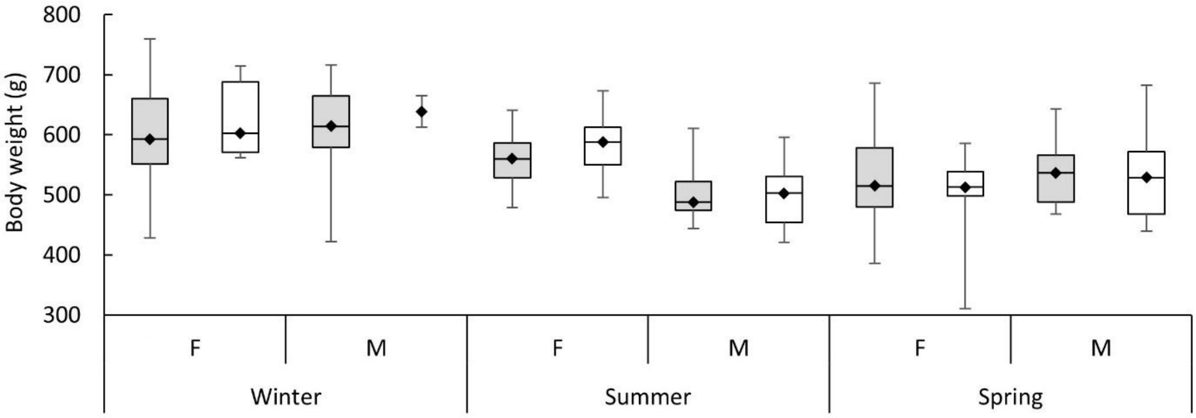

Figure 4.2. Body weights (g) of 88 male and 114 female PIT-tagged grey squirrels that were (grey boxes) or were not (white boxes) recorded visiting bait hoppers deployed in two woods in winter 2017/2018, two woods in summer 2022 and two woods in spring 2023. Median values are shown by diamonds, interquartile ranges (50% of the records for each group) are shown by boxes and minimum and maximum values are shown by whiskers

In summer, females were more likely to weigh more than males (Figure 4.2) and 13 of the 14 heaviest females PIT-tagged were not recorded visiting hoppers. The number of squirrels recorded for each sex and season group varied considerably for those that did and did not visit hoppers (2-29 squirrels). As a result, the inclusion of body weight in the model produced much larger standard errors for both the sex and season estimates (Supplementary Material). The analysis was therefore repeated with only the fixed factors of season, sex and squirrels per hopper, with an interaction between season and sex. The reduced model did not significantly differ in fit compared with the model including body weight ($\chi^2_{4, 195} = -0.263$, $p = 0.621$) and fitted the data significantly better than the null model ($\chi^2_6 = 43.89$, $p < 0.0001$).

The reduced binomial GLM predicted that the probability of a male or female squirrel visiting a hopper would significantly decline, in most scenarios by more than 25%, with an increase from 2 to 4.5 squirrels per hopper (Supplementary Material; Figure 4.3 a, $z_{6,\,195} = -3.989$, $p < 0.01$). Spring was the only season in which it was predicted that female squirrels would be more likely to visit hoppers than male squirrels. Squirrels were significantly less likely to visit hoppers in summer than spring ($z_{6,\,195} = -3.989$, $p < 0.0001$) and this was largely explained by the prediction that females were significantly more likely to visit hoppers than males in spring compared with summer ($z_{6,\,195} = -1.739$, $p < 0.0001$).

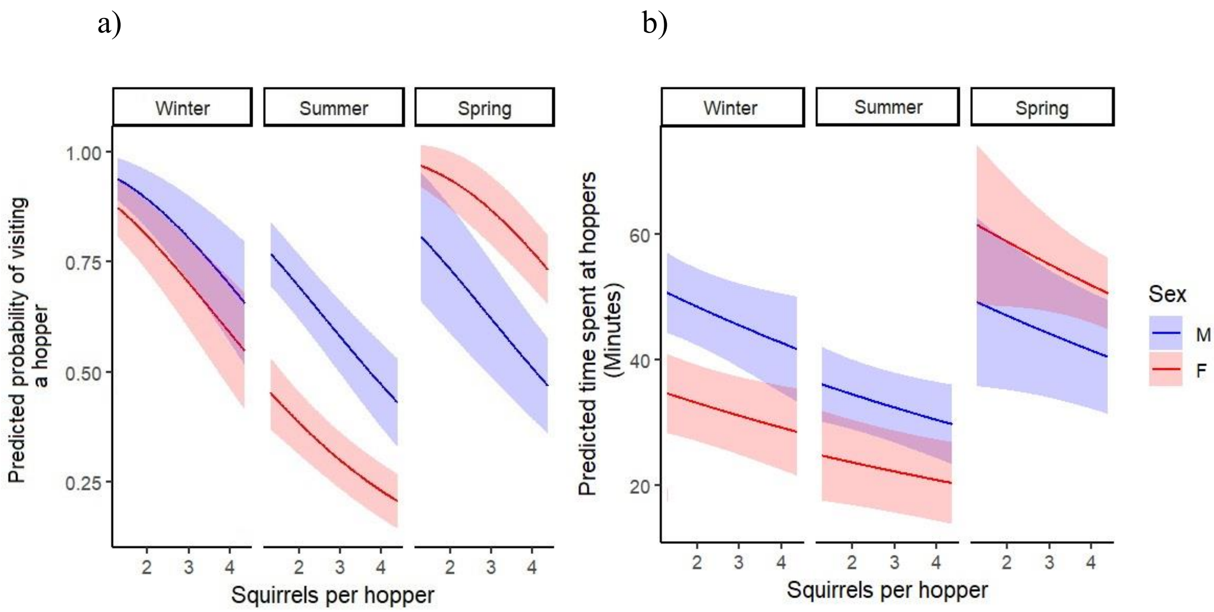

Figure 4.3. GLM predictions for the average probability (and associated SE) that male and female squirrels will enter a bait hopper (a) and the average time spent visiting bait hoppers (b) within four trial days in different seasons and for increasing numbers of squirrels per hopper. Data were collected in winter 2017/2018, summer 2022 and spring 2023 from 202 grey squirrels PIT-tagged in six independent woodlands (2 woods surveyed per season). A total of 21 to 24 hoppers per wood (3 hoppers/ha) were deployed in the winter and summer and 14 hoppers per wood (2 hoppers/ha) in spring.

The gaussian GLM predicted that squirrels would spend significantly more time visiting hoppers in spring than in summer (Supplementary Material; Figure 4.3 b; $t_{6,\,118} = -3.989$, $p < 0.0001$). No other variables were predicted to have a significant effect, though time spent visiting hoppers appeared to decrease with increasing numbers of squirrels per hopper. The data did not support the addition of body weight as a variable in the model. There was no obvious relationship between time spent visiting hoppers and squirrel body weight (Figure 4.4), however, any trends present are likely to have been masked by between-sex and between-season variation. Only 8 squirrels spent longer than 100 minutes visiting hoppers during the trial days: 7 females in spring and 1 male in winter.

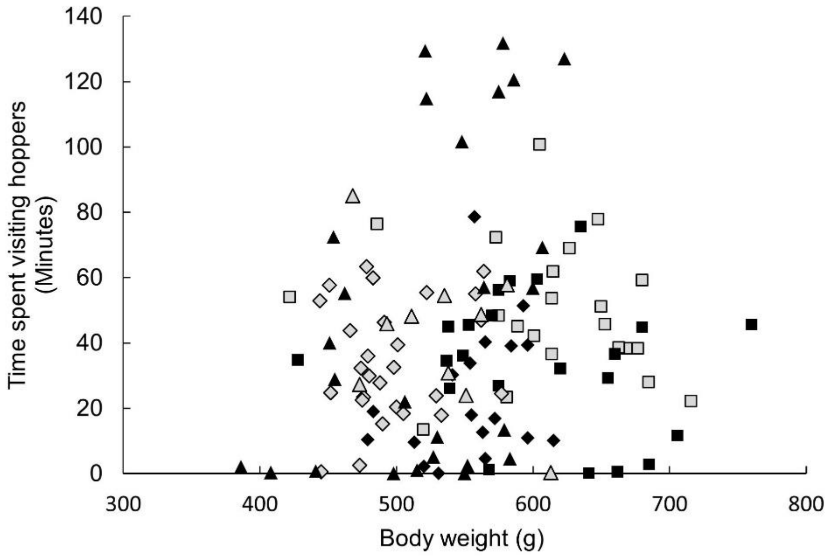

Figure 4.4. The relationship between body weight (g) and time spent visiting hoppers in four days for 59 male (black) and 66 female (grey) PIT-tagged grey squirrels. Data were collected in winter 2017/2018 (square), summer 2022 (diamond) and spring 2023 (triangle).

Duration of hopper visits versus weight of bait taken

Data for total bait taken in four days were recorded for 67 individual squirrels, including 15 females and 23 males monitored in summer 2022 and 19 females and 10 males monitored in spring 2023. There was a strong positive relationship between the number of seconds individuals spent visiting hoppers and the weight of bait taken per visit ($\tau = 0.815$; $p < 0.001$) with individuals taking an average of 1 g of bait per minute spent at hoppers (Figure 4.5). On average, female squirrels took 46.5 g of bait, with a maximum of 173.1 g, while male squirrels took an average of 38.8 g of bait, with a maximum of 83.2 g.

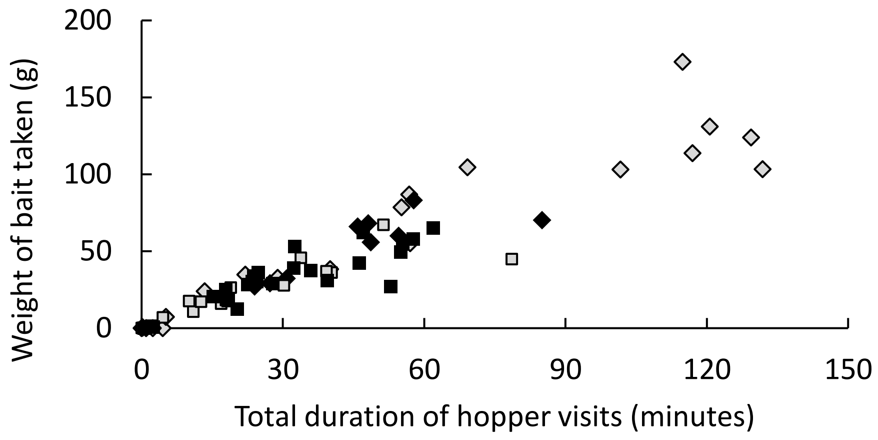

Figure 4.5. A comparison of the weight of bait taken from hoppers and duration of hopper visits in four trial days for 67 PIT-tagged male (black) and female (grey) grey squirrels. In total, 21-24 hoppers were deployed per wood in two woods in summer 2022 (diamonds) and 14 hoppers per wood were deployed in two woods in spring 2023 (squares). There was a strong positive relationship between the number of seconds individuals spent visiting hoppers and the weight of bait taken per visit ($\tau = 0.815$; $P < 0.001$) with individuals taking an average of 1 g of bait per minute spent at hoppers.

4.5 Discussion

This study assessed factors affecting bait uptake by six populations of grey squirrels, by monitoring the feeding behaviour of PIT-tagged squirrels using purpose-designed bait hoppers. I discuss these findings in relation to managing grey squirrel populations in the future using oral contraceptives in baits. The amount of bait taken by squirrels was significantly associated with the time that squirrels spent visiting hoppers in four woods assessed in spring and summer and was therefore used to infer the amount of bait taken from hoppers by squirrels in winter in a comparative analysis.

The bait hopper design used in this study was effective at delivering bait to grey squirrels in the six woodlands trialled, with 58% of PIT-tagged females and 67% of PIT-tagged males recorded visiting hoppers. This is consistent with grey squirrel average bait uptake results measured using the bait marker RB in this study (56%) and the trials conducted in Chapter 3, in three woods in summer and three woods in winter (67%).

For a contraceptive to be effective, particularly if the effects on fertility are limited to a few months, ideally it will be delivered to reproductively active female grey squirrels in early December and May, to ensure animals are infertile immediately prior to the peak mating periods (Hayssen, 2016). In the current study, female squirrels were more likely to visit hoppers and male and female squirrels were more likely to consume more bait in spring, followed by winter then summer.

Summer, along with spring, is a peak time for litter production for grey squirrels and the relative proportion of females that produce litters in spring, summer or both will be dependent on food availability related to the seed mast crop of the previous autumn and the severity of the previous winter (Gurnell, 1996). In good seed years, grey squirrel reproduction will start in winter, in bad seed years, it will be delayed until the following spring. The grey squirrel diet predominantly consists of tree seeds from oak, beech, hazel. In autumn 2021, the UK based Tree Council (2021) reported one of the lowest oak seed (acorn) crops in 7 years. It is therefore likely that squirrels will have cached less food over winter and may have delayed their breeding until summer 2022, when natural food availability improved. At the time of the summer 2022 PIT-tag trial, it is feasible that a greater number of females may have had reduced activity and thus less likely to feed from hoppers, as a result of having dependent young in the drey. This is supported by the fact that the heaviest females that were PIT-tagged at both woods in summer 2022, were not recorded by the hoppers 1 to 2 weeks later. The best female bait uptake results (62-75%) in the bait marker study were found in three woods in the summers of 2018 and 2019, when it is possible that there were fewer females breeding.

Recapture rates of male and female PIT-tagged squirrels in summer 2022 were lower than in winter and spring, while the RB bait uptake analysis was very similar to both winter woods and one of the spring woods. This suggests that an increase in movement of squirrels into and out of the study woods may have also contributed to the reduced hopper access by PIT-tagged squirrels. The trapping at both summer woods was delayed due to a heat wave and this may have motivated squirrels to move to another location. Overall, the findings of this study suggest that summer may not be the best time of year for bait deployment, due to inconsistencies in movement, activity and bait uptake amongst squirrel populations, particularly for female squirrels. Autumn was not included in the study as this is when tree seeds are at their most abundant, so supplementary feeding is unlikely to be successful and trapping rates are typically very low (Gurnell, 1996). In winter and early spring, many of the squirrel's main natural food resources are depleted and they are reliant on cached food (Steele & Wauters, 2016). This may explain why the squirrels in this study were highly likely to enter hoppers in winter and spring. With the resource confines of this study, it was only possible to conduct trials in two woods per season, conducted in separate years. To confirm these findings, additional trials are required in winter, summer and spring, to further understand any annual variation in squirrel feeding behaviour and confirm the best months for bait deployment.

Male and female squirrels visited hoppers multiple times and on most days of the four-day bait deployment, demonstrating that if oral contraceptives were deployed in baits, individuals that visited hoppers are likely to receive multiple doses within a short space of time. Although bait uptake was found to be slightly higher in male squirrels, male and female squirrels consumed very similar amounts of bait and exhibited similar bait uptake patterns. Variation in bait uptake between individuals of the same sex was greater than the variation seen between sexes. The findings from this study are comparable to those of Tiberi et al. (2024), who also concluded that there was no overall difference in bait uptake between individual grey squirrels of different sex, age and reproductive status, though they found that, on average, breeding females had the highest levels of bait uptake.

Bait uptake has also been found to be similar between male and female house mice and male and female Norway rats, with body weight influencing bait uptake between individual rats of the same sex (Inglis et al., 1996; Jacob et al., 2003). No evidence was found that body weight influenced bait uptake by grey squirrels, though it is recommended that trials are conducted on additional woods in different seasons and years to confirm this. Additional data are also required on the effects of squirrel age or female breeding status on bait uptake, due to seasonal variations in the difficulty in visually assessing the latter two factors and a more detailed assessment is required to confirm this.

In Chapter 3, I found that hopper density was a key factor in determining population level bait uptake in squirrels. Results from the PIT-tag trials conducted in winter and summer found that most squirrels visited multiple hoppers in both seasons. Consequently, it was decided that 2/ha should be trialled in spring, as a more efficient deployment, reducing the effort required for bait delivery. Lowering the hopper density did not affect the proportion of squirrels that visited hoppers, or the time squirrels spent feeding from hoppers, although the probability of visiting a hopper was significantly inversely related to the number of squirrels per hopper. Prior to any bait delivery campaign, it is recommended that an initial squirrel density estimate be obtained using a proven method, such as the camera trap index-based method described in Chapter 5; so that the density of hoppers deployed can be adjusted to ensure there are fewer than 3 squirrels per hopper.

In this study, most PIT-tagged squirrels visited hoppers within the first week, during the pre-bait period, suggesting that neophobia was not an important influence on bait delivery. The proportion of grey squirrels visiting hoppers decreased slightly for the four-day trial period. This could be because bait marker dye was added to the bait. As previously discussed, RB has been shown to be unpalatable to rodents at higher concentrations (Fernandez & Rock, 2011). In Chapter 2, the concentration used in this study was trialled in captive squirrels for its palatability, however, it is possible that some individuals within the populations may have been more sensitive to its taste, or that the taste may have become stronger under certain environmental conditions. The lack of spillage recorded at the hoppers during the trial, provided further evidence that the RB bait was consumed by squirrels at the hopper and was not removed and made available to nontarget species.

The average amount of bait taken in four days for male and female squirrels was 39 g and 47 g, respectively. Oral contraceptives are currently being trialled on captive grey squirrels to determine the contraceptive dose that would be required to render an individual infertile. Once the dose has been determined, data gathered in this study could be used to quantify the frequency of deployment, quantity per deployment and concentration to ensure that most of a squirrel population is treated effectively. However, it is recommended that additional field trials are conducted in additional woodlands in different seasons, different years and using both 2 and 3 hoppers/ha, to increase the confidence in the findings of this study. Additional work is also required to assess how reproductive status may affect grey squirrel bait uptake in different seasons, to ensure breeding animals are targeted. Spatial analysis of the PIT-tag data would also provide information on the distances moved by animals between hoppers, informing the minimum spacing distance to use when deploying bait hoppers.

The bait delivery and monitoring system, developed and tested for this study could be adapted to design delivery methods for other oral baits and other small mammal species. This could include the delivery of oral vaccines such as the plague vaccine for prairie dogs (Abbott et al., 2022). Further testing would be required to adapt the hopper to prevent nontarget access by other species relevant to the environment in which the feeders are deployed.

4.6 Conclusion

The bait delivery and monitoring system developed and tested in this study demonstrated that it was possible to deliver multiple doses on most days of a 4-day deployment to both male and female grey squirrels. Bait deployment is likely to be more effective in spring, immediately prior to the second peak in breeding, followed by winter, immediately prior to the first breeding season. The findings of this study will inform bait delivery methods to deploy oral contraceptives in the future for grey squirrel population management. This system could be adapted to design delivery for other baits and other small mammal species.

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4.8 Supplementary Material: GLM model summary outputs and goodness of fit tests

1) Binomial GLM for likelihood that a squirrel will enter a hopper for two different hopper densities in spring 2023

Global model structure

glm(formula = visited_spring ~ Period*Site + Sex + Site + Squirrels_hopper, family = binomial(link = cloglog), data = dat_spring, na.action = na.fail)

Analysis of Deviance Table

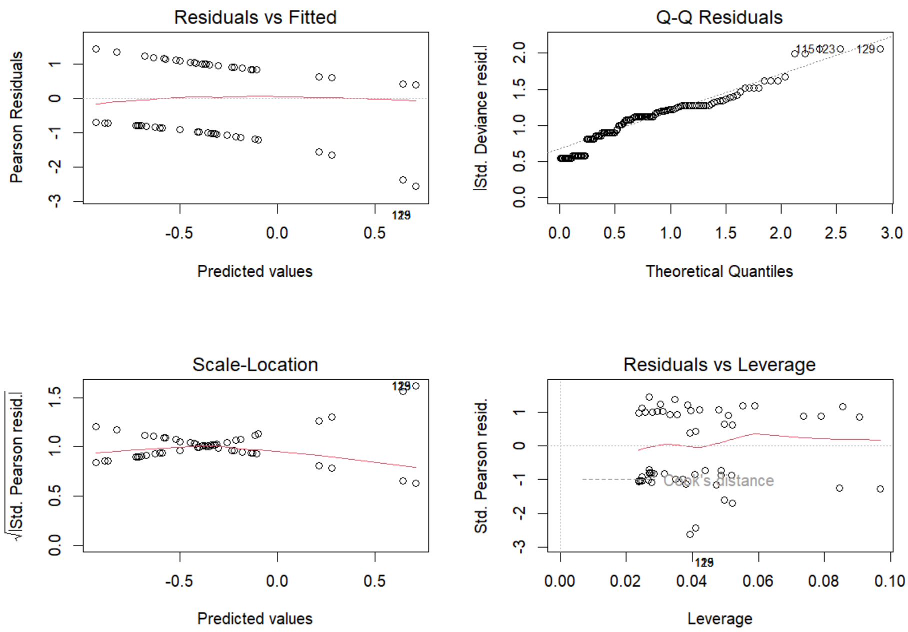
Model 1: visited_spring ~ Period * Site + Sex * Site + Squirrels_hopper
Model 2: visited_spring ~ Period + Site + Sex * Site + Squirrels_hopper

Model	Resid. Df	Resid. Dev	Df	Deviance	*P*
1	125	157.07			
2	127	158.04	-2	-0.974	0.6145

Model 2 summary

```
Coefficients:
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)      -0.625491   0.421306  -1.485 0.137637
Period            0.066147   0.291588   0.227 0.820538
SiteGE            1.175034   0.321070   3.660 0.000252 ***
SexM             -0.430114   0.260039  -1.654 0.098121 .
Squirrels_hopper  0.008883   0.015261   0.582 0.560519
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 179.95  on 131  degrees of freedom
Residual deviance: 158.04  on 127  degrees of freedom
AIC: 168.04
```

- The overdispersion parameter theta = 1.24
- Model fit compared to null model: $X^2 = 21.91$ on 4 degrees of freedom, P < .0001



2) Gaussian GLM for amount of bait uptake indicated by total time grey squirrels spent visiting hoppers for two different hopper densities in spring 2023

Global model structure

mod_spring_duration <- glm(duration_m ~ Period*Site + Sex + Squirrels_hopper, family="gaussian"(link = log),data = dat_springduration, na.action=na.fail)

Analysis of Deviance Table

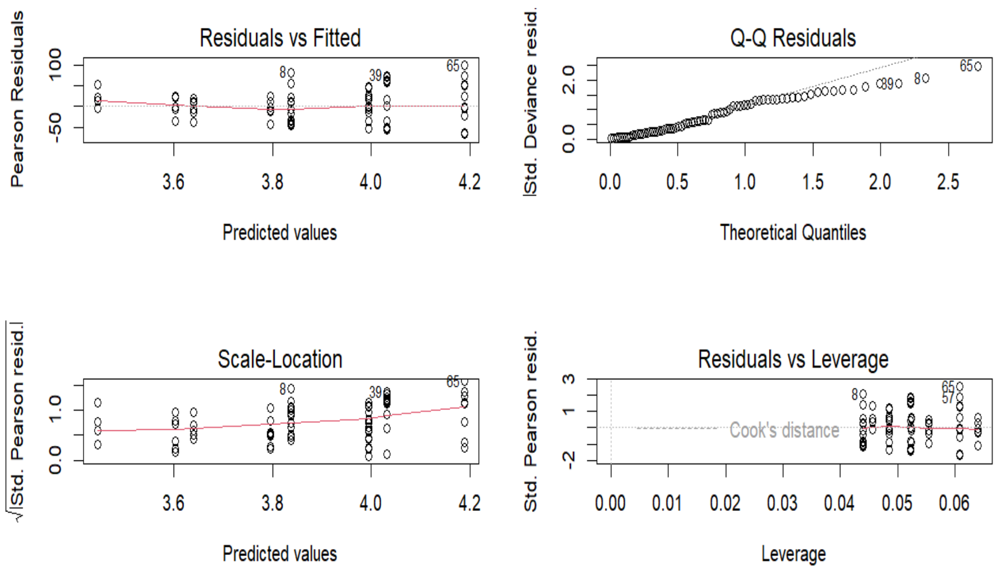
Model 1: duration_m ~ Period * Site + Sex * Site
Model 2: duration_m ~ Period + Site + Sex + Site

Model	Resid. Df	Resid. Dev	Df	Deviance	*P*
1	71	120629			
2	72	120805	-1	-176.25	0.7474

Model 2 (without interaction) summary

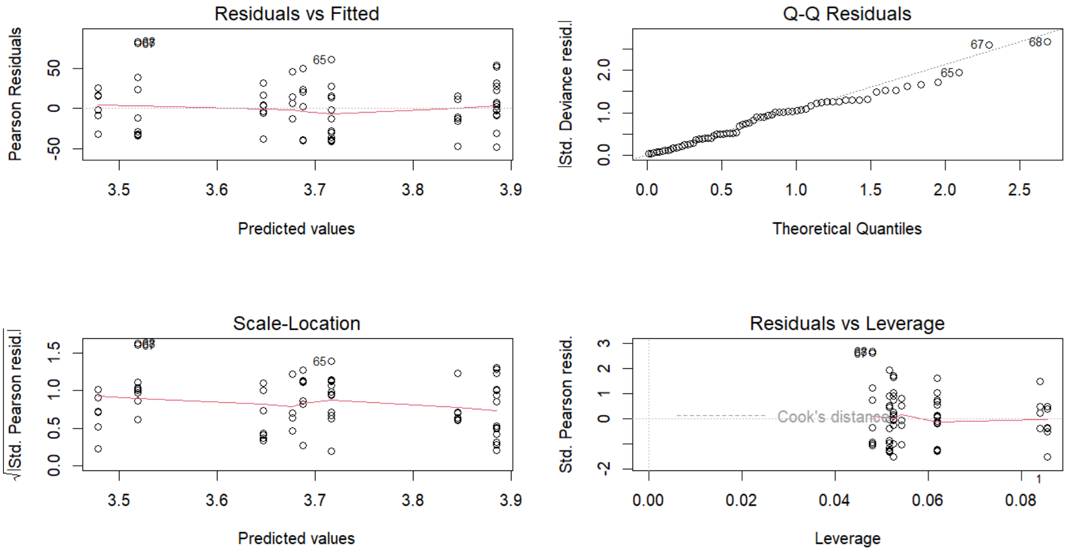
```
glm(formula = duration_m ~ Period + Site + Sex, family = gaussian(link = log), data
 = dat_spring_duration, na.action = na.fail)

Coefficients:
             Estimate Std. Error t value Pr(>|t|)    
(Intercept)   3.6812     0.3327  11.063   <2e-16 ***
Period        0.1569     0.1869   0.839    0.404    
SiteGE        0.1939     0.1873   1.035    0.304    
SexM         -0.3925     0.2382  -1.647    0.104    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 1677.885)

    Null deviance: 127670  on 75  degrees of freedom
Residual deviance: 120805  on 72  degrees of freedom
AIC: 785.89
```

- Model fit compared to null model: $X^2 = 3432.5$ on 4 degrees of freedom, P < .0001



Model 2 summary (with top 8 outliers removed)

```
glm(formula = duration_m ~ Period + Site + Sex, family = gaussian(link = log),data
= dat_spring_duration, na.action = na.fail)

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  3.54773    0.33977  10.442 1.87e-15 ***
Period       0.16877    0.19455   0.867    0.389
SiteGE      -0.19809    0.19865  -0.997    0.322
SexM        -0.04012    0.20455  -0.196    0.845
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 1020.54)

    Null deviance: 67180  on 67  degrees of freedom
Residual deviance: 65314  on 64  degrees of freedom
AIC: 669.96
```



3) Binomial GLM analysing factors affecting the likelihood that a squirrel will enter a hopper

Global model structure

glm(formula = Visited ~ Bodyweight_g * Sex + Season * Sex + Season * Bodyweight_g + Squirrel_hoppers, family = binomial(link = cloglog), data = dat_visited, na.action = na.fail)

Model summary

```
Coefficients:
                           Estimate Std. Error z value Pr(>|z|)
(Intercept)               0.6627461  1.8609070   0.356   0.7217
Bodyweight_g              0.0017841  0.0033031   0.540   0.5891
SexM                     -1.3061608  2.1129440  -0.618   0.5365
SeasonSummer              2.4086752  2.8696140   0.839   0.4013
SeasonWinter              1.7508038  2.5089569   0.698   0.4853
Squirrel_hoppers         -0.2995466  0.1009258  -2.968   0.0030 **
Bodyweight_g:SexM         0.0009913  0.0038778   0.256   0.7982
SexM:SeasonSummer         1.2726817  0.5736927   2.218   0.0265 *
SexM:SeasonWinter         1.0161256  0.6266740   1.621   0.1049
Bodyweight_g:SeasonSummer -0.0073965  0.0051147  -1.446   0.1481
Bodyweight_g:SeasonWinter -0.0039240  0.0042744  -0.918   0.3586
```

```
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 268.52  on 201  degrees of freedom
Residual deviance: 222.00  on 191  degrees of freedom
AIC: 244
```

Analysis of Deviance Table
Model 1: Visited ~ Bodyweight_g * Sex + Season * Sex + Season * Bodyweight_g + Squirrel_hoppers
Model 2: Visited ~ Season * Sex + Squirrel_hoppers

Model	Resid. Df	Resid. Dev	Df	Deviance	*P*
1	191	222			
2	195	225	-4	-2.632	0.6211

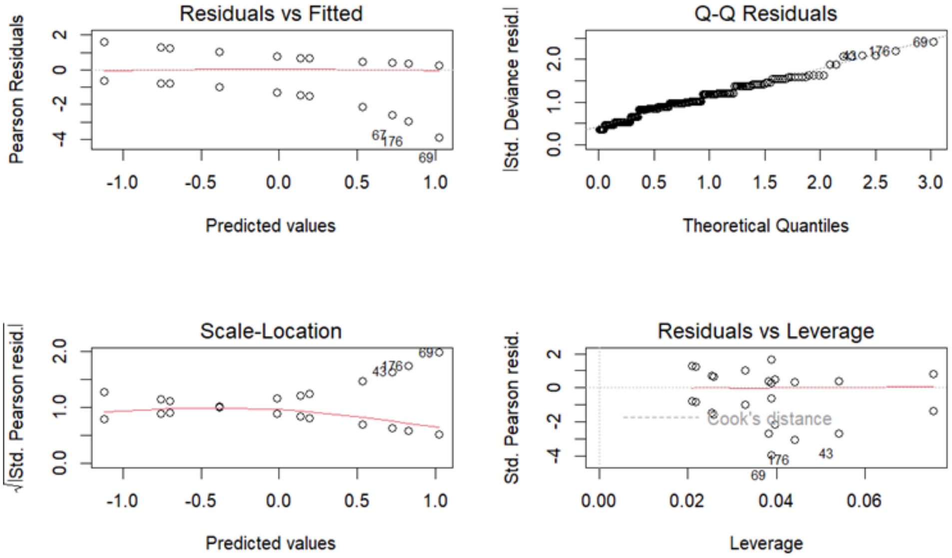
Model 2 (without bodyweight) summary

```
Coefficients:
                   Estimate Std. Error z value Pr(>|z|)
(Intercept)         1.63059    0.53819   3.030  0.00245 **
SeasonSummer       -1.73982    0.43612  -3.989 6.63e-05 ***
SeasonWinter       -0.50387    0.46526  -1.083  0.27881
SexM               -0.73366    0.38921  -1.885  0.05943 .
Squirrel_hoppers   -0.30988    0.09934  -3.119  0.00181 **
SeasonSummer:SexM   1.62404    0.49961   3.251  0.00115 **
SeasonWinter:SexM   1.03051    0.53582   1.923  0.05445 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 268.52  on 201  degrees of freedom
Residual deviance: 224.63  on 195  degrees of freedom
AIC: 238.63
```

Emmeans pairwise comparison summary

```
Season_pairwise estimate  SE     df     z.ratio p.value
Spring - Summer 0.9278     0.372  Inf    2.497   0.0125
Spring - Winter -0.0114    0.407  Inf    -0.028  0.9777
Summer - Winter -0.9392    0.244  Inf    -3.856  0.0001

Season_pairwise Sex_pairwise estimate    SE  df z.ratio p.value
 Spring - Summer F - M          1.624 0.500 Inf   3.251  0.0012
 Spring - Winter F - M          1.031 0.536 Inf   1.923  0.0545
 Summer - Winter F - M         -0.594 0.483 Inf  -1.229  0.2190
```

- The overdisersion parameter theta = 1.154158
- Model fit compared to null model: X^2 = 43.89 on 6 degrees of freedom, P < .0001



4) Gaussian GLM analysing factors affecting the time squirrels spent visiting hoppers

Model structure

glm(formula = Duration_m ~ Squirrel_hoppers + Sex * Season, family = gaussian(link = log),
data = dat_no_zero, na.action = na.fail)

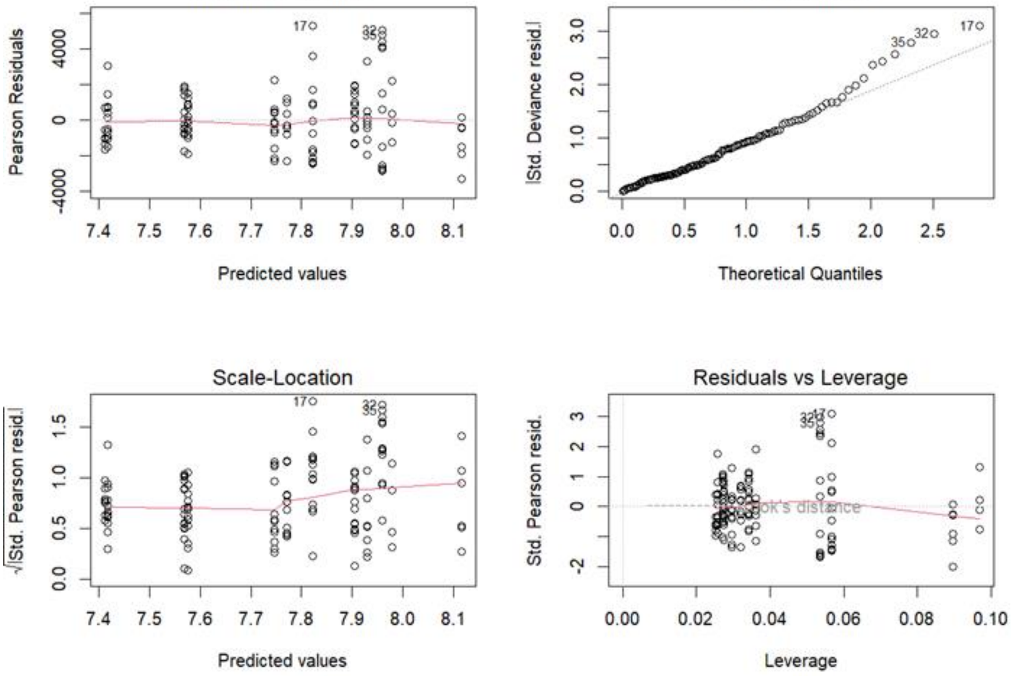
Model summary

```
Coefficients:
                  Estimate Std. Error t value Pr(>|t|)    
(Intercept)        3.292921   0.308568  10.672  < 2e-16 ***
Squirrel_hoppers  -0.062956   0.057708  -1.091  0.27753    
SeasonSpring       0.907494   0.336871   2.694  0.00809 ** 
SeasonWinter       0.340663   0.339612   1.003  0.31787    
SexM               0.377049   0.328926   1.146  0.25399    
SeasonSpring:SexM -0.600200   0.413668  -1.451  0.14945    
SeasonWinter:SexM  0.001176   0.395282   0.003  0.99763    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 837.8834)

    Null deviance: 110536  on 124  degrees of freedom
Residual deviance:  98869  on 118  degrees of freedom
AIC: 1204.9
```

Emmeans pairwise comparison summary

```
Season_pairwise Sex_pairwise estimate    SE  df t.ratio p.value
 Summer - Spring F - M        -0.60020 0.414 118  -1.451  0.1495
 Summer - Winter F - M         0.00118 0.395 118   0.003  0.9976
 Spring - Winter F - M         0.60138 0.333 118   1.804  0.0737

Season_pairwise estimate    SE  df t.ratio p.value
 Summer - Spring   -0.607 0.242 118  -2.512  0.0133
 Summer - Winter   -0.341 0.199 118  -1.719  0.0883
 Spring - Winter    0.266 0.220 118   1.207  0.2297
```

- Model fit compared to null model: $X^2 = 11667$ on 6 degrees of freedom, $P < .0001$


Pearson Residuals
Predicted values
|Std. Deviance resid.|
Theoretical Quantiles
Scale-Location
√|Std. Pearson resid.|
Predicted values
Residuals vs Leverage
Std. Pearson resid.
Leverage

Chapter 5. A camera trap method for estimating densities of grey squirrels

The findings of this chapter are published in:

Beatham, S. E., Stephens, P. A., Coats, J., Phillips, J., & Massei, G. (2023). A camera trap method for estimating target densities of grey squirrels to inform wildlife management applications. *Frontiers in Ecology and Evolution*, 11, 1096321. https://doi.org/10.3389/fevo.2023.1096321

The data for this chapter are published in Dryad digital repository:

Beatham (2023) Camera trap grey squirrel photograph data https://doi.org/10.5061/dryad.95x69p8q5

5.1 Abstract

Effective wildlife population management requires an understanding of the abundance of the target species. Camera traps are increasingly used to estimate animal abundance, and methods have been developed that do not require the identification of individual animals. Most of these methods have been focussed on medium to large mammal species with large range sizes and may be unsuitable for measuring local abundances of smaller mammals that have variable detection rates and hard to measure movement behaviour. The aim of this study was to develop a practical and cost-effective method, based on a camera trap index, that could be used by practitioners to estimate target densities of grey squirrels in woodlands to provide guidance on the numbers of traps or contraceptive feeders required for local grey squirrel control.

Camera traps were deployed in ten independent woods of between 6 and 28 ha in size. An index, calculated from the number of grey squirrel photographs recorded per camera per day had a strong linear relationship ($R^2 = 0.90$) with the densities of squirrels removed in the trap and dispatch operations conducted as part of the rhodamine B field trials described in Chapter 3 and other studies. There were no significant differences between the number of squirrel photographs per camera recorded by three different models of camera, increasing the method's practical application. This study demonstrated that a camera index could be used to inform the

number of feeders or traps required for grey squirrel management through culling or contraception. Results could be obtained within six days without requiring expensive equipment or a high level of technical input. This method can easily be adapted to other rodent or small mammal species, making it widely applicable to other wildlife management interventions.

5.2 Introduction

Effective wildlife population management requires an understanding of the abundance of the target species. Currently, the most widely used method of squirrel control in the UK is trap and dispatch (Mayle et al., 2007). Fertility control, using oral contraceptives delivered in baits via feeders, is currently being developed as an additional tool to reduce population sizes and to slow the rate of population recovery after culling (Croft, 2021; Massei et al., 2020). The density of traps or contraceptive hoppers deployed can significantly affect eradication times (Croft et al., 2021) and the trials described in Chapter 4 showed that for effective contraceptive delivery, there should be enough hoppers deployed to ensure there are fewer than three squirrels per hopper. Grey squirrel management is conducted by volunteers and practitioners throughout the UK. A practical and cost-effective method for estimating squirrel densities is therefore required to guide population control operations and to assess the impact of these interventions on a local scale.

Camera traps are increasingly used to estimate mammal population sizes (Jayasekara et al., 2021; Massei et al., 2018; Mason et al., 2022; Noss et al., 2012) and, in the last few decades, methods have been developed that estimate animal abundance based on the detection rates of animals by camera traps that, unlike traditional capture-mark recapture applications, do not require the identification of individual animals (Gilbert et al, 2021; Howe et al., 2017; Loonam et al., 2021; Moeller, 2017; Palencia et al., 2021). These methods often require strict sampling protocols, the provision of complex ancillary data on factors such as animal movement and most have been developed on medium to large mammal species with large range sizes, such as ungulates or big cats. Most of these models are based on passive detection rates of animals in their environment, with the assumption that the presence of the cameras should not affect the behaviour of the target species, so baits or lures should not be used. It is therefore challenging to apply these methods to obtain abundances for species with lower passive detection probabilities, such as those that are small in body size, that are quick moving or that spend a lot of their time in hard to monitor locations, such as fossorial or arboreal mammals.

Developments in camera trap technology, which include longer battery life, faster trigger speeds, higher sensitivity and greater memory capacity, have widened their application for monitoring the activity of rodents and other small, fast-moving mammals. To increase the detection probability of small mammals, some of these studies have focussed cameras on areas of animal activity, based on activity signs or by using bait, and indices calculated from the number of camera trap photos per unit effort have been found to be closely correlated with other measures of population size for Norway rats *Rattus norvegicus* (Lambert et al., 2018), red-backed voles *Myodes rutilus* and deer mice *Peromyscus maniculatus* (Vilette et al, 2016) and snowshoe hares *Lepus americanus* and red squirrels *Tamiasciurus hudsonicus* (Vilette et al. 2017).

In this study, I deployed camera traps in ten independent woods of between 6 and 28 ha in size. Piles of bait were used to lure squirrels in front of the cameras, to create areas of activity and increase detection probability. Indices were calculated for each wood based on the number of squirrel photographs recorded per camera per day and these were compared with the total number of squirrels removed through trap and dispatch, undertaken as part of local eradications for the trials described in Chapter 3. An index was selected based on the relative linear regression model fit, measured by R^2, its practical application and cost-effectiveness. Different time filters were tested; photos from the same camera that were recorded within a designated time from a previous photo were excluded from the analysis. The filter that produced the best model fit was selected. To improve the cost-effectiveness and practical application of the method, two cheaper models of camera were tested alongside the higher end model used to develop the method.

The aim of this study was to develop a practical and cost-effective camera trap method that could be used by practitioners to estimate target densities of grey squirrels in woodlands to determine the number of traps or contraceptive feeders required for effective control. I discuss how these methods could be adapted to improve their application to grey squirrel management methods and the assessment of other rodent and small mammal populations.

5.3 Materials and Methods

Study sites

The study was conducted in 10 mature woods at the same time of year, between mid-June and mid-July, from 2018 to 2021. Woods were located in two regions of the UK; eight in Yorkshire, England (54°N, 0°W) and two in Denbighshire, Wales (53°N, -3°W). Woods were between 6 ha and 28 ha in area and consisted of either broadleaf or a mix of broadleaf and conifer tree species. I measured the area of each wood from a satellite base map using a measure tool (Google My Maps 2018 to 2021). Each wood was sampled once. To ensure independence, woods sampled within consecutive years were not directly connected to each other via wooded corridors or hedgerows and were located at least 600 metres apart. The first seven woods, sampled in 2018 and 2019, were discrete areas of woodland with little connectivity to other woodland areas. The last three woods sampled were highly connected to other woodland areas.

Camera deployment

For these field trials, I led the study design and analysis and conducted most of the field work and data processing, with assistance from Animal and Plant Health Agency (APHA) colleagues that I had trained in the methodology or that were already experienced in some of the techniques. At each wood, camera traps (Reconyx™ HC500 or HS2X) were deployed at a density of 1/ha. Camera placement in the field was guided by a 1 ha grid generated in ArcGIS (version 10.7.1) overlayed onto a satellite map using the ArcGIS Collector mobile phone application and was adjusted according to accessibility; for example, steep slopes or thick vegetation were avoided (Figure 5.1 a).

Cameras were fixed to trees at approximately 1 meter above the ground and with the lens angled between horizontal and 45° below horizontal (Figure 5.1 b). A laser pen or 1-meter wooden pole, placed parallel to the base of the camera, was used to position a pile of bait at the centre of the camera field of view, between 1 and 2 meters away from the camera lens. The bait pile consisted of approximately 1.5 kg of 50:50 whole maize and peanuts. The cameras were set to take one photograph per trigger and the passive infrared sensor to high sensitivity. Cameras were deployed for 3-6 days and the bait in front of each camera was checked every 1-3 days (guided by a prior assessment of potential bait uptake by non-target species) and replenished, if required.

a) b)

Figure 5.1. Cameras were placed at a density of 1/ha, guided by a 1 ha grid, at random locations approximately evenly spaced across 10 woods. Camera placement was adjusted according to accessibility; for example, steep slopes or thick vegetation were avoided. A map of the camera locations (red squares) at wood SA (outline in grey) is provided as an example (a). Cameras were fixed to trees at approximately 1 meter above the ground and with the lens angled between horizontal and 45° below horizontal, focussed on a bait pile (b).

At the end of each deployment, the cameras were removed and all the photographs containing squirrels were digitally tagged using the Reconyx MapView Professional™ software (Figure 5.2). For the first five woods, photographs were also tagged with the number of squirrels present in each photograph. The resulting data were quality checked by a second observer re-analysing a sub-sample of the photographs to ensure there was no observer bias in the records. The final photographs taken by each camera in each wood were checked for the amount of bait remaining, as this is likely to affect squirrel activity and the number of photographs recorded. Photograph data were excluded from index analysis, and the number of cameras adjusted accordingly, for days when a camera ceased to work due to insufficient battery power or faults, when the bait had been completely removed, or when the camera was not focussed on any part of the bait pile. The latter occasionally occurred due to set up error or if the camera was subsequently knocked out of position by a person or an animal.

Figure 5.2. A photograph from a Reconyx™ HS2X camera-trap deployed in a wood and baited for 4 days, used to calculate an index to measure grey squirrel density

Grey squirrel trapping

Grey squirrel trapping and dispatch methods were conducted as part of other studies and were approved by the joint Fera and APHA Animal Welfare and Ethical Review Body (AWERB). Within two to eight weeks of camera deployment, squirrel live-capture cage-traps were installed in each wood, secured to 1-metre-high wooden stands, evenly distributed throughout each wood at a density of 3 traps/ha. The traps in each wood were left open and pre-baited with a mixture of maize, peanuts and several whole hazelnuts for three to 11 days, dependent on the availability of resources. Traps were then set and checked at least once every 24 hours. For animal welfare and health and safety reasons, traps were not set if heavy rain, high winds or high temperatures were forecast, and each trap was partly covered with a waterproof sheet. Trapping was conducted within a four-week period, typically Monday to Friday, for a minimum of 5 days, until squirrel capture rates were reduced to an average of less than one per day over three consecutive days. Lawton & Rochford (2007) found that most, if not all, grey squirrels in a population could be captured within 5 days of an intensive trapping regime.

Squirrels that were trapped were humanely dispatched using a UK Home Office approved (schedule 1) method, by a trained and competent person and the sex of the squirrel recorded.

Camera index design and selection

I considered four camera indices as candidates for estimating grey squirrel densities. All indices were based on the number of squirrel photographs per number of working cameras per trial day and were designed to be practical, cost-effective and representative of squirrel activity. Trial days consisted of consecutive 24 hours. The differences between indices concerned the time the first trial day began and which trial days were used for the photograph counts, that were modified to try to make the method more practical and accurate.

Index 1 used all squirrel photographs recorded during consecutive 24 hours from the time the last camera was deployed in each wood. Index 2 used all squirrel photographs recorded during consecutive 24 hours, from 24 hours after the last camera was deployed; this was to allow the squirrels time to find the bait piles before the assessment began. Index 3 used all squirrel photographs recorded within consecutive 24 hours, from 24:00 on the day the cameras were deployed until 24:00 on the day before they were collected. Starting and finishing at a standard time simplified the photo data selection process. Index 4 used all squirrel photographs from the 24 hours that recorded the maximum number of squirrel photographs from each consecutive 24 hours starting from when the last camera was deployed; this was to provide the maximum level of activity, which may have offered a more accurate representation of density.

For all four indices, I applied time filters of 0.5, 1, 2, 3, 4, 5, 10, 20 and 30 minutes, where any photographs that were recorded within the specified interval after the previous photograph were excluded from the photograph counts. The application of a time filter was used to moderate inflated counts caused by individuals that remain in front of a camera for extended periods of time (Tourani et al., 2020). This is especially applicable at bait piles, where some individuals may feed for longer than others. I used linear regressions to test whether the values calculated for each index could be used to predict the density of squirrels trapped and removed in each wood and I calculated the coefficient of determination (R^2) as a measure of fit (Nakagawa and Cuthill, 2007; Vilette et al., 2016; Villette et al., 2017). I assessed the statistical significance of the model with the greatest R^2 using an F-test. Data normality was confirmed using a Jarque-

Bera test and through plots of the residuals. To make the data processing methods more widely accessible to practitioners, all data analysis was conducted using Microsoft Excel®.

Camera model comparison

A method developed to be a practical tool available to a wide range of practitioners needs to be suitable for use with most of makes and models of cameras. The Reconyx cameras that were used to develop the method are one of the most technologically advanced models on the market, but they are also one of the most expensive, making them inaccessible to many grey squirrel management practitioners. The method was therefore trialled with two alternative types of camera, the widely used, mid-priced Browning® BT-5 and a lower budget camera, the Toguard H70A. Table 5.1 provides a comparison of the main parameters for each of the three cameras.

Table 5.1. A comparison of features for three different camera traps trialled to calculate a camera-based density index based on the number of squirrel photographs recorded camera/trial/day.

Camera Model	Approximate price (£)	Image resolution (megapixel)	Angle of detection (°)	Trigger speed (seconds)	Time between triggers (seconds)
Reconyx Hyperfire 2	400	3	45	0.2	0
Browning BT-5	200	4	55	0.7	5
Toguard H70A	50	20	130	0.3-0.5	15

In trial 1, I deployed seven Reconyx Hyperfire 2 cameras and seven Browning BT-5 cameras in a 7 ha wood in North Yorkshire in February 2020. Both camera models were placed next to each other vertically on a tree, angled towards a bait pile, using the same camera deployment method described above. The position of the cameras from each model (top or bottom) was alternated for each consecutive deployment within the same wood, to reduce any bias caused by camera position. In trial 2, conducted in August 2021, I worked with volunteers from the Westmorland red squirrel group, to deploy nine Toguard cameras and nine Reconyx camera in a 9-ha area of a woodland in Cumbria. The volunteers then baited the cameras. For both trials, cameras were baited for three days, then removed and the photographs containing squirrels tagged and counted. The total number of photographs per camera per trial day and the number of photographs per camera per trial day with a 5-minute filter applied were then compared between the Reconyx cameras and two other models.

5.4 Results

Camera index

A total of 89,011 squirrel photographs were recorded in the ten study woods, with a range of 48-22,673 photographs per wood (Table 5.2). For most woods, the camera deployment and photograph analysis were completed within 6 days. Out of the 31,031 squirrel photographs recorded in the first seven study woods, 98% contained one individual, rather than multiple individuals; therefore, it was decided that it would not be cost-effective to include the number of squirrels in photographs in the index analysis, as this would considerably increase the photograph processing time.

Table 5.2. The results of grey squirrel camera surveys and live trap and removal conducted at 10 woods. Trapping was conducted with a trap density of 3/ha and within 2 to 8 weeks of the camera surveys. Included are the number of working cameras, number of trap days, the number and density of squirrels removed. Values are included from index 3 with a 5 minute filter applied, selected as the best predictor for squirrel density trapped. Final trap rate was defined as the average number of squirrels removed on the last three days of trapping.

Wood Id	Year	Size (ha)	N cameras	N camera days	N squirrel photos	Index values	Trap days	N squirrel trapped	Squirrel density (N/ha)	Final trap rate (N/day)
BP	2018	18	15	4	2404	7.18	15	75	4.2	0.33
FP		9	5	4	48	12.09	7	16	1.8	0.67
LT		10	9	4	65	1.27	9	17	1.7	0.67
PA		6	6	4	2041	5.25	7	22	3.7	0.33
GE	2019	7	7	3	4204	9.19	5	17	2.4	0.67
HA		8	8	3	10492	0.96	8	39	4.9	0.67
ST		8	7	3	12320	8.33	8	38	4.8	0.67
EL		28	25	3	18543	20.79	15	129	4.6	0.67
SA	2020	15	15	6	22673	10.80	13	105	7.0	1.33
PE	2021	7	8	4	16223	8.19	11	74	10.6	0.33

The duration of grey squirrel feeding activity, taken from the photographs, was consistent across woods; the average time of day (24 hour clock) the first squirrel photograph was taken was 5:20 (SD = 38 minutes) and the average time of day of the latest squirrel photograph taken was 21:09 (SD = 46 minutes) producing an average duration of squirrel activity of 15 hours 49 minutes (SD = 1 hour 17 minutes). For all ten woods, the number of days between the completion of the camera survey and the start of the trap and dispatch was, on average, 29 days (range = 3-54 days). Between 16 and 129 squirrels were trapped at the ten woods (Table 5.1). On average, 53% of the squirrels caught were male (range = 43-65%) and on average, 86% of the squirrels trapped in each wood were trapped within the first 5 days (range = 67-100%). For nine woods, a trap rate of less than 1 squirrel/day for three consecutive days was achieved in 15 trap days or less. For wood SA, trapping was stopped on day 13 due to insufficient resources and the final trap rate was 1.33 squirrels/day, with the final three days' capture numbers 3, 1 and 0, respectively. The densities of squirrels trapped at each wood, were between 1.6 and 10.6 squirrels/ha.

Each of the four camera indices tested provided a good linear fit with the density of squirrels trapped at each wood, all achieving R^2 values of over 0.77 (Figure 5.3). Time filters of between 1 and 5 minutes improved the model fit for all indices. The regression model for index 3, with a 5 minute filter applied, had the highest R^2 (0.90, Figure 5.4) and was highly significant ($F_{1,8}$ = 71.4, $P < 0.001$).

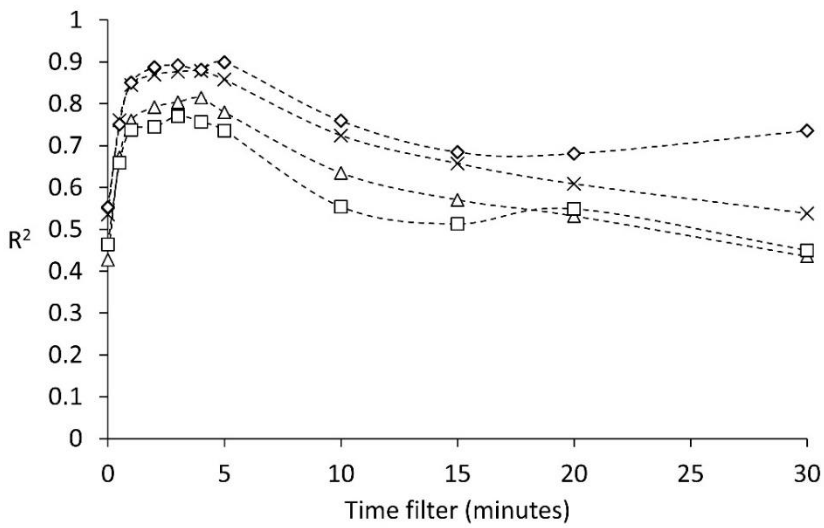


Figure 5.3. The relationship between four camera indices (based on the number of squirrel photographs/camera/trial day) and the density of squirrels trapped and removed in 10 woods. R^2 denotes the variability explained. A time filter ranging from 0.5 to 30 minutes was applied to each index. Index 1 (cross) = all squirrel photographs recorded during consecutive 24 hours from the time the last camera was deployed. Index 2 (square) = all squirrel photographs

recorded during consecutive 24 hours, from 24 hours after the last camera was deployed. Index 3 (diamond) = all squirrel photographs recorded within consecutive 24 hours, from 24:00 on the day the cameras were deployed. Index 4 (triangle) = all squirrel photographs from the 24 hours that recorded the maximum number of squirrel photographs from consecutive 24 hours starting from when the last camera was deployed.

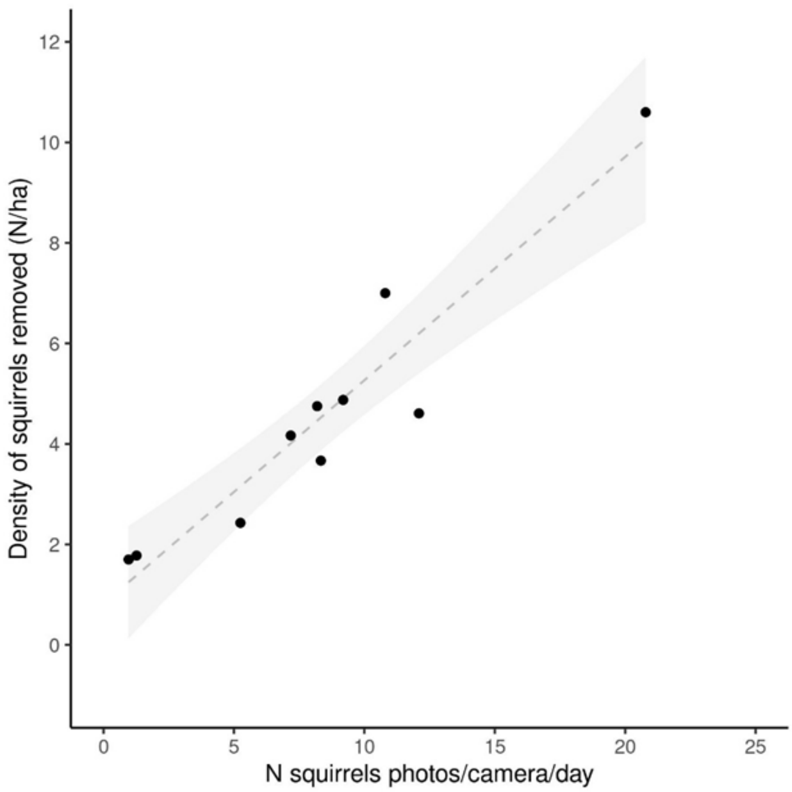


Figure 5.4. A camera index, based on the number of squirrel photographs taken/camera/trial day by baited cameras deployed at 1/ha, plotted against the density of squirrels trapped and removed in 10 woods. Only photographs recorded within consecutive 24 hours, from 24:00 on the day the cameras were deployed were used and any squirrel photograph obtained within 5 minutes of a previous squirrel photograph at the same camera was filtered out of the analysis. Line of best fit (dashed) is y = 0.4446x + 0.8203, 95% Confidence Intervals (grey shading) are also shown, R^2 = 0.90.

Camera model comparison

During trial 1, a Reconyx camera in one location stopped working on day 1, due to battery failure. The analysis was therefore conducted using photograph data from six locations in the wood. Analysis of the bait piles in the camera field of view showed that both camera models in each location were focussed on over 90% of the area each of the bait piles. The number of squirrel photographs recorded by the Reconyx cameras (Table 5.3) was significantly higher than the Browning cameras (Paired sample Wilcoxon signed rank two-tailed; $W(17) = 3$, $P < 0.05$). When a 5 minute filter was applied to the data from both cameras there was no significant difference between the two camera models (Paired sample Wilcoxon signed rank two-tailed; $W(12) = 26.5$, $P > 0.05$).

Table 5.3. The results of a comparative analysis between the total number of grey squirrel photos recorded and the number of grey squirrel recorded when a five minute filter was applied (excluding any photos taken withing 5 minutes of a previous photo) by three different camera models, placed in the same woodland locations focussed on the same bait piles for three days.

Trial	Camera location	N photos for each camera model		N filtered photos for each camera model	
1		Reconyx	Browning	Reconyx	Browning
	1	167	79	9	12
	2	1276	841	53	51
	3	2518	703	26	31
	4	2584	1895	50	51
	5	1	5	1	4
	6	1318	397	22	24
	Total	7864	3920	161	173
2		Reconyx	Toguard	Reconyx	Toguard
	1	6	20	5	8
	2	8	9	3	3
	3	18	20	4	5
	4	0	3	0	1
	5	1	2	1	1
	6	4	20	1	7
	7	1	13	1	5
	8	14	24	4	4
	9	11	12	2	3
	Total	63	123	21	37

In trial 2, nine Reconyx cameras recorded photographs for three trial days, with six cameras focussed on 100% of a bait pile, two on 75% and one on 25%. Nine Toguard cameras also recorded data for the duration of the trial, all focussed on 100% of a bait pile. The data were combined for the three trial days at each location, as there were many days with zero squirrel photograph counts. The number of squirrel photographs recorded by the Reconyx cameras (Table 5.3) was significantly lower $W(8) = 0$, $P < 0.05$) than the Toguard cameras. When a 5 minute filter was applied to the data, there was no significant difference in the number of photographs recorded by the two camera models (Paired sample Wilcoxon signed rank two-tailed; W(7) = 5, P > 0.05).

5.5 Discussion

The aim of this study was to develop and test a cost-effective and practical method that could be used by practitioners to estimate target densities of grey squirrels in woodlands to improve the efficacy of management applications. A camera index, based on the number of squirrel photographs per camera per day, had a strong linear association with densities of grey squirrels trapped and dispatched in ten woods in less than three weeks. The results from chapter 4 suggested that, for contraceptive delivery to be successful, enough hoppers should be deployed to ensure there are fewer than three squirrels per hopper. By deploying cameras at the start of a contraceptive campaign and using the methods described, a target number of hoppers could be calculated based on the index produced.

The index that provided the best relationship ($R^2 = 0.90$) applied a 5-minute filter to all squirrel photographs recorded from 24:00 on the day the cameras were deployed, for at least two consecutive 24 hours. The linear association found was strong and at the higher end of the scale when compared with other studies, which have reported R^2 values of 0.6 to 0.9, when analysing camera-trap indices and independent estimates of mammal density (Rowcliffe et al., 2008; Rovero and Marshall, 2009; Villette et al., 2016; Vilette et al., 2017). The relationship between the camera index and density of squirrels removed was consistent over different time periods, with all four indices accounting for a significant amount of variance in the densities of squirrels trapped ($R^2 > 0.77$) when a time filter of between 1 and 5 minutes was applied. The application of the time filter was important, as R^2 values for all four indices were less than 0.56 when all squirrel photographs were used unfiltered. This is presumably because some squirrels remained at the bait piles for some time and contributed to a greater proportion of the number of photographs.

Other studies have found that applying a filter of more than 1 minute, will reduce the proportion of photographs triggered by the same animal (Yasuda, 2004) and thus improve the relationship between camera indices and animal density values (Massei et al., 2018; Villette et al., 2016;

Villette et al., 2017). The optimum filter length is likely to be study specific, dependent on the species, environment and camera methodology used.

One advantage of using a time filter is that the photographic rate is not as sensitive to camera variables as results will be standardised between different camera models and locations. For example, locations that have a wider detection area and models that have faster trigger speeds are likely to record more photographs of the same individual for the same time period. A time filter will omit these extra photos, thus moderating the number of photographs per event. In this study, this meant that the number of photographs recorded by low, medium and high budget camera models tested, that had very different specifications, were not significantly different, making the method more practical and accessible to practitioners with specific resources. Another advantage is that the index will be more robust to observer errors made in the photograph processing, for example, if a squirrel photograph is missed and is not tagged. This is because only one photograph per individual per bait pile visit needs to be accounted for, making the index less subject to observer bias. This may also make the index more adaptable for use with machine learning automated image identification software, which can now achieve accuracies of over 90% (Tabak et al., 2019), thus offering a large reduction in processing time.

One issue with using camera traps to index relative abundance is the lack of suitable independent methods for comparison. Most published estimates of mammal abundance concern individually identifiable animals (Gilbert et al., 2021) and the use of capture-mark-recapture based models (Hayato, 2020). Live trapping and mark-recapture is one of the widely used methods for estimating small mammal abundance and has been shown to have a strong relationship with camera trap indices when estimating the density of red squirrels (Villette et al., 2017). For many scenarios, it is not practical to trap, mark and recapture animals to estimate abundance, as the process has cost, time and welfare implications. In addition, as the grey squirrel is an invasive species in the UK, it is an offence under section 14 of the Wildlife and Countryside Act (1981) to release grey squirrels into the wild without an appropriate licence (UK Government, 2023). This is likely to be the situation for other non-native invasive species throughout Europe.

This study exploited already planned local eradications as an opportunity to estimate squirrel density and to compare this estimate with camera trap indices. If conducted correctly, local eradications can provide accurate estimates of mammal density in a defined area, as every individual in the population should be accounted for. If there is uncertainty that 100% eradication has been achieved, then the minimum number alive (MNA) per unit area can be calculated and used to estimate a minimum density. For instance, MNA densities calculated from live trapping have been shown to have a strong relationship with camera trap indices for snowshoe hares *Lepus americanus* (Villette et al., 2017).

The mean number of grey squirrels trapped in the ten study woods was 4.6 squirrels/ha, with a range of 1.7 to 10.6 squirrels/ha. A mean density of 4/ha has been recorded for grey squirrels in broadleaf woodland in the UK, ranging from 0 to 13 per ha (Merrick et al., 2016). There are several reasons why the density of squirrels trapped should reflect the total density in each wood. Trap effort was high and standardised between each wood, with cameras and traps deployed at the same time of year, across discrete woodland habitats, that were larger than the size of an average grey squirrel home range (less than 5 ha; Lawton et al. 2016; Wauters et al, 2002). Trap density was high (3/ha) and trapping was conducted consistently for between 5 and 15 days, until no squirrels were caught.

Grey squirrels typically have a high capture probability and previous studies have confirmed, via capture mark recapture, that grey squirrel eradications in woods were achievable within 5 days, using trap densities of between 1 and 2 traps/ha (Lawton & Rochford, 2007). Thus, using 3 trap/ha with this methodology should enable the capture of every squirrel within 5 trap days for even the highest densities of squirrels recorded in the UK. In Chapter 4, using the same trapping methods as this study, I demonstrated that woodland eradications conducted in six woods produced density estimates that were agreeable with those obtained from capture mark recapture estimates based on PIT-tagged squirrels.

In all 10 woods, within 15 days the squirrel trap rate was reduced to 1 per day or less for 3 consecutive days and on average 86% of the squirrels trapped in each wood were caught in the first 5 days. Out of the total number of squirrels trapped, 53% were male and 47% were female, suggesting that capture probabilities were not significantly different between sexes and that both sexes were equally attracted to bait. Grey squirrels will quickly recolonise an area where numbers have been removed (Lawton & Rochford, 2007), therefore population closure was maximised to avoid an overestimate of density. This was achieved through a short camera survey time (less than 5 days), the time between camera survey and squirrel removal (average 29 days) and the intensity and short period of removal (less than 3 weeks). Closure was also likely to be maintained as studies on individually marked squirrels have shown they will typically move no more than 200 metres between baited traps or feeders within the timescale of the study (Beatham et al, pers. obs., unpublished) and less than 500 metres between traps over several years (Taylor et al., 1971).

The use of bait will increase the consistency of squirrel activity levels and the probability of detection. The effort used to detect squirrels was standardised between woods by using a set density of cameras and averaging the number of photographs by the number of cameras and days they were deployed. To ensure the attractiveness of the bait to squirrels remained consistent across woods, cameras were deployed in all woods early to mid-summer, at a time of year when natural food availability is relatively low. As bait piles are necessary for detecting squirrels, it is important to ensure that the bait pile is located in the centre of the camera field of view and that bait does not run out during the trial. At least one bait check is recommended within the 3 days following the first deployment of bait, depending on initial observations or knowledge of local bait uptake by non-target species such as badgers, deer or birds.

Overall, 98% of squirrel photographs contained only one squirrel. In all woods, there were more squirrels than cameras deployed, and it appears that, to avoid confrontation, squirrels might stagger their access to bait over the course of a day as a consistent duration of squirrel activity was recorded by the cameras at all woods. This has been found in previous studies where squirrel populations have demonstrated a hierarchy of dominance over feeding resources (Pack et al., 1967). One advantage of this is that it requires much less processing time if the number of squirrels in each photograph does not need to be counted and the density estimate will be more accurate if each squirrel can be detected discretely within different timeframes.

The camera index was found to be cost-effective for measuring a range of densities of squirrels in woodlands that could be targeted by traps of feeders, with estimates achievable within six days, however, further development is required to widen its application. For instance, the method needs to be tested on very low densities of squirrels, to ensure it can be used effectively to monitor the progress of eradications. Similarly, more data are required for woods in which squirrel density is over 7/ha, to test the relationship more thoroughly at the higher end of the scale. In addition, the method has so far only been tested in broadleaf or mixed woodlands in summer. Management through culling or contraceptives will likely be most effective when applied immediately before the grey squirrel's main breeding season, in late winter (Hayssen, 2016); consequently, the suitability of this method needs to be tested in late November/early December. It is likely that the relationship will be different in winter, as squirrel feeding activity is greatly reduced by restricted daylight hours (Thompson, 1977), affecting access to bait piles.

In all woods, cameras were deployed at 1/ha. To improve cost-effectiveness, it may be worth modelling lower densities of cameras. However, as each camera was associated with a bait pile, which will affect the distribution of squirrels in a wood and, thus, the detection of squirrels at other bait piles, cameras cannot be modelled independently, and field trials would have to be conducted with lower numbers of cameras and associated bait piles to assess the comparative effect of lowering camera density.

The method presented in this study is highly adaptable to other rodents and small mammal species in different environments, however, confirmation of estimate accuracy would initially be required with an alternative robust technique to measure population sizes. Once achieved, this camera trap method has the potential to be more cost-effective and more employable than other approaches.

5.6 Conclusion

This study demonstrated that a camera index based on the number of photographs per camera per day could be used estimate target densities of grey squirrels in woods, to inform the number of feeders or traps required for effective grey squirrel control. The method was cost-effective and practical, with density estimates achieved within 6 days, with low budget cameras, minimum equipment and a low level of technical input. Providing that estimate accuracy can be initially confirmed with an alternative reliable density method, this method could be adapted to other rodents and small mammal species in other environments.

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Chapter 6. Developing species-specific feeders for oral contraceptive delivery

6.1 Abstract

The oral contraceptives in development for grey squirrels in the UK are only mammal-specific, rather than species-specific. It is therefore important that a species-specific bait delivery system is developed and tested to ensure contraceptives will be administered in a targeted and cost-effective way, with minimal impact on the environment and on nontarget species. There are feeding devices available that are designed to target species and deliver toxins, vaccines and other pharmaceuticals, including oral contraceptives. These devices are based on basic principles, with nontarget access prevention achieved through a door or lid, a specific entrance shape and by taking advantage of specific behavioural traits or physical capabilities of the target species.

For most of the UK, the only squirrel species present is the grey squirrel and grey squirrels are often unique amongst other wildlife species in terms of their body size, colour, behaviour and locomotion. This means that, in most areas, a feeder with a weighted bait door may be sufficient to exclude most other species of UK wildlife. Due to the morphological and behavioural overlaps between grey and red squirrels, a more complex design will be required to distinguish between the two species in areas where they coexist. This study had two main objectives; 1. Test whether a feeder with a weighted door can be accessed by grey squirrels only in areas where red squirrel do not occur; and 2. Test whether a feeder can be developed that utilises body weight to allow access by most grey squirrels whilst excluding red squirrels.

I tested the bait hoppers I developed in Chapter 2 and utilised in the bait trials in chapters 3 and 4 for their species-selectivity in 10 woodlands in Yorkshire, with only grey squirrels, not reds, present. The bait compartment was contained within a metal tube, which was accessed by a weighted door. The hopper doors were recorded opening on 26,758 occasions, with 18,851 identified as grey squirrels and 9 occasions identified as wood mice.

For the second study, I used squirrel feeders with integrated automatic weighing platforms to gather data on red squirrel weights from 8 red squirrel habitats in Cumbria, Northumberland,

North Yorkshire and North Wales at different times of year. The maximum red squirrel weights recorded were compared to the weights of grey squirrels collected at the same time of year, as part of the bait uptake trials described in Chapters 3 and 4. From the data collected, a prototype feeder was developed, and tested in a small pilot trial, in a woodland in Cumbria where both red and grey squirrels were present. The results from the red and grey squirrel body weight comparison suggested that body weight could be used to select between the two species, with a 450 g weight threshold allowing over 90% of adult greys access to a bait, while excluding all reds. The red squirrel body weights were very consistent across sites, months and years, suggesting that one weight threshold could be used for different red squirrel habitats. In a three week field trial in Cumbria, a prototype selective feeder, with a 450 g weight threshold, allowed grey squirrels to access the bait for most attempts, but prevented red squirrels from accessing the bait. This provided an important proof of concept.

The analyses conducted for both of the objectives in this chapter suggested that it should be feasible to develop cost-effective species-selective contraceptive feeders for areas of the UK where only grey squirrels are present and areas of the UK where both red and grey squirrels are present.

6.2 Introduction

The oral contraceptives in development for grey squirrels in the UK are only mammal-specific, rather than species-specific (UK Squirrel Accord, 2024). It is therefore important that a species-specific bait delivery system is developed and tested to ensure contraceptives will be administered in a targeted and cost-effective way, with minimal impact on the environment and on nontarget species.

Contraceptives can be designed to be more target specific in several ways. For instance, a bait that is liquid or viscous in consistency will not be as easily removed from a feeder as a solid one, thus minimising the risk that the contraceptive is taken outside the feeder and made available to non-target species. This is particularly important for squirrels and other rodents that scatter food or that cache it underground for later consumption (Hopewell & Leaver, 2008; Smith & Reichman, 1984). Ensuring animals consume bait in situ also allows more accurate estimation of the contraceptive dose rates that could be expected in field conditions and will reduce bait waste, thus reducing associated costs and field effort required for delivering contraceptives. The taste of a bait and whether it is animal-based or plant-based, may ensure that it is more likely to be consumed by the target species over nontargets.

There are feeding devices available that are designed to target species and deliver toxins, vaccines and other pharmaceuticals, including oral contraceptives. These devices are based on basic principles, with nontarget access prevention achieved through a door or lid, a specific entrance shape and by taking advantage of specific behavioural traits or physical capabilities of the target species. For example, bait boxes designed for the delivery of the oral contraceptive, ContraPest®, encapsulate a short, narrow tunnel system, that can be easily navigated by rats, which are generally smaller, more nimble, more intelligent and less neophobic than many potential nontarget animals (Senestech, 2024). A contraceptive bait feeder for wild boar has also been developed that requires the animals to lift a conical-shaped lid with their head to access the bait. The lid is a weight and shape that is impossible to lift for most other wild mammal species that inhabit the European locations in which it was tested (e.g. Massei et al. 2010; Campbell et al. 2011; Ferretti et al., 2014).

Ideally the design of a species-specific feeder will be as basic as possible, as it will have to be cost-effective, easy to maintain and robust enough to use in different environments and weather conditions. It is likely that, to deliver oral contraceptives to grey squirrels, two feeder designs will be required. Most of the UK has only the grey squirrel present and in most of these areas, grey squirrels are unique amongst wildlife species in terms of their body size, colour, behaviour and locomotion. This means that, in most areas, a feeder with a weighted bait door may be sufficient to exclude most other species of UK wildlife. Due to the morphological and behavioural overlaps between grey and red squirrels, a more complex design will be required to distinguish between the two species in areas where they coexist.

In this study I tested the bait hoppers I developed in Chapter 2 and used in the bait trials in chapters 3 and 4 for their species-selectivity. The hoppers were designed to minimise nontarget access in several ways. The bait compartment was contained within a metal tube, which was accessed by a door. The positioning of the door was based on a previous hopper design, developed by the Forestry Commission. This was designed to deliver the anticoagulant rodenticide Warfarin to grey squirrels (Mayle et al., 2007), but the license to use it was revoked by the EU in 2014, due to welfare concerns. The door has a 70 g weight and pivots on a top hinge, angled to encourage a squirrel to push it open with its head to access the bait. The weight is designed to exclude smaller animals from consuming bait. To reduce nontarget access further, each hopper was fixed to a wooden stand (approximately 1-meter high). In each case, the bait used was a hazelnut butter: a thick, slightly fluid paste, that had been found to be attractive to squirrels in previous trials but was difficult to remove from the hoppers and had to be eaten in situ. In the bait trials in Chapters 3 and 4, very little bait spillage was recorded during daily checks. The hoppers recorded, via a magnetic door switch, the date and time the bait door was opened and each bait hopper was monitored using a camera trap, to identify which animals had opened the door.

I conducted trials to assess whether squirrel body weight could be used to select between grey and red squirrels so that a bait hopper could be developed that would allow most adult greys access to a bait, while excluding all red squirrels. The maximum red squirrel body weight is reported to be 350g, while the weight range for adult grey squirrels is reported to be 440-650g (Mammal Society, 2024). By setting a minimum weight threshold required to open a feeder door, therefore, body weight should provide a relatively simple way to distinguish between the two species. To assess how effective this would be, more detailed data were required on the comparative bodyweights of the two species in different seasons and locations.

For the assessment, I commissioned the design and construction of squirrel feeders with integrated automatic weighing platforms. This was conducted by a practitioner with expertise in this area. These devices were used to gather data on red squirrel weights from a number of red squirrel habitats in Cumbria, Northumberland, North Yorkshire and North Wales. The maximum red squirrel weights recorded were compared to the weights of grey squirrels collected at the same time of year, as part of the bait uptake trials described in Chapters 3 and 4. From the data collected, a prototype feeder was developed and tested in a small pilot trial, in an area of Cumbria where both red and grey squirrels were present.

This study had two main aims; 1. Test whether a feeder with a weighted door can be accessed by grey squirrels only in areas where red squirrel do not occur; and 2. Test whether a feeder can be developed that utilises body weight to allow access by most grey squirrels whilst excluding red squirrels.

6.3 Materials and Methods

Field trials assessing the species-specificity of a bait hopper with a weighted door

Trials were conducted between 2018 and 2023 in 10 independent woods (4 woods in winter, 4 woods in summer and 2 woods in spring) that were located within 25 km of York, England (53.96ºN, -1.09ºE). In all these woods grey squirrels, but not red squirrels, were present. Data for these trials were collected simultaneously with data collected for most of the bait uptake trials described in Chapters 3 and 4, or as part of other studies. Wood selection was dependent on landowner permission and restricted to those that had a good level of access for field operatives but very low levels of public access. For these field trials, I led the study design and analysis and conducted most of the field work and data processing, with assistance from Animal and Plant Health Agency (APHA) colleagues, practitioner or volunteers, that I had trained in the methodology or that were already experienced in some of the techniques.

Between 14 and 30 hoppers were deployed, approximately evenly spread across each wood. For hopper design and setup, see Chapter 2 and Figure 1. Each hopper was monitored for 4 days (approximately 96 hours), during which it was baited daily with 40 g of 100% hazelnut paste mixed with the bait marker dye Rhodamine B (Merck Life Sciences UK Limited, Dorset, UK) at a concentration of 0.18% (see Chapter 3). The bait was manually weighed in and out of the hoppers each day. Hoppers recorded the date and time the bait door was opened, through the engagement and disengagement of a magnetic switch (Chapter 2). Hoppers were monitored with Reconyx™ HC500 or HS2X cameras set to high sensitivity, fixed to a tree and focused on the hopper entrance (Figure 6.1).

For the first year, HC500 cameras were primarily used, fixed within 4 meters of the hopper and set to record three photos per trigger. To improve the detection probability of animals entering the hoppers, from 2019 onwards, HS2X cameras were primarily used, the distance between the camera and hopper was reduced to within 2 meters and the cameras set to take one photo and a 10 second video per trigger. To reduce the amount of processing time required, only photos and videos recorded within 60 seconds of the hopper door opening were analysed, to identify the animal that opened it. This interval was used as the camera trigger often did not coincide exactly with the door switch activation and the clocks on both devices were manually set, so were not exactly syncronised.

Field trials assessing whether body weight could be used to distinguish between grey and red squirrels to design a selective contraceptive feeder

For this study, a squirrel feeder with an integrated automatic weighing platform was designed and developed by a practitioner, Barry Bickerton, with some guidance on the design provided by myself. The feeder consisted of a wooden bait compartment fixed to a 1-metre-high wooden stand. In front of the compartment was a load cell covered by a 12 x 20 cm metal weighing platform, connected to a battery pack and SD card reader, contained within a plastic, waterproof box (Figure 6.2). When an animal stood on the weighing platform, the load cell recorded 20 weights every 2 seconds, to minimise weight variation caused by animals moving around. Data transfer was managed by an Arduino microcontroller board, which read data from the load cell and converted it into grams. The weight in grams was then saved to an SD card, together with the time and date read from a real time clock module. A metal mesh tunnel with a clear plastic roof covered the weighing platform. This was designed to prevent more than one squirrel from sitting on the scales at once and to prevent rain or debris falling onto the scales and affecting the readings or function.

Between 2021 and 2023, 1-5 weighing devices were deployed in 8 areas of woodland during 12 separate trials conducted in Northumberland, Cumbria, North Yorkshire and North Wales. All of these woodlands were known to be inhabited by red squirrels. Trials were conducted at some areas twice, in different months of the year, to assess weight variation throughout the year. Some areas were chosen as they were known to have grey squirrel present, to test whether competition between the two species would affect red squirrel bodyweights. For most trials, I deployed the weighing devices with the help of local red squirrel volunteer groups or practitioners. In each location, the devices were positioned at least 200 meters apart, to record weights from as many different red squirrels as possible.

Each weighing device was monitored with a Reconyx™ HS2X camera, fixed to a tree and focussed on the weighing platform. When the weighing device was first set up, a 200 g check weight was placed on the weighing platform, to check the accuracy of the scales. The bait compartment of the weighing device was filled with approximately 500 g of bait (peanuts, sunflower seeds and hazelnuts with an approximate mix ratio of 45:45:10), except for one trial, where only whole hazelnuts were used as the preferred bait choice of the squirrels in the area. The volunteers/practitioners rebaited the feeders every 2-4 days for between 7 and 16 days, dependent on volunteer/practitioner availability. All the photos and videos recorded within 60 seconds of each weight recorded were used to identify the animal weighed. As with the door switch data in objective 1, this interval was used as the camera trigger often did not coincide exactly with the weight record and the clocks on both devices were manually set, so were not exactly synronised.

I first compared the maximum weights recorded for red squirrel at each site to grey squirrel weights collected in Chapters 3 and 4, to assess the degree of overlap between the two species. As the weight data collected in these studies were limited in terms of season and location recorded, I then requested additional weights from practitioners undertaking grey squirrel control across northern England and Scotland, in areas where there were red squirrels present. I also compared these weights with the maximum red squirrel weights.

From the body weight comparison (see Results), I assigned a weight threshold of 450 g and commissioned the design of a prototype selective feeder based on this weight. The selective feeder consisted of a passive infra-red sensor, which, when it detected movement, activated a weighing platform, similar in design to the automatic weighing devices (Figure 6.3). If the weight recorded was at least 450 g, a signal was sent to a radio control servo to open a door to the bait compartment. If the weight fell below 450 g, the door closed. I deployed the prototype in a woodland in Cumbria, where both red and grey squirrels were present.

The feeder was baited for 26 days with whole hazelnuts and a few grams of 100% hazelnut paste, either by myself or a local red squirrel practitioner. The feeder used the same mesh tunnel design as the weighing devices, to restrict access to one squirrel at a time. All videos were analysed and each animal at the feeder identified, along with whether or not they had opened the door.

a)

b)

Figure 6.1. A Reconyx™ HS2X camera-trap set up to monitor bait hopper use by grey squirrels and nontarget animals (a) and photographs used to identify a grey squirrel and a wood mouse that had opened the hopper door, based on the photos or videos recorded within 60 seconds of the door switch being activated (b).

a)

b)

Figure 6.2. A red squirrel feeder with integrated automatic weighing scales, designed to record the body weights of red squirrels that feed from it (a). The weights, together with the date and time they were recorded, were stored on an SD card. Animals using the feeder were identified from photos and videos recorded by a Reconyx™ HS2X camera focused on the entrance of the feeder (b). Although multiple red squirrels were recorded around the feeders, the weighing platform and tunnel size meant that no more than one individual was ever recorded on the weighing platform at one time.

a)

b)

Figure 6.3. A grey squirrel selective feeder based on body weight (a). When a passive infrared sensor detected movement, the weighing platform was activated. If the weight was at least 450 g, the electronic bait door opened and stayed open for as long as the weight was maintained on the platform. The feeder entrance was monitored using a Browning^{TM} Dark Ops Pro X 1080 camera trap, set to record one photo and one 10 second video per trigger, to identify animals using the feeder.

6.4 Results

Field trials assessing the species-specificity for a bait hopper with a weighted door

The 172 hoppers analysed from the 10 trial sites recorded 81,410 photos or videos, with all hoppers recording grey squirrels. A number of potential nontarget mammalian and avian wildlife species were recorded by the cameras at each site (Table 6.1). Grey squirrels, wood mice, field voles, great tits, robins and greater spotted woodpeckers were the only species observed entering the hoppers i.e. were recorded placing at least one foot inside the entrance.

Table 6.1. Wildlife species recorded by camera traps monitoring of 172 bait hoppers deployed between 2018 and 2023 at 10 different woodland sites in Yorkshire, UK. Included is whether the animal entered the hopper (was recorded placing at least one foot inside the entrance).

Species recorded		N Sites	N hoppers entered
Mammalia	Grey squirrel *Sciurus carolinensis*	10	164
	Roe deer *Capreolus capreolus*	10	0
	Wood mouse *Apodemus sylvaticus*	7	14
	Badger *Meles meles*	6	0
	Red fox *Vulpes vulpes*	6	0
	Rabbit *Oryctolagus cuniculus*	4	0
	Brown rat *Rattus norvegicas*	3	0
	European hare *Lepus europaeus*	2	0
	Field vole *Microtus agrestis*	1	1
	Hedgehog *Erinaceus europaeus*	1	0
Aves	Great tit *Parus major*	8	6
	Robin *Erithacus rubecula*	8	3
	Common pheasant *Phasianus colchicus*	6	0
	Jay *Garrulus glandarius*	6	0
	Tit (other) *Paridae* spp.	4	0
	Greater spotted woodpecker *Dendrocopos major*	2	1
	Other	7	0

The hopper doors were recorded opening on 26,758 occasions (Table 6.2) with 18,851 identified as grey squirrels, 9 occasions identified as wood mice and the remainder not identifiable (Figure 6.1). Three videos recording wood mice entering one hopper at one site suggested that the door was only briefly nudged open by the mice but also that it was unlikely that any bait was taken.

Table 6.2. Results from hopper species-specificity trials conducted at 10 woods in Yorkshire, UK. Between 9 and 22 hoppers were deployed in each wood. Each hopper was monitored with a camera trap, to allow the identification of any animal that opened a door to access the bait compartment. The hoppers recorded the date and time the door was opened and the this was matched to either grey squirrels or nontargets, from photos or videos taken within 60 seconds of the door opening.

Year	Month	Wood ID	No. hoppers	Total no. photos/ videos	No. times door opened	No. matched to grey squirrel	No. matched to nontargets	No. trigger not identifiable
2018	January	HW	19	12,633	1,782	1,074	0	711
		SC	22	13,416	3,909	2,165	0	1,744
	July	LT	29	5,387	1,617	1,259	0	268
		PA	17	1,452	1,355	681	0	674
		FO	9	951	1,408	652	0	1,069
	August	BP	15	3,022	2,577	1,254	0	1,323
2022	June/July	PW	18	6,941	4,299	3,571	0	723
		SC	18	5,609	2,747	2,022	0	615
2023	May	GE	13	15,743	3,652	3,449	0	203
		BW	11	16,256	3,419	3,037	9*	373
Total			172	81,410	26,765	19,164	9	7,703

* Wood mouse

For the rest of the times the door was opened, the animal could not be identified as there was no associated photo or video within 60 seconds of the door switch activation. In the first year of the trials, 46% of times the door was opened but the animal could not be identified. From 2019 onwards, when cameras were positioned closer to the hoppers, the newer model of camera was used and video function was added, the number of occasions where the door was open but the animal was not identified was reduced to 12%.

Objective 2. Field trials assessing whether body weight could be used to distinguish between grey and red squirrels to design a selective contraceptive feeder

The maximum red squirrel weights collected from 10 trials were 378-442 g (Table 6.3). Weights recorded were consistent (within 50 g) between squirrels from the same areas at different times of year and between areas that did and did not have grey squirrels present surveyed at the same time of year. The heaviest weights recorded in each area were those from red squirrels, or grey squirrels if present.

In total, 205 grey squirrels were weighed as part of the bait uptake trials conducted in six woods in Yorkshire, described in Chapter 4. In two woods studied in winter 2018, 96% of 50 squirrels weighed were at least 450 g, in two woods studied in summer 2022, 94% of 89 grey squirrels in two woods weighed at least 450 g and in two woods studied in spring 2023, 92% of the 66 grey squirrels weighed were at least 450 g. In total, 3.4% of the grey squirrels weighed in all six woods were aged as juvenile (see Chapter 4).

Table 6.3. The maximum red squirrel weight ranges recorded by automatic weighing devices deployed in different red squirrel woodlands in the north of England and north of Wales. Weighing devices were deployed and baited for 7-16 days at each location. Some devices could not be analysed due to faults with the weighing system or the associated camera trap. The total number of weighing devices analysed, number of days analysed and number of weight records matched to red squirrels are included.

Location	Year	Month	N days	N weighing devices analysed	N days analysed		N records	Max. red squirrel weights (g)
					Mean	Range		
Horsley, Northumberland	2021	Nov/Dec	13	5	10	2-13	1164	424
	2022	July	9	3	9	9	373	378
Grasmere, Cumbria*	2022	Aug	8	5	6	3-8	307	429
Otterburn, Northumberland	2022	Jan	11-14	5	12	11-14	1946	397
		July/Aug	7	5	7	7	3271	429
Snaizeholme, North Yorkshire	2022	June	8	1	3	3	497	410
Beckermonds, North Yorkshire	2023	June/July	7	4	3	2-4	5899	435
Clocaenog, Denbighshire*	2023	August	8	4	8	8	88	431
Hepple, Northumberland	2023	August	16	4	16	16	2563	442
Newby Bridge, Cumbria*	2023	July/Aug	15	1	15	15	230	429

*Locations with grey squirrels present, confirmed on cameras

From the comparative body weights of red and grey squirrels collected, a 450 g weight threshold was selected for the selective feeder. During the 26 day monitoring period for the grey squirrel selective feeder, the camera trap recorded 23 visits by grey squirrels. On 7 occasions, the grey squirrels activated the bait door to open and on six occasions the door remained closed; 10 occasions were inconclusive as the full visit was not captured on video. On 2 of the occasions the door did not open, the squirrel was only very briefly on the platform. The camera recorded 4 visits by red squirrels. On one occasion, the red squirrel was chased off the feeder by a grey squirrel, on two occasions the red squirrel was on the weighing platform and the door was not activated and on one occasion it is was inconclusive, as the full visit was not recorded. The only other animals to be recorded on the weighing platform were wood mice, on 24 visits, none of which activated the door.

6.5 Discussion

The analyses conducted for both of the objectives in this chapter suggested that it should be feasible to develop cost-effective species-selective contraceptive feeders for areas of the UK where only grey squirrels are present and areas of the UK where both red and grey squirrels are present. Ideally, an oral contraceptive feeder would be 100% selective, only delivering bait to the target species, to ensure no nontarget impact and to minimise bait waste. In reality, some bait consumption by nontargets might be unavoidable; although it would be particularly important to minimise access to oral contraceptives for species of conservation importance or for domestic animals.

The trials conducted demonstrated that a relatively simple mechanical feeder with a weighted bait door was very effective in delivering baits to grey squirrels, while woodmice were the only nontarget species recorded opening the door, but on only 9 of the 19,062 occasions that the animal opening the door was identified and at only one of the ten sites trialled. The associated videos also suggested that it was unlikely that the mice were able to access the bait on these occasions. Wood mice are highly abundant, widespread rodents which have a high reproductive rate, a life span of under 1 year and a conservation status of ‘Least Concern’ for the UK and the rest of the world (Mammal Society, 2024). It is therefore unlikely that the administration of a contraceptive to some individuals would have any meaningful impact on local populations.

Wood mice are, however a prey species for many other animals including foxes, weasels, tawny owls and domestic cats. It is therfore important that any residual biological effect caused by the consumption of prey species that has eaten a contraceptive is assessed before a contraceptive is registered for general use.

Although the data gathered provided compelling evidence for the bait hopper's high degree of species selectivity, the locations at which the trials were conducted had a narrow geographical range, with all ten sites located within 25 km of York, England. Also, all sites were broadleaf or mixed broadleaf woodlands. The range of wildlife species detected at these sites were typical of UK woodlands; however, further trials are required in other habitats and regions of the UK to ensure that no variations in widlife behaviour, species distribution or abundance will affect the selectivity of the feeder.

UK species not present at the trial sites included larger ungulates such as red deer *Cervus elaphus*, fallow deer *Dama dama* and wild boar *Sus scrofa*. It is unlikely that the bait hoppers would be robust enough to prevent access from these species or larger livestock animals such as cattle. Placing the bait hoppers in trees or other elevated locations would, however, prevent access from these animals. Some UK mustelids such as the pine marten *Martes martes* and pole cat *Mustela putorius*, were not present in the trial locations. Mustelids are typically good climbers and opportunistic feeders, so could pose a challenge in terms of nontarget prevention. A more sophisticated approach would therefore be required, potentially using physical barriers and/or an upper body weight limit, to prevent access. It is, however, important that any further intervention does not deter grey squirrels from entering feeders.

The results from the red and grey squirrel body weight comparison suggested that body weight could be used to select between the two species, with a 450 g weight threshold allowing over 90% of adult greys access to a bait, while excluding all reds. The red squirrel body weights were very consistent across sites, months and years, suggesting that one weight threshold could be used for different red squirrel habitats. However, if there were concerns that there were heavier reds in an area where a contraceptive was to be deployed, the feeders could be monitored with a non-contraceptive bait first, using a similar camera trap set up to the selective feeder trial and the weight threshold adjusted accordingly.

The minimum grey squirrel weights recorded were also consistent across the sites, years and months sampled. Additonal grey squirrels weights are required from different sites, years and months, to fully understand what proportion of grey squirrels would be excluded from a contraceptive feeder for a given weight threshold. It is likely that the grey squirrels with the smaller body weights were juveniles or sexually immature animals. As male and female grey squirrels reach sexual maturity after 10 months (Hayssen, 2016), it is unlikely that delivering a contraceptive to juvenile grey squirrels would influence population reduction, unless the longevity of a contraceptive effect extends beyond this time. If this is not the case, any selective feeder that excludes juveniles may offer more cost-effective delivery, as the contraceptive will be targetted towards breeding individuals, thus minimising bait waste.

The selective feeder trial provided an important proof of concept; through the use of a bodyweight threshold, a bait could be remotely delivered to grey squirrels and that red squirrels could be prevented from accessing a bait. Additional trials are required to test the feeder in different habitats, regions and times of year to fully assess the effectiveness of the feeder. This design could be used in comparative scenarios, to deliver baits targetted towards small mammal species, in areas where co-occurring nontarget species do not have the same body size of grey squirrels.

6.6 Conclusion

From the trials conducted in this study, I found that a feeder with a weighted bait door proved to be successful in excluding most other species of UK wildlife, in areas where there are grey squirrels, but no red squirrels present. I also found that body weight could be used to develop a feeder that allows access by most grey squirrels whilst excluding red squirrels. This was confirmed from data collected from a prototype selective feeder where both red and grey squirrels were present. Additional trials are required to test the feeder in different habitats, regions and times of year to fully assess the effectiveness of the feeder. This design could be used in comparative scenarios, to deliver baits targetted towards small mammal species, in areas where co-occurring nontarget species do not have the same body size of grey squirrels.

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Chapter 7. Discussion

7.1 Study background

An oral contraceptive vaccine is being developed for the control of grey squirrels in the UK. For practical applications, it is crucial that a suitable bait delivery system is available to ensure oral contraceptives can be delivered to enough grey squirrels, with minimal impact on nontarget animals. There are over 2.5 million grey squirrels distributed across the UK (Croft et al., 2017; Mathews et al., 2018). The successful management of grey squirrels will therefore require landscape-level coordinated control. Oral contraceptives deployed on this scale will require a delivery system that is effective, practical, targeted and economically viable.

This study focused on the development and testing of a system to deliver oral contraceptives to the invasive, non-native grey squirrel *Sciurus carolinensis* in the UK. The findings suggest that oral contraceptives could be delivered in baits via species-specific feeders to most grey squirrels in most woodlands with minimal impact on non-target species, using practical and cost-effective methods. In this chapter I provide a background to the study, discuss the key findings, limitations of the methodology used, further work required and the wider applications of the results.

7.2 Measuring individual bait uptake in grey squirrels

To maximise effectiveness, oral contraceptive delivery should predominantly target the reproductively active females in a population (Massei. 2023; Massei & Cowan, 2014). In UK woodlands, there are two main peaks in grey squirrel mating: December to January and April to May; some females produce two litters within the same year (Gurnell, 1996). The average size of a grey squirrel home range is less than 5 ha (Lawton et al., 2016; Wauters et al., 2002) and average grey squirrel density is reported as 4-5 squirrels/ha, ranging from less than 1 to over 13 squirrels/ha (chapter 4; Merrick et al., 2016). In group-living rodents, such as the grey squirrel, high reproductive rates and high population densities may result in intraspecific competition for resources, particularly between individuals of different sex and breeding status.

Bait uptake by wildlife can be affected by many factors, such as sex (Whisson & Salmon 2009), the time of year at which bait is deployed (Robinson, Cushman, & Lucid 2017), bait density and distribution (Jacob et al., 2003), the availability of alternative food sources (McRae et al. 2020) and the density of the target species (Haley, Berentsen, & Engeman 2019). Understanding how bait uptake differs between individuals is important to optimise delivery methods and ensure enough animals receive an effective dose. This will also help minimise bait waste and the associated effort and cost of bait delivery in the field. To collect these data, animals need to be individually identifiable, and a suitable system designed to accurately record the weight of bait consumed.

In Chapter 2, I demonstrated that a novel design of feeder, or bait hopper, with integrated PIT-tag reader and strain gauge, could accurately measure feeding visits and the amount of bait taken per visit. In Chapter 4, I demonstrated that this device could be used remotely in the field to accurately measure the amount of bait taken by of individual squirrels, with minimal human interference and a high identification accuracy. The unique modifications to the hopper design described in Chapter 2 resulted in improved data collection, practicality, field robustness and battery life in the field, when compared to other similar devices designed to monitor feeding behaviour using PIT-tags (Kenwood et al., 2005).

Automatic strain gauges are used to measure food consumption from feeders in laboratory rat trials, however, to link the data to individuals, animals are usually singularly housed (Farley et al., 2003). This means that bait consumption is less likely to be representative of free-living animals, as factors such as interspecific competition, alternative food availability and weather conditions, will be absent. When singularly housing captive animals, there will be also additional ethical considerations and economic costs, which are likely to limit the numbers of animals that can be assessed. Remote devices with strain gauges have been used to measure the body weights of animals in the field (Bosch et al., 2015), but none have demonstrated the accuracy of weighing necessary to calculate the dose rates of contraceptives or other drugs.

Neophobia towards bait stations may affect bait uptake in rodents (Inglis et al., 1996). In this study 58% of PIT-tagged females and 67% of PIT-tagged males, visited hoppers within the first week of hopper deployment, suggesting that neophobia did not prevent bait uptake in most squirrels. The attractiveness of the bait used, 100% hazelnut butter, may have encouraged squirrels to overcome any neophobia of devices. Bait or lure development is an important aspect of wildlife control that uses devices such as bait dispensers or traps, as attractive baits or lures can lead to a large increase in the numbers of individuals targeted (Takács et al., 2018). Most of the male and female grey squirrels PIT-tagged visited hoppers multiple times and on most days of the four-day bait uptake monitoring demonstrating that, if oral contraceptives were deployed in baits, individuals that visited hoppers would likely receive multiple doses within a short space of time. This is important for immunocontraceptive vaccines or synthetic hormones, that require multiple doses to ensure infertility (Chen et al., 2022; Massei et al., 2020).

The PIT-tag trials suggested that social interactions like bait monopolisation or aggression are unlikely to reduce oral contraceptive delivery effectiveness, providing the ratio of hoppers to squirrels is sufficient. The number of feeding visits and number of hoppers visited was slightly higher in male squirrels then females; overall, however, the sexes consumed very similar amounts of bait and exhibited similar bait uptake patterns. Importantly, there was no indication that males monopolised the bait and prevented access by females, as found in other studies on rodents (Whisson and Salmon, 2009). However, the probability of squirrels entering hoppers declined considerably with increasing numbers of squirrels per hopper and some displacement of individuals from hoppers by other squirrels was observed. This indicates that density-related social interactions may influence feeding behaviour in grey squirrel populations, which has been found in other studies on the grey squirrel (Gurnell, 1996). Body weight has been found to influence bait uptake between individual Norway rats of the same sex (Inglis et al., 1996); however, no evidence was found that body weight influenced bait uptake by grey squirrels. Similarly, there was no evidence that reproductive status or age affected bait uptake, from additional analyses conducted on some of the grey squirrel PIT-tag datasets from this study (Tiberi et al., 2024).

Season is another important factor affecting bait uptake and contraceptive delivery, particularly if the duration of the effect of a contraceptive is relatively short, making it necessary to treat females immediately prior to mating. There are little published data on the long-term effects of contraceptive vaccines and the results will be highly specific to the species investigated. Infertility from treatment with injectable GnRH based contraceptives has been shown to last over a year for most individuals post-treatment in wild boar *Sus scrofa* (Massei et al., 2012), white tailed deer *Odocoileus virginianus* (Gionfriddo et al., 2011) and black tailed prairie dogs *Cynomys ludovicianus* (Yoder & Miller, 2010), while infertility has been observed in laboratory rats up to approximately 4 months (Pinkham et al., 2022).

The effects of the oral contraceptives currently available for rodent control, EP-1 and Contrapest, are reversible requiring multiple doses to achieve and maintain an effect (Witmer et al., 2017; Witmer & Raymond-Whish, 2021). The harshness of the gastrointestinal environment, leading to degradation of fragile antigens and the requirement of adequate doses to generate immunity instead of vaccine tolerance, mean that achieving a consistent and effective dose for an oral contraceptive vaccine is very challenging (Ramirez et al., 2017). The effects of oral contraceptives may reverse if antibody titre levels decline over time (Massei et al., 2020; Pinkham et al., 2022). It is therefore likely that the impacts of oral GnRH based contraceptives will last less than a year and it may be necessary to deploy a contraceptive in different seasons to maintain effectiveness.

Findings from the PIT-tag field trials (chapter 4) suggested that spring is likely to be the best season to deliver oral contraceptives to female squirrels, as this was the only season where female squirrels were more likely to visit hoppers and had a higher bait uptake than males. Male and female squirrels were also likely to consume more bait in spring. The next best bait uptake was observed in winter, followed by summer. Bait uptake may be greater in winter and spring, as many of the squirrel's main natural food resources are depleted and they are reliant on cached food (Steele & Wauters, 2016). Summer may not be the best time of year for bait deployment, due to inconsistencies in movement, activity and bait uptake amongst squirrel populations, particularly for female squirrels. Autumn was not included in the study as this is when tree seeds are at their most abundant, so supplementary feeding is unlikely to be successful as demonstrated by low trapping rates (Gurnell, 1996).

In this study, all animals PIT-tagged under anaesthesia and under a UK Home Office Project License, using specialist pharmaceuticals and equipment. The PIT-tag readers used were bespoke and the PIT-tagging process required extra weeks in the field. All of these factors meant that the PIT-tag trials were more expensive and labour-intensive than other data collection methods used. With the resource confines of the study, it was only possible to conduct trials in two woods per season, conducted in separate years. Grey squirrel feeding and breeding behaviour is subject to annual variation, with the relative proportion of females that produce litters in spring, summer or both dependent on food availability related to the seed mast crop of the previous autumn and the severity of the previous winter (Gurnell, 1996; Wimpenny et al., 2021). In good seed years, grey squirrel reproduction will start in winter, in bad seed years, it will be delayed until the following spring. To confirm the findings of the PIT-tag trials, additional trials are required in winter, summer and spring in multiple years and in a range of different habitats and locations, to assess any annual or regional variation in squirrel feeding behaviour and optimise bait deployment.

7.3 Measuring population level bait uptake in grey squirrels

The effectiveness of a contraceptive is determined by the number of individuals it is delivered to, together with the proportion of individuals rendered infertile. In Chapter 2, rhodamine B (RB) already used as bait marker in small mammals, including those of the Sciuridae family (Fernandez & Rocke, 2011), was tested in grey squirrels. It is important to trial different concentrations of RB on the target species, as the detection probability and palatability of RB are dependent on the concentration used, which is species-specific and related to body weight (Fisher, 1999).

The results from captive squirrel trials showed that a concentration of 0.18% RB mixed in hazelnut butter was as palatable to squirrels as hazelnut butter alone and that an individual needed to consume only small amounts of bait (<5 g) for it to be detected in the flank hair. These trials also estimated that RB detection was achieved at less than 17 mg/kg body weight. These findings are comparable to a study by Fernandez & Rocke (2011), who found that concentrations of 0.05% to 0.30% of RB in bait were readily accepted by prairie dogs and that RB concentrations of 12 mg/kg bodyweight were sufficient for detection in whiskers under UV light. Squirrel flank hair appeared to be more reliable than tail hair for RB detection, as tail hair was found to have more natural fluorescence that made lower levels of RB harder to identify.

The findings from the RB field trials (Chapter 3), showed that it was possible to deliver bait to the majority of grey squirrels in six out of ten woods within four days. Hopper density and squirrel density were important factors affecting bait uptake, with a higher proportion of the population consuming bait from three feeders per hectare rather than one per hectare. This is consistent with the results from the PIT-tag trial, that found that at least 1 hopper per 2 squirrels should be deployed to maintain a high level of bait uptake. Season also affected bait uptake, with more squirrels consuming bait in summer than in winter. Autumn was not included in the study due to natural food availability and spring was also not included, as it was initially hypothesised that bait deployment for an oral contraceptive would need to be every 6-12 months, to maintain a year-round effect. The results from Chapter 4 suggest that bait uptake should be more equal in summer and winter. The difference between the RB and PIT-tag results may have been caused by differences in sample sizes between the two studies and annual variation in summer bait uptake, as described before. In the PIT-tag study, two woods were trialled in summer 2022, while in the RB study, six woods were trialled in the summers of 2018 and 2019.

Fertility control models for ungulates have suggested that more than 50% of fertile females will need to be maintained infertile to achieve meaningful reductions in numbers, even when fertility rates are low (Hobbs et al., 2000). Comparatively, models for grey squirrels have suggested that a threshold of 75% contraceptive efficacy is required for effective management (Croft et al., 2021; Croft & Massei, 2024). Sterilisation studies conducted on rice field rats (*Rattus argentiventer*) and house mice have found that approximately 50–75% of the females in the population must be rendered infertile (Jacob et al. 2003 and 2008). The findings of both Chapters 3 and 4, suggest that these thresholds could be achievable through optimised bait delivery, providing the contraceptive has a high level of efficacy. For the woods where there was at least 1 hopper per 2 squirrels, the average number of male and female squirrels (66%) and females only (57%) that consumed RB from hoppers were comparable to the percentage of PIT-tagged male and female squirrels (62%) and female only (57%) that entered hoppers in the PIT-tag trials (Chapter 4).

It is also likely that the results from the RB assessment are conservative and may have underestimated bait uptake. Although the palatability of RB was assessed in captive squirrels, there were only 12 squirrels per treatment group, so it is possible that some individuals under field conditions may have found the RB bait unpalatable. This study and others have also suggested that humidity may reduce RB bait palatability in the field (Tolkachev, 2019). Research will be required to ensure an oral contraceptive is highly palatable to the target species.

The RB assessment was based on individuals trapped after bait deployment, so may have included individuals that were not present at the time of bait delivery, or that had moved into the area as a response to the removal, as observed in other studies (Lawton & Rochford, 2007). The movement of squirrels into the study woods was minimised by the short time period (less than three weeks) between the deployment of RB baits and the start of squirrel removal. Conversely, the PIT-tag assessment was based on only a proportion of the squirrel populations it was possible to trap and tag. The sample size used in this study was small and the number of woods was not balanced across seasons.

What constitutes effective control is subject to interpretation and is highly dependent on management goals. For example, there is some evidence that reducing grey squirrel densities to under 4 squirrels per ha, may significantly reduce tree damage (Beatham et al., unpublished data; Mayle & Broome, 2013). Models have also shown that fertility control may be most cost-effective when delivered after lethal control, once the target population density has been reduced, maintaining low densities and preventing recovery (Croft et al., 2020 and 2021).

Additional trials are required in different seasons, years, location and habitats, to further investigate the factors affecting population level bait uptake by grey squirrels, to optimise delivery methods. The used of two hoppers per ha should also be explored further, as a potentially more cost-effective method for delivering bait and different distribution patterns of hoppers my facilitate delivery.

7.4 Estimating densities of grey squirrels for population management

The findings from chapters 3 and 4 suggested that, to maximise bait uptake in grey squirrel populations, it is important to ensure there are at least 1 hopper per 2 squirrels deployed. The aim of the trials described in chapter 5 was to develop and test a cost-effective and practical method that could be used by practitioners to estimate densities of grey squirrels in woodlands and to improve the efficacy of management applications. The camera index developed in Chapter 5, had a strong linear association with densities of grey squirrels trapped and dispatched in ten woods in less than 3 weeks. The index that provided the best relationship ($R^2 = 0.90$) applied a 5-minute filter to all squirrel photographs recorded from 24:00 on the day the cameras were deployed, for at least two consecutive days. The linear association was strong, at the higher end of the scale compared with other studies (R^2 value range = 0.6 to 0.9) when matching camera-trap indices with independent estimates of mammal density (Rowcliffe et al., 2008; Rovero and Marshall, 2009; Villette et al., 2016, 2017). Other studies have found that applying a filter of at least 1 minute, will reduce the proportion of photographs triggered by the same animal (Yasuda, 2004) and thus improve the relationship between camera indices and animal density values (Villette et al., 2016, 2017; Massei et al., 2018). The optimum filter length is likely to be study specific, dependent on the species, environment and camera methodology used.

Indices based on photographic capture rates per effort are commonly used as proxies for population abundance (Palmer et al., 2018) and have been shown to provide accurate estimates of relative abundance for a range of mammal species in a variety of environments (Rowcliffe et al., 2008; Villette et al., 2016, 2017; Lambert et al., 2018). However, this continues to be an area of contention in ecology (Stephens et al., 2015). Studies that assume a direct relationship between detection rates and abundance often do not account for other factors that may affect this relationship, such as animal movement or activity levels, probability of detection and the effort employed to detect the animal (Pollock et al., 2002; Sollmann et al., 2013; Broadley et al., 2019).

Camera-trap based methods rely on random sampling and assume that animal movement and behaviour are not affected by camera trap presence; therefore, camera traps must be unbaited (Palencia et al., 2021). Although such methods have been used to measure landscape level densities of grey squirrels (Mason et al., 2022), as grey squirrels are largely arboreal, fast moving and relatively hard to detect compared with larger mammals, achieving accurate local densities of grey squirrels can be challenging. By luring animals to the camera field of view using bait, it was possible to capture a meaningful index of the resident squirrel population within 4 days.

The use of bait, to some extent, addresses some of the issues associated with indices, by increasing the consistency of squirrel activity levels and the probability of detection. The effort used to detect squirrels was standardised between woods by using a set density of cameras and averaging the number of photographs by the number of cameras and days they were deployed. To ensure the attractiveness of the bait to squirrels remained consistent across woods, cameras were deployed in all woods during early to mid-summer, at a time of year when natural food availability is relatively low. Overall, 98% of squirrel photographs contained only one squirrel. Previous studies on grey squirrels demonstrated a hierarchy of dominance over feeding resources (Pack et al., 1967). In all woods, squirrels outnumbered cameras deployed, and it appears that, to avoid confrontation, squirrels might stagger their access to bait over the course of a day as a consistent duration of squirrel activity was recorded by the cameras at all woods.

The use of a time filter helped to standardise animal detection rates between different camera models and locations. For example, locations that have a wider detection area and models that have faster trigger speeds are likely to record more photographs of the same individual for the same time period. In this study, this meant that the number of photographs recorded by low, medium and high budget camera models tested, that had very different specifications, were not significantly different, making the method more practical. Another advantage is that the index will be more robust to observer errors made in the photograph processing, for example, if a squirrel photograph is missed and is not tagged. This is because only one photograph per individual per bait pile visit needs to be accounted for. This also means the method may be more adaptable for use with machine learning automated image identification software, which can now achieve accuracies of over 90% (Tabak et al., 2019), thus offering a large reduction in processing time.

The camera index was found to be cost-effective for measuring a range of densities of squirrels in woodlands that could be targeted by traps or feeders, with estimates achievable within 5 days, however, further development is required to widen its application. For instance, the method needs to be tested on very low densities of squirrels, to ensure it can be used effectively to monitor the progress of eradications. Similarly, more data are required for woods in which squirrel density is over 7/ha, to test the relationship more thoroughly at the higher end of the scale. In addition, the method has so far only been tested in broadleaf or mixed woodlands in summer. Management through culling or contraceptives will likely be most effective when applied immediately before the grey squirrel's main breeding seasons, in spring and winter (Hayssen, 2016); consequently, the suitability of this method needs to be extensively tested in late November/early December. It is likely that the relationship will be different in winter, as squirrel feeding activity is greatly reduced by restricted daylight hours (Thompson, 1977), affecting access to bait stations piles.

This study exploited local eradications as an opportunity to estimate squirrel density and to compare this estimate with camera trap indices. If conducted correctly, local eradications can provide accurate estimates of mammal density in a defined area, as every individual in the population should be accounted for. If there is uncertainty that 100% eradication has been achieved, then the minimum number alive (MNA) per unit area can be calculated and used to estimate a minimum density. For instance, MNA densities calculated from live trapping have been shown to have a strong relationship with camera trap indices for snowshoe hares (Villette et al., 2017). The mean number of grey squirrels trapped in the ten study woods was 4.6 squirrels/ha, with a range of 1.7–10.6 squirrels/ha. As noted above, a mean density of 4/ha has been recorded for grey squirrels in broadleaf woodland in the United Kingdom, ranging from 0 to 13 per ha (Merrick et al., 2016).

There are several reasons why the density of squirrels trapped should reflect the total density in each wood. Trap effort was high and standardised between each wood, with cameras and traps deployed at the same time of year, across discrete woodland habitats, that were larger than the size of an average grey squirrel home range (5 ha; Wauters et al., 2002; Lawton et al., 2016). Trap density was high (3 ha^{-1}) and trapping was conducted consistently for between 5 and 15 days, until no squirrels were caught. Grey squirrels typically have a high capture probability and previous studies have confirmed, via capture mark recapture, that grey squirrel eradications in woods were achievable within 5 days, using trap densities of between 1 and 2 traps ha^{-1} (Lawton & Rochford, 2007). Thus, using 3 traps ha^{-1} with this methodology should enable the capture every squirrel within 5 trap days for even the highest densities of squirrels recorded in the United Kingdom. The density estimates produced from the trapping records also reflected those obtained in chapter 4, from capture mark recapture estimates based on PIT-tagged squirrels.

Grey squirrels quickly recolonise an area from which they have been removed (Lawton & Rochford, 2007). A short camera survey time (less than 5 days), the time between camera survey and squirrel removal (average 29 days) and the intensity and short period of removal (less than 3weeks) helped to maintain population closure. Closure was also likely to be maintained as studies on individually marked squirrels have shown they will typically move no more than 200 metres between baited traps or feeders within the timescale of the study

(Beatham et al., pers. obs., unpublished) and less than 500 metres between traps over several years (Taylor et al., 1971).

In all woods, cameras were deployed at 1 ha^{-1}. To improve cost-effectiveness, it may be worth modelling lower densities of cameras. However, as each camera was associated with a bait pile, which will affect the distribution of squirrels in a wood and, thus, the detection of squirrels at other bait piles, cameras cannot be modelled independently, and field trials should be conducted with lower numbers of cameras and associated bait stations to assess the comparative effect of lowering camera density.

7.5 Developing and testing the species-specificity of contraceptive delivery

Ensuring any bait delivery system is species-specific is crucial, particularly when bait containing contraceptives could be consumed by native species, like the red squirrel, similar in size and feeding habits to the target species. The bait used in the trials in chapters 3 and 4 had a 100% hazelnut butter base which had the consistency of a viscous paste. This bait type was chosen to ensure that it was attractive to squirrels yet difficult to remove from the hoppers. This, together with the use of a weighted door that had to be lifted to access the bait, meant it was more likely to be consumed in situ, minimising waste and availability to nontarget species. The lack of spillage recorded at the hoppers during the trials suggested that this was the case.

For areas where only grey squirrels were present, which is currently the case for most of the UK, the bait door and associated remote camera records analysed in Chapter 6 suggested that, on over 99% of occasions, the door was opened by grey squirrels. The bait delivery system based on a weighted bait door and on the hopper raised above the ground excluded common species of UK wildlife such as badger, roe deer, rats, mice, voles and various bird species. Although the data gathered provided compelling evidence for the bait hopper's high degree of species selectivity, the locations at which the trials were conducted had a narrow geographical range, with all ten sites located within 25 km of York, England. Also, all sites were broadleaf or mixed broadleaf woodlands. The range of wildlife species detected at these sites were typical of UK woodlands; however, further trials are required in other habitats and regions of the UK to ensure that no variations in wildlife behaviour, species distribution or abundance will affect the selectivity of the feeder.

The results from the red and grey squirrel body weight comparison suggested that body weight could be used to select between the two species, with a 450 g weight threshold allowing over 90% of adult greys access to a bait, while excluding all reds. Those animals lower than 450 g are likely to be immature animals. Male and female grey squirrels reach sexual maturity after 10 months (Hayssen, 2016) and it is unlikely that delivering a contraceptive to juvenile grey squirrels would influence population reduction unless the longevity of a contraceptive effect extends beyond this time. If this is not the case, any selective feeder that excludes juveniles may offer more cost-effective delivery, as the contraceptive will be more likely delivered to breeding individuals, thus minimising bait waste. The prototype tested provided an important proof of concept for the use of body weight as a selective tool. Additional red and grey squirrel weights are required from different sites, years and months, to confirm the weight threshold on a national scale and in additional seasons, years and environments.

7.6 Recommendations for practitioners based on study findings

Based on the findings of this study, to deploy an oral contraceptive in woodlands for effective and targeted grey squirrel management, I would recommend following the guidelines:

1. Deploy the contraceptive in spring, to ensure breeding females are targeted immediately prior to the breeding season and to minimise the field effort required. Also deploy hoppers in winter if there expected to be a lot of grey squirrel movement into and out of the area and/or the contraceptive is unlikely to remain effective after two months.
2. Estimate the density of grey squirrels in the target woodland. Deploy remote cameras (any brand) across the area at a density of 1 camera ha^{-1}. Bait the cameras and leave for 5 days, replenishing the bait after 2-3 days. Check cameras for the presence of red squirrels. If photos contain only grey squirrels, use a bait hopper with a 70 g weighted bait door to deliver the contraceptive. If red squirrels are present, use a feeding hopper that can select between the two species (and exclude red squirrels) based on body weight, with a 450 g threshold to open the bait door.
3. From the density estimates calculated, deploy enough hoppers, spread evenly across the wood at a density of approximately 1 ha^{-1}, to ensure there is at least one hopper per two

grey squirrels. Fix the hoppers to 1-metre-high wooden stands to reduce nontarget access.

4. Pre-bait the hoppers with hazelnut paste (or similar) every 2-3 days for one week with the bait doors propped open with a stick, to encourage the squirrels to use them. Monitor each hooper with a remote camera, to provide information on nontargets in the deployment area.
5. If the bait uptake from the hoppers is good, proceed to deploy the contraceptive via the hoppers, for 7 days, closing the hopper bait doors so that they have to be pushed open. Assume that each grey squirrel will consume approximately 43 g of bait or less and adjust the contraceptive concentration accordingly to ensure each grey squirrel receives an effective dose.
6. At 6-month intervals, deploy the remote cameras to calculate an updated grey squirrel density estimate, to monitor grey squirrel population size over time and to measure the effectiveness of the contraceptive.

7.7 Wider applications for the findings of this study

The methods and devices developed in this study could be adapted to other small mammals and for other wildlife management purposes. The hopper design has wider applications for wildlife management - in particular, for efficacy studies on bait-delivered drugs in the context of wildlife disease control and/or population reduction. Research requiring marked individuals has traditionally relied on external tags, which have the potential to be lost, damaged or misread, and often require some kind of human intervention, through recapture or tracking, to gather data (Gibbons & Andrews, 2004). PIT-tags have minimal welfare impacts, are relatively inexpensive and easy to apply and generally have a lower tag loss rate than external tags (Smyth & Nebel, 2013). PIT-tags can be used on a wide range of species and have been employed widely; wood ducks *Aix sponsa*a entering nest boxes (Bridge et al., 2019), wood mice *Apodemus sylvaticus* using bait stations (Godsall et al., 2014) and den use by edible dormice *Glis glis* (Kukalová et al., 2013). Low rates of tag loss and mortality have been found when PIT-tagging bats (van Harten et al., 2019) and ground squirrels (Schooley et al., 1993) and this study provides further evidence that PIT-tagging is a reliable method for gathering behavioural data on small mammals in their natural environment. The 3D-printed insert upon which the electronics are fixed allows the hoppers to be easily modified; therefore, the hoppers can be adapted to record other types of data from different animal species. By assessing patterns of bait consumption in representative population samples, adapted hoppers could be used to model the efficacy of bait delivery campaigns and inform bait delivery strategies at a larger scale.

The hoppers were relatively robust, with few failures, despite wet and cold weather conditions during the trials. The few hopper faults that were recorded were likely caused by wet conditions affecting the electrical circuits and can be easily mitigated against with improved weather-proofing. Additional adaptations to increase hopper robustness are likely to be required for environments where temperature and precipitation levels are more extreme. The battery management system meant there were no issues with battery life during the trials. Other studies have not been able to demonstrate the same degree of longevity, despite using larger batteries such as car batteries to power bait stations (Kenward et al., 2005). Larger batteries reduce the portability and practicality of devices for use in the field and the regular monitoring and changing of batteries can be labour-intensive, especially difficult in remote environments and could potentially disrupt the focal species' natural behaviour.

This study provided additional evidence that bait markers such as rhodamine B (Baruzzi et al. 2017; Fisher, 1999) can provide data on the proportion of a population that has consumed a bait and the effectiveness of bait delivery systems. These markers are less labour intensive, more cost-effective, and less impactful on animal welfare and produce results that are more representative of natural behaviour, than more invasive techniques such as trapping and marking (Tolkachev, 2019). This can allow a greater number of trials to be conducted simultaneously and the use of more robust analysis methods. In previous studies, hair samples for RB analysis have been washed and mounted on slides in preparation for analysis (Weerakoon, Price, & Banks 2013). In this study I found that hair samples could be analysed effectively whilst inside transparent plastic sample bags that they were placed in when taken in the field, thus, making the process much more time efficient and less susceptible to labelling errors or sample contamination. Bait marking can also be used to assess distances that animals move, from an area where the bait is deployed, to areas where they are sampled (Beatham, 2021), and this can provide information on the potential scale of impact of a bait deployed for wildlife management.

A common issue for bait-delivered rodent control campaigns is the impact on nontarget animals. Even when bait boxes are used to target bait delivery to specific species, nontarget small mammals may access bait, which wastes bait, reduces cost-effectiveness, but also increases the likelihood of secondary impacts on predators that feed on those small mammals (Brakes & Smith, 2005). By using a magnetic switch on the bait door together with a remote camera, it was possible to monitor the hopper access by non-target animals and it is recommended that a similar system is installed for devices delivering baits to other wildlife for other purposes.

A high level of species-specificity was achieved using a basic feeder design for the woodlands trialled. These woodlands, located in Yorkshire, UK, had only one squirrel species present and a lack of other arboreal or climbing mammals. It is likely that other bait delivery campaigns will require a more sophisticated system. Placing the bait hoppers in trees or other elevated locations or placing them in burrows or tunnel systems, may make bait more accessible to small mammal target species, while reducing access by larger animals. The use of a body weight threshold aided species-specificity and this may be one option, depending on the comparative body weights of nontarget species. The technology developed has wider applications and is being used to develop a grey squirrel selective cage trap, for control in areas where there are reds present.

An Artificial Intelligence (AI) system is currently being developed to identify and tag photographs of grey squirrels, to reduce the time and effort required for processing photographs for estimating squirrel densities (Beatham et al., unpublished). Advances in technology have meant that an increasing number of AI based systems are being developed to aid species-specific bait deployment. An AI system with 90% accuracy has been developed for the selective delivery toxic baits to wild dogs and foxes (Charlton et al., 2023) and an AI system has been proposed for the delivery of oral contraceptives to grey squirrels (BBC, 2024). In the future, it is likely that algorithms will be developed so that these systems will be able to deliver bait to a specific species with up to 100% accuracy and precision. These systems will need to be trained extensively on target mammal species in different scenarios; however, currently the main issues with AI are the prohibitive costs required to develop prototype systems and adapt prototypes to robust and marketable products.

The findings of chapters 3 and 4 suggested that to maximise bait uptake in grey squirrel populations it is important to ensure there are enough feeders per individual and this will be particularly important for target species that are territorial. It is therefore important that robust methods to estimate population sizes of the target species are available. The main advantage of using camera-trap data over traditional capture mark-recapture methods is that they do not require multiple captures of animals. This means that wildlife population estimation can be more cost-effective, practical and with fewer negative animal welfare implications.

Methods for estimating animal abundance have to be specifically adapted to take into account the life history of the focal species (Gilbert et al., 2021). Methods used to validate camera indices estimates have included distance sampling (Rovero and Marshall, 2009), direct counts (Rowcliffe et al., 2008), mark-recapture live trapping (Villette et al., 2016, 2017), tracking plates (Lambert et al., 2018) and models to simulate animal movement and photographic rate (Nakashima et al., 2018; Luo et al., 2020). It is recommended that, if applying the methodology presented here to estimate the abundance of other mammalian species, an alternative reliable method to validate abundance is initially required.

7.8 Conclusion

Invasive non-native rodents and other small mammals cause a large number of negative economic and environmental impacts worldwide, including losses to the food industry, damage to property and the transmission of diseases (Stenseth et al., 2003; Witmer et al., 2017). It is important to have practical, cost-effective tools for their management and the mitigation of their impacts. In addition to the grey squirrel, key species listed on the 100 world's worst invasive alien species list the IUCN include; the black rat *Rattus rattus*, the house mouse *Mus muscola*, the small Indian mongoose *Herpestes javanicus* and the stoat *Mustela ermina* (Lowe et al., 2024). The methods and devices developed in this study could be adapted to optimise contraceptive delivery methods for the management of these species in the future. The methods could also be adapted to optimise the delivery for other bait-delivered substances, including vaccines to control wildlife disease such as an oral rabies vaccine for the small Indian mongoose (Vos et al., 2013), an oral plague vaccine for prairie dogs (Abbott et al., 2012).

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