The Validation of Novel Ecological Survey Methods for Use in Describing Harvest Mouse *Micromys minutus* Autecology

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Abstract

According to much of the literature relating to *Micromys minutus* (harvest mouse) the species has historically presented many challenges to researchers, particularly when attempting to collect sufficient data to describe their ecology, life history and responses to the ever-increasing threat of habitat loss and fragmentation. Methodological improvements are needed which provide sufficient species-specific data to underpin conservation and which are of sufficient quality to allow their movement ecology to be quantified. Here two novel methods were developed and tested, which included remote scent surveys using a detection dog and Radio Frequency Identification (RFID) trapping. After validation, RFID trapping was then used to quantify *M. minutus* movement in fragmented habitats. A preliminary study was carried out which assessed the ability of a dog to be trained to indicate the scent of *M. minutus.* Here positive reinforcement training methods were used and the dog's effectiveness was evaluated in a training environment using scent samples collected from controlled and uncontrolled situations. Secondly, RFID trap effectiveness was compared to the results of live trapping. Data were maximised by releasing individually tagged *M. minutus* into a suitable semi-natural enclosure on the Moulton College estate. After validation a further release was undertaken to investigate *M. minutus* movement ecology. Here gaps of differing widths were incorporated into the release enclosures and movements between the habitat patches were measured. Individuals included in each release cohort were exposed to an Open Field Test prior to

release, and thus, their behaviour in relation to trapping and movement was also assessed. There is strong evidence that a dog can be trained to detect *M. minutus* and discriminate their scent from other sympatric non-target species in a controlled training environment. When applied to uncontrolled field situations, the remote scent survey proved more effective than nest search surveys by volunteers during the autumn months, providing preliminary evidence that olfactory indicators could be more efficient than visual clues when establishing presence of *M. minutus*. Additional validation in uncontrolled settings is still required. Encouraging results were also seen during validation of the use of RFID trapping with better results in terms of raw trapping rates over live trapping being observed. Furthermore, findings indicate that *M. minutus* have sufficient navigational and motion capacity to successfully move over gaps ≤2m, but gaps greater than 2m could limit their movement with possible implications for population persistence. The findings also suggest that individuals that explore more slowly may have an advantage when inhabiting a fragmented habitat. Thus, movement propensity is likely to be an individual behavioural trait and may vary across situations; this provides a novel perspective on their conservation and may support conservation decisions being based on behaviour rather than density. The data collected for this thesis demonstrates that progress has been made in terms of monitoring *M. minutus* and the findings presented are entirely novel for this species. Nevertheless, they remain a challenging species and more questions have been asked than can be answered within the thesis. However, the sum of this work has provided a clear direction for future research on *M. minutus.*

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1.1 Introduction

Currently it is thought that the sixth mass extinction event is imminent (Barnosky *et al.*, 2011) and if current trends in increased natural resource requirements continue, biodiversity will continue to decline (Kok *et al.*, 2018). The drivers behind these declines are mostly linked to meeting human demand for resources, in particular related to the need to support an ever-increasing human population (Foley *et al.*, 2005; Rounsevell and Reay, 2009). Yet, without the services provided by diversity within an ecosystem (e.g., buffering and filtration, nutrient regulation, pollination and soil retention) (Costanza *et al*., 1997), it is unlikely that humans could persist. The effect of biodiversity loss is not uniform across habitat types and losses impact ecosystems differently, and in some cases the interaction between drivers have a synergistic effect (Mantyka-Pringle *et al.*, 2012). The effects begin at the primary level, with the removal of habitat and reduction in plant biomass and diversity, with a cascade effect up the trophic levels potentially undermining the entire ecosystem (Fischer and Lindenmayer, 2007). In high diversity ecosystems, the loss of a species can be compensated by the presence of another. Therefore, inter-species competition, niche overlap and interactions at different trophic levels are fundamental for stabilising ecosystems (Pires *et al.*, 2018). Furthermore, the presence of species that are able to disperse and re-colonise can act as an insurance policy for ecosystem resilience in agricultural landscapes

(Tscharntke *et al.*, 2005; Pires *et al.*, 2018). As a synergistic effect between these drivers is likely to exist this protective buffer may be essential for conserving ecosystem services (Pires *et al.*, 2018). By 2100 changes in land use and climate change have been predicted to have the greatest impact on biodiversity, and thus, it is vital to implement conservation measures now to minimise the impact of these drivers (Sala *et al.*, 2000).

Understanding the spatio-temporal dynamics of how species interact with their biotic and abiotic environments remains vital for the management of species within a changing environment (Sutherland *et al.*, 2013). While species may adapt to changes in an environment if allowed sufficient time (Fahrig, 2003; I-Ching *et al.*, 2011), the rate of change that has occurred in recent years has put pressure on many species, particularly those that are susceptible to disturbance and reliant on the agricultural landscape such as *Micromys minutus* (harvest mouse) (Bence *et al*., 2003; de la Peña *et al.*, 2003; Svenning Petersen, 2007; Rödel *et al.*, 2015)

The major driver of changes in land use in Britain is agriculture, which accounts for over 70% of the surface area (DEFRA, 2011; Ollerton *et al.*, 2014). So, improving the quality of these habitats is vital for the conservation of biodiversity in the UK. UK Biodiversity Partnership (2010) noted that 73% of the identified priority habitats were declining due to changes in agricultural practice. Many of these changes have occurred after the Second World War with higher production demands on agricultural systems brought about the increased socio-economic pressures (Heroldová *et al.*, 2007).

Traditional land management for agriculture has had many functional benefits for biodiversity (habitat, connectivity, shelter, corridors) and has been vital for the provision of ecological networks, allowing the flow of genes between populations. Modern farming practices, however, have substantially altered the framework of the agricultural habitat (Robinson and Sutherland, 2002; Sutherland *et al*., 2013) and the resulting degradation has seen a related decline in biodiversity (Robinson and Sutherland, 2002).

During the post-war timeframe, there have been changes in community morality. The traditional motivation of social and moral equity gained by behaving responsibly towards ecology within a community is no longer effective within a globalising world (Gatzweiler, 2006). Fiscal motivators are now favoured within the agricultural industry and have existed under a number of guises since the early 1980s; currently in England, Natural England (NE) facilitate Environmental Stewardship at various levels (Natural England, 2011). Within the framework of Environmental Stewardship, the creation of margins and beetle banks within field systems are popular measures for biodiversity conservation. These have proven to be beneficial habitat for many taxa including *M. minutus* and provide significant connectivity within the agricultural landscape (Bence *et al.*, 2003; Shore *et al.*, 2005; Meek, 2011) (although such margins can be mismanaged).

Financial support for farmers is also provided by the European Union (EU), in the form of the Common Agricultural Policy (CAP). This has reportedly been the EU's most inefficient policy, with its primary focus to provide social

security for farmers (Helm, 2017). The link between income provision and production has led to ever greater intensification, resulting in the loss of vital wildlife habitats and loss of ecosystem services gained from a diverse agricultural landscape (Van Zanten *et al.*, 2014). While changes in the environmental provisions have been integrated in the CAP since 2015, Brexit has provided scope to reform CAP and subsidies in the UK. The possibility to promote more environmentally friendly practices where payments could be linked to the provision of environmental benefits rather than land ownership could be realised (Helm, 2017). However, the ecological knowledge of some species dependent on these habitats is limited thus conservation measures are rarely based on empirical knowledge (Barton *et al.*, 2015). With appropriate planning these reforms have the potential to re-establish networks of ecological importance to wildlife, but autecological understanding needs to be developed to underpin these reforms and to prevent counter-productive conservation practices (Diffendorfer *et al.*, 1995).

M. minutus was once an iconic species of the British agricultural landscape, yet they remain one of the native species that are missing basic life history information, mainly due to challenges related to methodology (Riordan *et al.*, 2009). While they were once abundant in many of Britain's traditional landscapes, changes in infrastructure and management has meant their numbers have reportedly fallen, which is particularly evident in the agricultural landscape (Rowe and Taylor, 1964; Perrow and Jowitt, 1995; Sargent *et al.*, 1997; Bence *et al.*, 2003). Ironically, the landscape that *M. minutus* has been so strongly associated has changed so rapidly that at

present *M. minutus* is predominantly considered a wetland species - whether this is because survey efforts in these areas have increased, or whether it is an adaptive response to changing land practices remains unclear (Agnelli and Lazzeretti, 1995; Meek, 2011). Habitat fragmentation occurring within these landscapes may prove to be limiting in terms of movement and dispersal for *M. minutus*, as dispersal opportunities may be limited (Kuroe *et al*., 2011; Meek, 2011) with isolated habitats being less likely to support *M. minutus* despite the presence of a suitable environment (Meek, 2011). With a reported 95% mortality rate over winter, it is inevitable that small, isolated populations cannot persist without sufficient ecological connections (Trout, 1976 in Trout 1978b).

M. minutus are underrepresented in the literature compared to other small mammal species, thus little is known about their population dynamics, movement ecology or basic ecology in the wild (Robertson and McKenzie, 2015; Kettel *et al.*, 2016), and are therefore the focal species for this thesis. While they are underrepresented in the literature, their ecological requirements are not entirely unknown (Trout, 1978b; Harris, 1979b; Harris, 1979c), yet, with the habitat changes they have experienced since these studies were undertaken, it is possible that what is known needs updating. The following section describes the current knowledge of this species.

1.2 *M. minutus* ecology

M. minutus have been described as a generalist, omnivorous species that consume insects, seeds, fruits, pollen and it is likely that they will scavenge

on the remains of other small mammals (Dickman, 1986; Okutsu *et al.*, 2012). A uniquely adapted semi-prehensile tail allows them to utilise resources within the stalk zone of seasonally available monocotyledonous vegetation, which may not necessarily be accessible to other sympatric small mammal species, and thus is a key habitat requirement. The availability of this type of vegetation dictates where they are found, which includes a variety of habitats, from cereal fields, hedgerows, field margins and wetlands to newly planted woodland (Rowe and Taylor, 1964; Harris, 1979a; Dickman, 1986; Bence *et al.*, 2003; Moore *et al.*, 2003; Hata, 2011; Meek, 2011).

Grassy vegetation is used to build aerial nests, serving two purposes: rearing young and providing shelter. Breeding nests will be larger than shelter nests, and females may build several nests per litter, but can remain in one nest for up to 25 days (Warner and Batt, 1976; Harris, 1979c). The insulative properties of the nest will be vital when rearing young as they critically reduce metabolic output and energy requirements (Pearson, 1960). Thus, in the UK breeding commences in May which coincides with suitable habitat availability (Harris, 1979c). The height of the nest will depend on the surrounding vegetation with an apparent preference for taller grasses (Hata, 2011). Height preferences however need to be interpreted with caution as nests are easily overlooked (Poulton and Turner, 2009) and are more conspicuous in taller vegetation when it dies back during the winter.

In mild weather, breeding can continue until December. However, 80% of breeding nests encountered between September and December fail due to

low temperature and precipitation (Harris, 1979c). Gestation takes between 17 and 19 days and the average litter size is 5.3, with 74% of young being born in August and September (Harris, 1979c). New born mice weigh <1g and gain weight rapidly compared to other rodent species and can climb after only a short period of maternal care (Harris, 1979c; Ishiwaka and Mori, 1999). By day nine most young mice will have gained sight and will be fully furred, and at day 16 they will be independent from their mother (Harris, 1979c). Young males will become sexually mature before females and able to reproduce earlier in the breeding season (Harris, 1979c); females become fecund at around two and a half months old (Sleptsov, 1947 in Trout, 1978).

In captivity females will choose to mate with males they are familiar with however mate preferences in the wild are undescribed (Brandt and Macdonald, 2011). If this behaviour occurs in the wild, there may be fitness implications related to inbreeding, particularly if dispersal opportunities are limited; high mortality over winter may mean inbreeding is inevitable regardless of mate selection.

M. minutus have an unfavourable surface area to volume ratio and their Daily Energy Budget (DEB) is proportionally higher than larger small mammal species, such as mice or voles (Gorecki, 1971). In evolutionary terms their unique semi-arboreal existence offer fitness advantages, which has enabled them to meet their DEB with less interspecies competition. However, their fur has poor insulative properties and adverse weather conditions can put pressure on their metabolism which often results in mortality (Trout, 1978b).

Based on data collected on captive individuals there are two main peaks of activity, after dusk and around dawn although activity was also recorded during daylight hours (Cross, 1970). In captivity they can adjust their activity depending on day-length, suggesting that there is flexibility in the activity patterns of *M. minutus* (Cross, 1970). In the wild there also appear to be two main peaks of activity which correspond with Cross (1970), the first around dusk and the other two to three hours after sunset, although the accuracy of the data collection method, namely tennis ball feeders and an activity monitor, may be somewhat unreliable as any such activity cannot conclusively be attributed to *M. minutus* (Warner and Batt, 1976).

1.3 Conservation status of *M. minutus*

In terms of distribution, *M. minutus* are found within the temperate zone throughout Europe and Asia (Harris *et al.*, 1995). In GB, *M. minutus* is at the edge of its natural range, with most of the populations thought to be in southern England (Sargent, 1997). In global terms being a peripheral population may mean GB's population could hold high adaptive significance to the species due to potentially unique genetic characteristics, thus having conservation importance (Lesica and Allendorf, 1995).

The International Union for the Conservation of Nature (IUCN) describe *M. minutus* as of "least concern" (IUCN, 2012). However, in the UK the results of a census in 1997 showed that there had been a 24% reduction in habitat availability, with a 71% decline in *M. minutus* numbers since Harris' national survey undertaken in 1979 (Harris, 1979a; Sargent *et al.*, 1997). This led to validating its inclusion as a priority species in the 2007 Biodiversity Action

Plan (BAP), updated in 2010 (JNCC, 2010). The BAP outlined recommendations for the conservation of the species at a national level. The Natural Environment and Rural Communities Act (2006) list *M. minutus* as a species "*of principal importance for the purpose of conserving biodiversity*"; one of only three rodents on the list, meaning that public bodies have a duty to "*have regard*" for this species when carrying out their functions, with particular attention when considering planning.

In the early part of the last decade *M. minutus* was the focus of a reintroduction project implemented by Chester Zoo, their aim being to establish a reintroduction protocol. Using a combination of hard and soft release, 128 mice were released in 2002 and 268 in 2003. Although this reintroduction was considered successful based on anecdotal evidence, the long-term monitoring methods were not sufficient to establish success (Kean, 2006).

1.4 Monitoring methods

The lack of detailed information about this species is attributed to the indeterminate effectiveness of current standardised survey techniques (Harris *et al.*, 1995; Poulton and Stone, 2008; Poulton and Turner, 2008; Riordan *et al*., 2009). There are two avenues of monitoring; presence/absence (using indicators of presence) and Capture Mark Recapture (CMR), where individuals can be identified over time.

1.4.1 Presence/absence and distribution data

Establishing presence of *M. minutus* has often proved challenging; such methods are generally labour intensive and thus reliant on volunteers to carry out field work. As with other elusive species their field signs are limited and for *M. minutus* include only their distinctive aerial nests. The current Mammal Society census protocol uses a nest search method to attempt to elucidate distribution of *M. minutus.* Other methods include collection of genetic material in bait pots (Morris *et al*., 2013), collecting hair samples from bait tubes, analysis of the contents of *Tyto alba* (barn owl) pellets and observing activity in tennis ball feeders (Warner and Batt, 1976; Buckley, 1977; Poulton and Turner, 2009; Meek, 2011). None of the mentioned methods have proven to be consistent and reliable spatially or temporally and some required considerable labour and cost to achieve sufficient results (Buckley, 1977; Hare, 2005; Poulton and Turner, 2009; Riordan *et al*., 2009; Meek, 2011).

Poulton and Turner (2009) carried out a pilot study to undertake field trials of methods of surveying *M. minutus*, including bait tubes and nest searches. They found these methods did not provide sufficient data and suggested the lack of results could prove de-motivating for volunteers if a national survey was established. However, in 2013 the Mammal Society embarked on a national nest search survey using volunteer surveyors. After one year this was cancelled, albeit not for the reasons predicted by Poulton and Turner (2009), but for reasons related to the substantial resources required and concerns over the successful delivery of a large-scale survey. This indicates the difficulties encountered when conserving a little understood

species such as *M. minutus.* Notwithstanding these issues, conservation measures which focus on *M. minutus* will inevitably contribute to the conservation of biodiversity within the target ecosystems (Hata, 2011).

1.4.2 CMR – Data collected at an individual level

Answering many fundamental questions in animal ecology relies on effective marking methods to establish identifiable individuals, which is essential for population estimates (Davis *et al*., 2017). Marking can be carried out in several ways, including fur clipping, the easiest method, albeit temporary. Longer term options include Passive Integrated Transponder (PIT) tags (microchips) and toe clipping, both offering long-term identification. Toe clipping is a less common practice at present due to ethical implications, particularly in scansorial species and can thus affect recapture and survival rates (McCarthy and Parris, 2004; Gannon and Sikes, 2007)

Microchipping has been used for identification of *M. minutus* and facilitates accurate lifelong recapture data (Kean, 2006). The microchipping procedure has only been carried out on anaesthetised *M. minutus*. It was thought not to be possible without anaesthetic. Undertaking this procedure without anaesthetic is possible in terms of the law. However, it is thought their size meant it was not practical (Animals (Scientific Procedures), 1986; Rudd, 2012).

Some marks will be lost or will fade while others can be made invalid by the addition of further marks, either intentionally or due to injury to the individual (Silvy *et al.*, 2005). Once successfully marked there needs to be an effective method of recapturing/recording individuals. The current

methods used to mark *M. minutus* do not connect with an interactive and automated system of data collection required for effective movement modelling (Nathan *et al.*, 2008; Baratchi *et al.*, 2013).

Some of the methods developed for monitoring mammals have not required the animal to be caught, and would be achieved either by natural marking or using a device to leave a mark/attach a collar. However, to gather reliable spatial/temporal data catching and restraint is normally necessary (Silvy *et al.*, 2005). The tried and tested method for small mammal CMR studies is live trapping whereby mammals are trapped with sufficient resources to survive for up to eight hours (Flowerdew *et al.*, 2004). Live traps are not as effective at trapping *M. minutus* as other sympatric small mammal species and trapping sufficient numbers for meaningful analysis to be carried out is difficult (Poulton and Turner, 2009; Williams, 2015). Reasons for this range from trap placement, inter-species competition, seasonal differences in trapping success and their crypsis (Warner and Batt, 1976; Trout, 1978b; Williams, 2015). Kettel *et al.* (2016) reported that *M. minutus* were the only species to be exclusively trapped when traps were located in the stalk zone. It is therefore likely that trapping success at the ground and in the stalk zone may depend on habitat type and *M. minutus* numbers (Williams, 2015; Kettel *et al.*, 2016).

External temperature affects the activity of some small mammal species. For example, *Microtus pennsylvanicus* (meadow vole) is normally considered a crepuscular species, yet when the temperature reached 20^{o}C a nocturnal preference was observed, potentially impacting trapping rates (Getz, 1961). Small mammal activity increases during wet weather and

therefore increases trapping success (Gentry *et al.*, 1966; Vickery and Bider, 1981). However, Lambin *et al.* (2000) found that seasonal fluctuations in vole populations occur, with reported losses during the summer. Trapping success in *M. minutus* may be impacted by their flexible activity patterns when exposed to differing day lengths (Cross, 1970).

Radio telemetry offers range data on tagged individuals and has been used widely across taxonomic groups. However, this method requires a large amount of effort and for a relatively small number of animals (Baratchi *et al.*, 2013). When tested on *M. minutus* there was an issue with tag retention over the monitoring period (Kean, 2006). Furthermore, to get an accurate location the individual must be located visually and with smaller species this is problematic, particularly with *M. minutus* due to their preferred habitat and small range movements (Trout, 1978b).

Global Positioning Systems (GPS) allows location data to be recorded and transmitted instantaneously or stored for offline transmission. Therefore, a great amount of detail on tagged individuals can be collected (Baratchi *et al.*, 2013). However, the battery needs to be of sufficient size to power units to collect adequate temporal data, which limits its use in smaller vertebrates (Kenward, 2000).

A key drawback with the aforementioned methods is a reliance on human accuracy since in a field setting human error has the potential to confound a data set and skew results (Baratchi *et al.*, 2013). Furthermore, invasive marking methods can affect behaviour and compromise welfare (Gannon and Sikes, 2007). Even when using non-invasive methods, the presence of

humans has an impact on behavioural and physiological states (Smith *et al.*, 2003).

Between the benchmark studies of Riordan *et al.* (2009), Poulton and Turner (2009), Meek (2011) and Williams, (2015) there is a consensus that traditional methods of monitoring are not sufficient nor entirely successful for this cryptic species. Although there is widespread concurrence that current methods are not suitable, little methodological progress has been made, thereby hindering the investigation of their movement ecology.

1.5 Movement and dispersal

The field of movement ecology is still relatively new and the development of species-specific knowledge is dependent mainly on the ability to gather sufficient data on a species, coupled with the statistical methods and computational power to analyse large data sets (Nathan *et al.*, 2008; Baratchi *et al.*, 2013). Typically, the process of dispersal consists of multiple movements (departure, transience and settlement) and is a specific class of movement from a natal to a breeding site or from breeding to another breeding site (Clobert *et al.*, 2009). However, other movement occurs which is not classed as dispersal but is critically connected to survival and reproduction (foraging, within-patch movement, or station-keeping) (Fahrig, 2007; Nathan *et al.*, 2008).

Dispersal ability is not simply a species attribute and intra-species variations are likely to occur in response to their environment (Clobert *et al.*, 2009). The movement framework presented by Nathan *et al.* (2008) suggested

that there are internal and external factors which influence movement and there is a cyclical feedback between these factors.

Movement and dispersal propensity is a complex process linked to evolution, previous experiences, learning ability, habitat type, personality and behavioural adaptations (Fahrig, 2007; Cornelius *et al.*, 2017). Thus, establishing the movement profiles of species, particularly those that present monitoring challenges, can be problematic. The evolution of movement and dispersal propensity, particularly inter-individual variation in *M. minutus,* is little understood, yet this is a key factor when considering conservation measures (Cornelius *et al.*, 2017; Schuster *et al.*, 2017a; Doherty and Driscoll, 2018).

Based on trapping data, the estimated mean movement for *M. minutus* is 8m, yet no timescale was provided with this estimate, with their dispersal range from natal nests at around 90m (Trout, 1978b). Thus, there is great potential for localised habitat changes to prove highly detrimental to *M. minutus*. Fragmentation of habitats poses a threat to the persistence of populations, and understanding the movement responses of *M. minutus* in fragmented habitats is a priority in terms of their successful conservation in the future (Cresswell *et al.*, 2012).

1.5.1 Motion capacity and perceptual range

The ability of an individual to move is dependent on many internal and external factors, including motion capacity (the physical ability to move), internal state (motivation to move), and navigational capacity (obtaining and using information to aid movement) (Nathan *et al.*, 2008; Doherty and

Driscoll, 2018). These capabilities in modified landscapes may become non-optimal, particularly if perceptual range (distance over which suitable habitat can be located) and resources are limited (Fahrig, 2007). There is evidence that *M. minutus* have the motion capacity to successfully move and disperse in continuous habitats (Trout, 1976 in Trout 1978b). However, there is also evidence that poorly connected habitat is detrimental for the populations of *M. minutus* although movement in fragmented habitats has yet to be quantified (Meek, 2011).

Perceptual range can vary with body size, habitat structure and environmental condition (Doherty and Driscoll, 2018). The small size of *M. minutus* which enables a scansorial lifestyle aids movement in the stalk zone. However, this may inhibit movement across open gaps at ground level as their perceptual range may be limited (Doherty and Driscoll, 2018). Nevertheless, the height gained by living in the stalk zone may extend their navigational and perceptual range, particularly when faced with a barrier, thus aiding risk assessment and movement success (Prevedello *et al.*, 2011).

1.5.2 Risks and benefits of movement

Fahrig (2007) presented two theories relating to the evolution of movement propensity within species. Firstly, higher risk of mortality associated with movement should result in the evolution of lower movement probabilities. The second, suggested that the increased probability of local extinction due to environmental stochasticity increases the risk of remaining in the current patch. Therefore, benefits of moving to locate alternative unexploited

habitat may be observed (Fahrig, 2003). Thus, higher rates of local extinction driven by environment pressures should select for a higher probability of movement. These evolutionary responses could lead to different dispersal strategies (bimodal), with individuals expressing either low or high movement propensity (Parvinen, 2006; Fahrig, 2007).

Movement choices involve balancing the risks and potential benefits gained by moving (Larsen and Boutin, 1994), the main risk being mortality, and thus, effective assessment of risk is vital for making appropriate movement decisions (Fahrig, 2007). Risk assessment can be associated with previous learning experience of an individual, habitat type, genetics and behavioural syndromes. Consequently, risk assessment may vary between individuals and therefore their movement success is likely to vary likewise (Cornelius *et al.*, 2017). For individuals to assess the risk of moving or dispersing they need a sufficient perceptual range. The inter-patch distance may prove too far to effectively assess risk, thus limiting dispersal success. It is possible for adaptations to occur at a behavioural rather than genetic level in response to fragmentation. Thus, personality could also influence risk assessment and movement success.

1.5.3 Personality, behavioural syndromes and ecology.

An extensive review of the integration of animal temperament/personality in ecology was presented by Réale *et al.* (2007). While some might argue the difference between temperament and personality, Réale *et al.* (2007) considered them to be synonyms. Thus, the same definition will be used in this thesis and will be referred to as 'personality' hereafter. The

aforementioned paper concluded that individual personality is likely to be linked to several ecological topics, including dispersal, movement and responses to the changing structure of landscapes.

Recent developments in testing the concept of personality in *M. minutus* found that the consistency of behavioural responses to various stimuli indicated that they do display personality traits (Schuster *et al.*, 2017a). Additionally, *M. minutus* display a consistent suite of correlated behaviours across situations (within and between individuals, characteristic of a given population); i.e. a behavioural syndrome (Sih *et al.*, 2004; Schuster *et al.*, 2017b). Within the behavioural syndrome correlations, two behavioural types (within individual variation, individual characteristic (Luttbeg and Sih, 2010)) of *M. minutus* were recorded, proactive (fast) and reactive (slow). These are likely to be linked to individual fitness advantages in changing environments in the wild (Cornelius *et al.*, 2017) and are also likely to differ in their time budget allocation, with impacts on exploring and foraging (Cote and Clobert, 2012).

The proactive/reactive axis is important in terms of ecology as it impacts how an individual interacts with its environment (Sih *et al.*, 2004). Schuster *et al.* (2017b) hypothesised that individual *M. minutus* that are classified in the fast-behaviour type (proactive) have higher fitness in summer and autumn, where risky behaviours could afford more access to resources during times of environmental stability. Whereas slow-behaviour (reactive) *M. minutus* have a fitness advantage during the winter and spring when their habitat is less stable and lower exploration activity saves energy and reduces interactions with conspecifics (Schuster *et al.*, 2017b). This bimodal

(reactive/proactive) behavioural model has persisted as both are beneficial in a changeable environment and may provide an insight into how *M. minutus* adapt in fragmented habitats. When assessing temperament traits, Réale *et al.* (2007) noted that these can be misinterpreted as bimodal (categorical) variables, when rather they should be assessed along a continuum.

Behavioural types have been broken down further into fast (**FE**) and slow (**SE**) explorers (Cornelius *et al.*,2017). **FE** classified individuals are likely to cross fragmented habitats more quickly but have lower survival and dispersal success due to their increased risk-taking behaviour (Rödel *et al.*, 2015; Cornelius *et al.*, 2017). However, **SE** spend longer in each fragment and display higher risk-assessment behaviours compared to **FE**, leading to higher dispersal success.

As Spiegel *et al.* (2017) suggested, based on modelled data, it is likely that the ability to obtain information on personality-dependent movement can generate predictions on spatial patterns in ecology, particularly if sufficient movement data can be obtained. However, movement ecology is a relatively young field of ecology and is largely dependent on technological developments facilitating data collection. Thus, understanding the links between personality and movement in *M. minutus* would greatly aid their conservation in an ever-unstable environment, potentially allowing innovative approaches to conservation to be developed for the species (Doherty and Driscoll, 2018).

1.5.4 Movement and barriers in fragmented habitats.

The importance of ecological connections in general are well documented in maintaining habitat quality, dispersal, and geneflow (Ahlroth *et al.*, 2010; Lawton *et al.*, 2010). Cresswell *et al*. (2012) highlighted the need to identify the barriers to dispersal for *M. minutus*, with a key question being to understand more about their motion and navigational capacity with particular reference to roads.

1.5.4.1 Effect of roads on mammals

Roads present a significant but not absolute barrier to small mammal movement (Oxley *et al*., 1974; Richardson *et al*., 1997; Underhill, 2002). Lack of cover, road size and high traffic density appear to deter crossings, as does road surface (Oxley *et al*., 1974; Richardson *et al*., 1997; Underhill, 2002). Merriam *et al*. (1989) found that only 7.9% of marked *Peromyscus leucopus* (white-footed mice) crossed a road, with the majority being males, and this would be in line with the findings of Wolff (1994); generally male mammals were less philopatric than females and may be an inbreeding avoidance strategy. Merriam *et al*. (1989) also found in their study that 55% of the mice that crossed the road subsequently died in the traps, but it is not known whether this was a potential methodological issue or whether road crossings were a behavioural response to an illness or other negative internal state.

There is potential for roads to benefit small mammals, particularly if their predators are negatively affected (Fahrig and Rytwinski, 2009). When considering the range of species that are reported to predate *M. minutus*

(owl spp, corvid spp, *Phasianus colchicus* (pheasant), *Mustela nivalis* (weasel)*, Felis catus* (domestic cat) and *Vulpes vulpes* (fox) (Sleptsov, 1947 In: Trout, 1978; Darinot, 2016), it is unlikely that they would benefit from this suggested effect. There is a general consensus that roads act as a barrier to small mammal dispersal. However, the UK-based studies looking at the effects of roads on small mammals were unable to draw conclusions on *M. minutus* as encounters were rare (Richardson *et al*., 1997; Bellamy *et al*., 2000).

1.5.4.2 Barriers in agricultural landscapes.

While roads present a significant physical barrier to dispersal and movement, other barriers exist within the agricultural landscape, such as gateways, footpaths, open fields and monocultures, as well as the habitat loss through annual mowing regimes (de la Peña *et al.*, 2003). Movement between habitat fragments will result in higher predation and it is likely that as distance between fragments increases movement decreases (Andreassen and Ims, 1998). The typical behaviour of similar species outlined in the section above suggests that movement may be thwarted when the distance is too large, even when they are not faced with a road surface or traffic. Meek (2011) found that good connectivity was one of the most important factors for the persistence of breeding *M. minutus*, and they were notably absent in intensively farmed habitats. These results clearly suggest that fragmentation affects *M. minutus*, but the extent has yet to be quantified.

1.5.5 Developing the knowledge on M. minutus movement.

For a species to move it must have sufficient capacity to do so, and this relates to navigation, motion and internal state (Nathan *et al.*, 2008). These are unknown parameters in *M. minutus*, yet this knowledge is vital when managing and restoring habitats (Nathan *et al.*, 2008). Thus, developing movement ecology knowledge in *M. minutus* is a priority, particularly when the impact from environmental pressures imposed on their habitat in recent times has caused a dramatic decline (Sargent *et al.*, 1997; Nathan *et al.*, 2008). However, before ecological developments can be made, suitable methodological developments are required, current methods do not work sufficiently well to elucidate movement data in *M. minutus*. The development of new technology should facilitate the basic movement data required in this species (Fahrig, 2007). There are untested methods that have been used successfully to overcome monitoring challenges in other species. These include Radio Frequency Identification (RFID) and establishing presence using a detection dog. These will be discussed in the subsequent sections.

1.6 RFID and wireless sensor networks

RFID is a passive system of data collection used globally and is particularly useful when monitoring cryptic species (Becker and Wendeln, 1997; Reichling and Tabaka, 2001). Scheibler *et al.* (2013) used RFID on wild *Phodopus roborovskii* (desert hamsters) and noted that behavioural observations using RFID were more accurate than video observation due to the finer temporal scale. To successfully implement an RFID system, two

components are required - an individually fitted PIT tag and an electronic reader. The PIT tag has no internal power source but is powered inductively when in close proximity to the electronic reader's antenna, facilitating indefinite and continuous data collection. An electronic RFID reader can be linked with other readers to create a network. The data collected from each read can be instantaneously transmitted via a Wireless Sensor Network (WSN) to a central data logger, which stores the data (Baratchi *et al.*, 2013).

The use of RFID in monitoring some cryptic species has become invaluable and the size of the PIT tags compared to GPS trackers is favourable for smaller species (Rehmeier *et al.*, 2006). For example, PIT tags were successfully fitted to honey bees which allowed the local effects of pesticides to be quantified (Henry *et al.*, 2012). However, many of the studies find the financial implications restricting and the cost of the reader and WSN prohibitive.

Attachment of monitoring devices over 5% of the individual's body weight is normally considered to be too heavy, with potential increase of mortality (Dyo *et al.*, 2009). PIT tags vary greatly in size, but are available from 30mg, well within the 5% range of the *M. minutus*. If the use of RFID were to prove effective, it would allow key questions relating to *M. minutus* movement ecology and autecology to be addressed.

1.7 Detection dogs and conservation

There is a variety of industries that focus on using dogs for their olfactory abilities (Gazit and Terkel, 2003). One of the developing sectors is the use

of dogs to detect animal scents for various purposes, including conservation, and there is increasing evidence to support this application (Browne *et al.*, 2006). The use of dogs has enabled presence surveys for cryptic species using olfactory indicators to be implemented. Furthermore, they have been used to collect a greater number of biological samples, which is of particular importance in mammal conservation (Rolland *et al*., 2006; Long *et al*., 2007; Fukuhara *et al*., 2010; Orkin *et al.*, 2016). Rolland *et al*. (2006) used a dog to detect scat of *Eubalaena glacialis* (North Atlantic right whale). This method generated a fourfold increase in samples when compared to using human surveyors. Similarly, Wasser *et al*. (2004) found that detection dogs were a better method for gathering data on individual *Ursus arctos* (brown bear) and *U. americanus* (black bear) when compared to hair-snags.

Dogs are not only used for relatively large fauna such as bears and whales but have successfully been used to detect invertebrates such as *Cochliomyia hominivorax* (screwworm) and *Reticulitermes flavipes* (Western subterranean termite) and are a popular method of *Cimex lectularius* (bed bug) detection all of which are more comparable in size to *M. minutus* (Pfiester *et al.*, 2008). Schulz and Wilks (2017) mentioned the use of a detection dog for monitoring *Pseudomys fumeus* (smoky mouse) and Duggan *et al.* (2011) compared detection dogs and live trapping in *Poliocitellus franklinii* (Franklin's ground squirrel). While these are larger species than *M. minutus*, these papers offer examples of the use of dogs to detect and differentiate between rodents.

Dogs can discriminate between similar species both taxonomically and geographically (Hurt *et al.*, 2000; Smith *et al.*, 2003) and can generalise scents when trained using samples from a limited range of individuals (Oldenburg *et al.*, 2016). This enables them to search for individuals of a species that they have not encountered during their training (Cablk and Heaton, 2006).

The effects of biotic and abiotic variables need to be considered when assessing the effectiveness of using dogs for conservation purposes. The dispersal of scent can rise and fall with changes in temperature and humidity. Thus, the detection ability can alter with changes in these abiotic factors, but may depend on target odour collection method (Wasser *et al.*, 2004; Long *et al.*, 2007). Detection distance varies depending on the local topography, habitat structure, wind speed and direction, and age of the sample (Wasser *et al.*, 2004). A detection dog's performance can be equally effective across habitats and vegetation density as demonstrated by Reed *et al.* (2011), where target scent was identified up to 10m with 75% accuracy, although more time should be allowed in more complex habitats (Leigh and Dominick, 2015).

There are several caveats to using detection dogs. Firstly, remote detection, where samples are taken from a habitat where presented to a detection dog in a controlled environment, was used to detect the presence of the invasive *Dreissena bugensis* (quagga mussel) (DeShon *et al.*, 2016). This method is beneficial as it does not require the dog to travel to a habitat, and samples could be tested in a central location. However, the repetitive experimental design can frustrate the dogs and their handlers, yet effective training

should overcome this. Secondly, the cost of training and caring for the dog can prove prohibitive and can be exacerbated if substantial travel is required (O'Connor *et al.*, 2012; Orkin *et al.*, 2016). However, if there is a limited survey window, dogs can prove more efficient as larger areas can be covered compared to traditional methods (Duggan *et al.*, 2011).

There is also conflicting evidence as to whether dogs are better than human surveyors at locating samples. O'Connor *et al.* (2012) concluded that humans were just as efficient as dogs when locating bumble bee nests (*Bombus* spp.). Whereas Orkin *et al.* (2016) found that dogs were not only better at locating samples, they were also more effective at detecting viable primate faecal samples when compared to the results of humans.

Lastly, identifying the odour signature that a dog is indicating on is often not possible. This is particularly problematic when unknown and unidentifiable contaminants may be present and may impact the dog's ability to discriminate non-target scent (Willis *et al.*, 2004). This is challenging when undertaking empirical studies, in many cases the target scent parameters are undefined and uncontrolled. This effect is amplified when dealing with wild animal scent as the exclusion of ingested contaminants normally controlled by experimental design in humans is not possible (Willis *et al.*, 2004). However, Orkin *et al.*'s (2016) findings suggest that diet did not impact the dog's ability to locate target scent.

The evidence suggests that the use of a *M. minutus* detection dog could provide a rapid and relatively cost-effective method to establish presence, particularly in areas where they have been overlooked. The primary

indicator of presence has traditionally been their distinctive nests; a visual clue. However, olfactory indicators are present and to a sensitive nose they could be a better and more reliable method of establishing presence.

1.8 Rationale

The functional value of *M. minutus* should not be underestimated. As secondary consumers, they may be affected by the bioaccumulation of localised residues and contaminants utilised in the agricultural industry (Jefferies *et al.*, 1973). This, coupled with their response to changes in habitat quality due to their high DEB requirement throughout the year, may make them an indicator species for the health of small mammal communities and the ecosystem (Gorecki, 1971; Perrow and Jowitt, 1995). Therefore, an in-depth understanding of their behaviour, habitat utilisation and motion capacity in fragmented habitats would aid the implementation of conservation measures, benefiting biodiversity and consequently ecosystem stability (Hata, 2011; Pires *et al.*, 2018).

Their unique life history, coupled with the monitoring challenges presented by *M. minutus*, could imply that they respond to internal and external factors differently to other sympatric species. Thus, generalisation about the impact of fragmentation across species should be avoided as there is potential to employ counterproductive conservation measures (Diffendorfer *et al.*, 1995). Therefore, improvements are needed to develop effective monitoring methods in *M. minutus* populations, with particular focus on developing knowledge on the motion and navigational capacity and ecology of the species to allow appropriate management and conservation.

1.8.1 Proposed methods

To address the monitoring challenges described, the initial task was to validate novel monitoring methods, these being electronic trapping using RFID technology and detection dog scent surveys.

1.8.2 Aim:

The overarching aim of the thesis is to develop and validate novel ecological survey methods that can be used for describing the autecology of *M. minutus*.

1.8.3 Objectives

Objective I: To evaluate the effectiveness of a detection dog at indicating *M. minutus* presence.

Objective II: To compare the effectiveness of remote scent surveys compared to standard nest searches at identifying *M. minutus* presence.

Objective III: To estimate capture probability of monitoring *M. minutus* using Radio Frequency Identification (RFID) compared to live trapping to assess the effectiveness of both.

Objective IV: To investigate the inter-individual behavioural differences on the survival and movement propensity of *M. minutus*.

Objective V: To investigate the effects of fragmentation at different scales on *M. minutus* movement.

The chapter outlines and related objectives are described below.

Chapter Two: Methods

Chapter two outlines the materials and methods utilised to validate and test the use of a detection dog for monitoring *M. minutus*, RFID trapping comparisons with live trapping and motion capacity in relation to behavioural type, sex and abiotic factors. Specific details of data analyses undertaken within Chapters three, four and five are described.

Chapter Three: Remote Scenting

Results of data analyses covering the temporal effectiveness of the detection dog during continuation training and the impact of abiotic factors on the detection ability of the dog are presented and discussed. Furthermore, the results of the discrimination testing are outlined and justified progression to uncontrolled field testing, the results of which are also presented in conjunction with the nest search survey outcomes.

Objectives: **I** and **II**

Chapter Four: RFID in comparison with live trapping and behavioural variations

Results of the RFID trapping in comparison with live trapping are described and discussed as are results relating to the temporal effectiveness and the impact of abiotic factors on trapping efficiency. Additional analyses relating to *M. minutus* behavioural type are presented, specifically relating to anxiety and subsequent impacts on survival over the course of the experiment.

Objectives: **III** and **IV**

Chapter Five: Movement of *M. minutus* between habitat patches

Chapter Five assesses movement of *M. minutus* between habitat patches using the RFID trapping method presented in Chapter Four. Furthermore, the movements are analysed in relation to other factors such as behavioural type and sex.

Objectives: **IV** and **V**

Chapter Six: Conclusion

Chapter Six summarises the key findings from Chapters Three, Four and Five and suggestions for the future applications of the methods are described in conjunction with the key limitations.

2 Chapter Two – Methods

Two experimental approaches were utilised within this thesis. Firstly, a dog was acquired and trained using positive reinforcement methods to remotely detect *M. minutus* from samples collected in uncontrolled field conditions. There were four steps to achieving this: initial training, where the dog was taught to indicate the target scent; continuation training, which was the same format as initial training but the primary purpose was to reinforced the target scent over several months, all runs were recorded to allow the dog's detection ability to be assessed over time; discrimination testing formally assessed the dog's ability to discriminate target scent from non-target species; and lastly, uncontrolled field testing where scent samples were collected in the field and presented to the detection dog remotely, allowing the discrimination ability of the dog to be assessed when unknown distraction scents may have been present. The scent sample collection method was similar to Morris *et al*. (2013), where feeders were set up in suitable habitat. Morris *et al.* (2013) collected faeces samples for genetic analysis to determine presence, whereas in this study *M. minutus* scent was used. The contents of the feeders were presented to the dog. The dog would not visit the habitat, nor was the intention to train the dog to detect *M. minutus* nests. A positive indication was given if *M. minutus* scent (combination of faeces and urine) was detected. The initial training and continuation training was undertaken within a fenced, outdoor area based at Moulton College. However, from approximately October 2015 training sessions were relocated to a controlled indoor environment based at

Moulton College. This minimised the presence of external distractions which may have impacted results.

The second experimental approach utilised a semi-natural release enclosure. Here, captive bred *M. minutus* were released to assess the effectiveness of Radio Frequency Identification (RFID) traps compared to live traps, with a subsequent release utilising a modified version of the release enclosure. The initial release focused on concurrent data collection between live trapping and RFID trapping. Therefore, two experimental plots were created within the release enclosure. The second release facilitated the collection of *M. minutus* movement data in fragmented habitat. Thus, habitat gaps were incorporated into the experimental design. Prior to release each individual *M. minutus* was identifiable by a subcutaneously fitted passive integrated transponder (PIT) tag and had been behaviourally assessed using an Open Field Test (OFT).

The RFID traps worked with a PIT tag and RFID technology to autonomously collect location data of chipped individuals. The RFID trap effectiveness was compared to the results of live trapping over the same timeframe. Data were analysed statistically to establish the most favourable method. This had a twofold benefit. Firstly, the released individuals provided a tagged, and therefore individually identifiable sample, and secondly the data collected were used to address the ecological questions, thus maximising potential for data collection. This release adhered to the IUCN guidelines for population reinforcement (IUCN, 1998).

2.1 Weather data

All weather data used within this thesis were obtained from historical weather records from the Pitsford weather station (Pitsford, Northamptonshire) which was located <1km from the release site.

2.2 Funding the *M. minutus* detection dog training

Alongside the funding provided by the Thomas Harrison Trust, additional funding was secured from the People's Trust for Endangered Species (PTES). This totalled £1120.00, of which £720 was allocated to 20 hours of formal dog training at Skylark Animal Management; the remaining funds were used for training consumables.

2.3 Dog selection

Smith *et al.* (2003) noted that their dog selection was based on an obsession for either a toy or food. Choosing the most suitable dog was vital since not all dogs have the natural drive and motivation to work. Therefore, a working type, female flat-coated retriever was chosen for this project. The dog was acquired at 11 weeks of age from a Kennel Club registered breeder and vaccinated and microchipped as per veterinary advice. The dog selected did not display traits to an obsessive level. However, it was important to maintain the dog's role as a balanced pet in addition to its working potential.

2.4 Detection dog training

All training methods were implemented as per advice from a detection dog training specialist at Skylark Animal Management. Training commenced when funding became available. The dog was five and a half months old at this point (September 2014); 36 hours of formal training were undertaken with a professional trainer from Skylark Animal Management, normally between one and two hours per week. Additional training was undertaken between the formal sessions to reinforce behaviours.

During the early stages of training, a medium sized red Kong® (Figure 2.1) dog toy was used as the target scent. The Kong provided a consistent and distinctive scent for the initial training which could also be used as reward for a correct indication. The dog's motivation to play was increased by adding rope to the Kong allowing tug of war play with the researcher. The dog would receive a reward (play) when a positive indication was given to the correct location of the hidden Kong. Teaching the dog games was an important element of the initial training and focused around the dog giving some form of indication to the location of the toy. Various other items were used to conceal the Kong, including plastic flower pots (Figure 2.2), milk bottles (Figure 2.3) and 380ml plastic bottles (Figure 2.4). The items used to conceal the target scent were moved from the ground to being fixed higher from the ground, as flat-coated retrievers are an air-scenting breed this was a more natural search pattern. Initially the indication was to look at the handler when the Kong was located; as the training progressed the dog was taught to sit when indicating (Figure 2.5).

Figure 2.1 – Red Kong used during detection dog training.

Figure 2.2 - Plastic flower pots used in training, the Kong was hidden under a pot, but it was not visible to the dog. Flags kept flower pots in position and prevented the dog from moving them. The dog would sniff each pot and if a positive indication was given a play reward was immediately provided.

Figure 2.3 – Plastic milk bottles with a hole cut in the front used to conceal the Kong. The dog would sniff each bottle and if a correct positive indication was given a play reward was immediately provided.

Figure 2.4 – The detection dog carrying out a search. One bottle contained *M. minutus* scent the remainder were clean bottles with blank scent. Upon correct indication a play reward was immediately provided (Image provided by Upton, 2015a).

Figure 2.5 – Detection dog correctly indicating *M. minutus* scent during a training activity (image provided by Upton, 2015b).

Pairing the Kong and *M. minutus* scent took place after ten formal training sessions. Small parts of Kong were placed in *M. minutus* scented bottles. The *M. minutus* scent was gathered by providing captive *M. minutus* food (Johnson and Jeff cockatiel mix) in the same plastic bottles that were used for detection. The mice would spend time in the bottles feeding, eventually defecating/urinating; they would also climb and urinate on the outside, which provided a stronger scent for initial training. However, as the dog progressed, the scented food was transferred to clean bottles, this meant the dog had to be more accurate in the scenting method. The size of the Kong was eventually reduced until the indication was given on *M. minutus* scent only.

To prevent boredom and maintain motivation the ways in which the samples were presented to the detection dog were varied (Kerley and Salkina, 2007; DeShon *et al.*, 2016). The variation helped to keep the dog interested in the training, which was quite repetitive. Kerley and Salkina (2007) reported that boredom was an issue in their study and the demotivation that resulted affected the working efficiency of several dogs involved.

A line of bottles checked once by the detection dog was classified as a "run" (Porritt *et al.*, 2015) and a single bottle within the run was defined as a "stand". Each run would have a single outcome either false, missed or correct. A false outcome involved the dog indicating at a stand where there was no target scent. A missed outcome was given when target scent was present, but the dog failed to detect it. Finally, a correct outcome was achieved when the dog indicated at the appropriate stand. There could be between zero and two target scent samples in each run. A run with zero

target scent samples was referred to as a blank run. The outcome for each run undertaken between February and June 2015 (continuation training) was noted, as was the number of bottles in a run and the number of *M. minutus* samples present in each run. These data were essential for temporal assessment of the detection dog's ability.

2.4.1 Scent handling procedure

Non-latex gloves were worn when handling any of the training equipment during a training session. Clean gloves were used when handling *M. minutus* scent and changed when handling the 'clean' training equipment; gloves were not used twice. Any used gloves were disposed of immediately to avoid contamination. These measures minimised *M. minutus* scent contaminating clean equipment, preventing confusion for the detection dog. The same procedure was followed when handling non-target scents. All training equipment was cleaned using hot water and bicarbonate of soda after each training session to remove all scents.

2.4.2 Scent discrimination training

Discrimination training involved teaching the detection dog to ignore non-target scents. Initially this involved hiding non-target scent (tea, coffee, flowers) as a distraction within plastic drinks bottles, if the dog was overly interested in the non-target scent or attempted to indicate on anything that was the correct scent a "no" command was given and was led to the next bottle (Smith *et al.*, 2003). The dog was given exuberant praise and rewarded with play when correctly indicating *M. minutus* scent. Gradually this method began to incorporate faeces and urine of other non-target small

mammals, mainly *Clethrionomys glareolus* (bank vole). The detection dog was rewarded for indication of *M. minutus* scent but never any non-target scent.

A single blind fully randomised discrimination test was carried out to assess whether the detection dog was effective at identifying *M. minutus* scent when a distraction scent of a non-target species was present.

2.4.3 Formal discrimination testing procedure

The discrimination testing procedure was adapted from Porritt *et al.* (2015). To gather scent from *M. minutus* and *C. glareolus*, an individual of each species was placed in a separate, open topped plastic box within a 380 ml plastic bottle. Food was provided in each bottle as a medium to gather their scent. They remained in the box for 30min before the discrimination test began. The scent handling procedures described in section 2.4.1 was followed throughout the discrimination testing.

The test was undertaken in the familiar training room, plastic drinks bottles were used to contain the scent, which was either blank (non-scented cockatiel mix), *C. glareolus (distraction scent and cockatiel mix) or M. minutus* (target scent and cockatiel mix). The bottles were placed on a shelf approximately 30 cm above the ground. Two sets of blank bottles were available to use. Only one set were used per run and a random number generator selected which set of blank bottles would be utilised for a run. This accounted for any variables related to the familiarity of the blank bottles. A total of six bottles were used per run and six runs were undertaken. In addition to the blank bottles, each run contained a target

scent and a distraction scent. The position of the bottle containing the *M. minutus* scent was noted as this was required for data analysis. The positions of the bottles were randomly selected before the test using a random number generator. The location of the *M. minutus* scent or order in which the runs were undertaken were not disclosed to the handler. A second person set up the runs while the detection dog and handler were out of the room. Each of the positions within the run were cleaned between each run and were labelled between A and F to allow for the runs to be set up easily by the second person.

During the test the dog wore a harness and was kept on a lead by the handler. The handler would ask the dog to "check" and upon this command the dog would sniff the bottles and indicate accordingly. The positive indication was the same as in training. The handler did not know the location of target scent, this avoided the handler providing hidden clues about the location of the target scent to the dog. The person setting up the runs confirmed whether the dog had indicated correctly. If the dog did not give an indication on the first run, a second was attempted. No more than two runs were needed. However, if a correct or false indication was given a second run was not undertaken.

2.4.4 Uncontrolled field test

Uncontrolled field tests were undertaken to test the method in field conditions and to assess the dog's ability to detect *M. minutus* with the presence of non-target sympatric species.

The same type of plastic bottles described in section 2.4.3 were used as feeders and facilitated the collection of *M. minutus* scent samples. These feeders (n=20) were placed in a transect or grid formation within confirmed *M. minutus* habitat with a 2m spacing and remained *in situ* for between two and six days. They were secured to bamboo canes for support using sewing elastic. Each feeder was baited with cockatiel mix. Over the entrance a 13mm gauge wire mesh was fitted to reduce access from non-target small mammals, the centre square was stretched slightly to ensure larger *M. minutus* could gain access. *Sorex minutus* (pygmy shrews) and juvenile *C. glareolus*, *Microtus agrestis* (field voles), *Apodemus sylvaticus* (wood mouse) and *Apodemus flavicollis* (yellow-neck mouse) could still gain entrance. The height of the feeder on the bamboo cane varied depending on the surrounding vegetation and whether this would facilitate *M. minutus* entering the feeder. Where there was no supportive vegetation the feeders were located on the ground. Due to the dieback in grassy vegetation when some of the surveys took place there were many occasions when the feeders were at ground level. However the height was not measured for all the feeders and this would be recommended for any future replications. The scent handling procedure described in Section 2.4.1 was followed at all stages of the uncontrolled field testing.

Eight surveys were carried out in field conditions at three different locations (Table 2.1). One of the surveys was discounted in the results as the feeders were left *in situ* for six days and a high level of urine and faeces from non-target species was observed on the outside of the feeder and could cause confusion to the detection dog. Another of the surveys undertaken was split

in two with half of the samples being collected after three days and the rest were collected after six, with a lower amount of visible contamination than the discounted survey.

Table 2.1– Locations of scent survey sample collection for uncontrolled field tests and subsequent nest searches.

Location Number	Location	Number of Surveys
1	Butcher's Lane, Moulton College estate, Boughton, Northamptonshire	5
2	Cottage Field, Moulton College estate, Pitsford Northamptonshire	2
3	Althorp Estate, Northamptonshire	1

2.4.5 Sample testing procedure

Samples were presented to the dog for checking normally within 48 hours of collecting from the field. Ideally this should be carried out immediately after collection. However, this was not always possible as the dog became tired during the session and in some cases conflicting time and resource requirements restricted data collection.

The samples were presented to the dog in the same room and at the same height as described in Section 2.4.3. However, samples were presented one at a time. There was potential to cause confusion if multiple positive samples were present in a run since during training normally only one or two was present. Practice runs using captive *M. minutus* scent took place to assess whether this change affected the dog's ability and no negative effect was evident.

The dog would sit when *M. minutus* scent was detected and the feeder number was noted, so its location could be mapped. If a weak indication was given by the dog, the feeder was retested and if a second indication was given this was considered a positive *M. minutus* sample. There was no conclusive method of testing the samples for *M. minutus* and therefore it was not possible to know if the sample contained *M. minutus* scent. However, the results of the continuation training and discrimination testing indicate that the dog demonstrated a sufficient reliability rate (Porritt *et al.*, 2015).

2.4.6 M. minutus nest search surveys

To replicate realistic nest searching conditions, nest searches were carried out by volunteers, higher education students based at Moulton College. They all had received training in the method and how to locate nests (i.e., using hands to search in the stalk zone of tall grasses and lower to the ground where tussocky grasses were present) (Mammal Society, 2013). Training also included describing the basic ecology of *M. minutus*, identifying suitable habitat and identifying nests *in situ*. Volunteers were also provided guidance on how to search and advised on how long to spend searching based on the Mammal Society's guidelines and all volunteers were trained by the researcher. Between two and four volunteers carried out nest searches per location.

Nest search surveys were undertaken in two of the three locations (locations 1 and 2). The margins and hedgerows were cut in location 3 before the nest survey could be undertaken and therefore a nest search was not possible.

During each survey, an area of 200m^2 was covered, over a maximum of 1hr, this being either a 10m x 20m or 100 x 2m search area. If there was more than one volunteer searching, then the time and area searched were divided accordingly (Mammal Society, 2013). The search area was measured using a tape measure (50m) and the volunteers were advised to follow appropriate hygiene practices (i.e., hand washing, no eating, drinking or smoking during the survey and using anti-bacterial hand gel). The approximate location, diameter and height of nests were recorded as well as nest category (shelter or breeding) and where possible the grass species utilised. The researcher kept an approximate count of nests encountered while setting up the feeders for the scent surveys. However, formal nest searching by the researcher was not undertaken to avoid damaging and disturbing the habitat, with potential effects on feeder usage by *M. minutus*. Additionally, there was the potential to bias nest search results carried out by volunteers.

2.4.7 Data analysis

With only one trained dog available for training and testing the scent survey method, the amount of data collected was limited compared to equivalent studies that may have used four or more dogs (Willis *et al.*, 2004). Thus, the use of inferential statistics was limited. To overcome this, the evaluation of the dog's detection ability was assessed using a scoring system. An equation originally presented in Porritt *et al.* (2015) (Eq.(1)) was adapted and a pass or fail could be determined for each training session. The result of Eq.(1)) is hereafter referred to as the 'detection score'. The detection score expresses the proportion of correct indications by the dog of a positive

scent target relative to a specified pass rate (*pa*), adjusted by the proportion of false indications. The results of the continuation training sessions that took place between February and June 2015 (described in section 2.4) were applied to Eq. (1) (Porritt *et al.*, 2015). When reporting the results, the pass rate was set lower than described by Porritt *et al*. (2015) as in the current study there were no potential life-threatening outcomes from the dog missing a target. Therefore, this figure was reduced from *pa*70 to *pa*60. It is important to note that the scoring system was not a statistical indication of performance and subsequent statistical test were undertaken on the detection scores.

$$ds = \left(\frac{x}{n} - r\right) - \left(\frac{y}{m}\right) \quad \ldots 1$$

ds = detection score
x = number of indications on a true target identified
n = total number of targets in test
r = pass rate (*pa*) / 100 (percentage the detection rate must be greater than the false indication rate to pass)
y = number of indications when there is no target (false indications)
m = total number of blanks in test calculated as (number of runs) × (number of stands) – *n* (adapted from Porritt *et al.*, 2015)

If data were not normally distributed the Johnson transformation in Minitab Versions 17 and 18 was undertaken to allow parametric analysis. Non-parametric alternatives were selected where normalisation could not be achieved. Parametric difference tests utilised included One-way ANOVA (*F*) and One-tailed t-test (t) and non-parametric alternatives included the Kruskal-Wallis (H) and Mann-Whitney (W). When testing associations, the Pearson's correlation (*r*) was utilised where data were normally distributed

and Spearman's Rank (Rho) correlation was used as the non-parametric alternative. The detection score was also calculated using the outcomes of the formal single blind discrimination testing, which provided a definitive outcome for the dog's discrimination ability and thus justified continuation of the proposed remote scent survey method.

Furthermore, the dog's ability to ignore true negatives (specificity, Eq. (2)) and indicate true positives (sensitivity, Eq. (3)) was calculated from the outcome of the formal discrimination testing (Willis *et al*., 2004).

$$\frac{\boldsymbol{d}}{\boldsymbol{c+d}} \qquad \ldots 2$$

$$\frac{\boldsymbol{a}}{\boldsymbol{a+b}} \qquad \ldots 3$$

Specificity and sensitivity calculations where a = true positive, b = false negative, c = false positive and d = true negative.

2.4.8 Scent surveys vs nest searches

The comparative data collected during the nest search surveys and scent surveys were too small to apply inferential statistics. Therefore, detection probabilities were calculated, which also allowed the seasonal effectiveness of each method to be assessed. Firstly, the raw detection rate (Eq.(4)) was calculated as per Long *et al.* (2007).

$$\frac{\mathbf{d}}{\mathbf{n}} \qquad \ldots 4$$

Raw detection - d=number of detections and n= number of sites surveyed (Long *et al.*, 2007).

Secondly, using the occupancy modelling function in program MARK (White, 2016) detection rates were calculated (Long *et al*., 2007). The unequal number of surveys between nest searches and scent surveys did not present an issue here, as the analyses were not carried out at sample level, but survey level. Furthermore, program MARK treats detections as independent of one another (Campbell, 2004). It is important to note here that the use of occupancy modelling in this sense is not to elucidate ecological inferences, but simply to establish methodological parameters. This approach was utilised in addition to the raw detection rate as there was a possibility of two types of error: the detection dog may have missed the target scent, or the scent was not present. Therefore, the probability of occupancy (proportion of sites where *M. minutus* visited a feeder) and true probability of detection (the proportion of sites with target scent that the

dog detected) was estimated using occupancy modelling in program MARK (White, 2016).

2.4.9 Data handling procedure for occupation modelling

The surveys were divided into site survey histories (*k*=8), for example, a history of 11000011 (where 1=species detected and 0=species not detected) with the first four digits relating to the scent surveys using a detection dog, and the remaining four digits being nest search surveys. An unequal survey history was accounted for by using a (.) instead of a 1 or 0 (110…00). The parameter estimates for p(t) overall capture probability was estimated for each survey method.

2.5 Methods: Chapters Four and Five

Releasing populations of captive reared *M. minutus* was a fundamental element of being able to test the RFID traps and compare their effectiveness to live trapping results. Once tested and validated the RFID traps were fundamental for allowing observations of the effect of fragmented habitats and behavioural type on *M. minutus* movement.

2.5.1 Husbandry, identification and breeding of the reintroduced M. minutus populations

M. minutus were donated by Shepreth Wildlife Park, the New Forest Wildlife Park, the Chestnut Centre, Battersea Park Zoo, Newquay Zoo, Berkshire College of Agriculture and from the personal collection of Miranda Krestovnikoff. *M. minutus* donated by the Chestnut Centre were of wild origin, all others were descendants of Chester Zoo's captive population.

Rudd's (Undated) methods of husbandry were followed for the care and welfare of the mice. Males and females were housed separately in related groups where possible. Breeding pairs were housed separately from the release population to minimise infanticidal behaviours and disturbance by conspecifics.

Housing varied throughout the captive rearing phase, with single sex group housing preferred (maximum n=25), for ease of husbandry and to maximise pre-release training (IUCN, 1998). Notwithstanding this, some individuals were housed singly, particularly at the beginning of the study. Tail biting can be a problem within captive *M. minutus* populations (Rudd, 2012), but was found to be reduced when housed as per Figure 2.6. This was a large square enclosure with indoor and outdoor areas which appeared to provide sufficient space to avoid encounters. Individuals that displayed persistent tail biting were housed individually to minimise damage to conspecifics; damaged tails would result in an individual being excluded from the release population. Pre-release training included acclimatisation to seasonal weather conditions, inclusion of wild food resources and enrichment for climbing and nest building.

Figure 2.6 *M. minutus* housing for single sex groups.

2.5.2 Microchipping

M. minutus were microchipped for identification purposes, which subsequently allowed autonomous collection of data for released individuals using RFID. ISO 11784 certified Passive Integrated Transponders (PIT) tags (134.2 kHz) measuring 1.35 x 7 mm (Loligo Systems, Denmark) injected subcutaneously using a suitable hypodermic implanter (Figure 2.7).

No licenses were required under the Animals (Scientific Procedures) 1986 Act for the microchipping procedure as no anaesthetic was being used (Whitehead, 2012); a novel method for this species. A pilot assessment was

carried out to monitor the effects of microchipping and no adverse effects were recorded. Behaviour and health was monitored by the researcher post-microchipping and no ill effects were noted, with normal feeding and locomotive behaviours returning within minutes, suggesting they had not been adversely affected by the procedure (Reichling and Tabaka, 2001). Chipping records were kept in a Microsoft Excel spreadsheet, along with number of implants each hypodermic needle had carried out as disposal was recommended after 100 uses.

2.5.3 Microchipping protocol

The microchipping protocol was developed in conjunction with a trained veterinarian. The protocol was piloted, firstly on dead *M. minutus* and then on a sample of the population. Three mice were chosen at random. These individuals were scruffed as per normal small mammal handling methods. The researcher selected to use their weaker hand for scruffing as control of the dominant hand was required for injecting the chip. The needle was inserted under the triangle of skin naturally formed between the thumb and forefinger of the handler when scruffing the mice. When the needle was inserted, the chip was injected using the implanter's plunging mechanism. If the handler thought the procedure was not going to be successful they would stop and allow the individual to recover. The needle and PIT tag were soaked in ethanol and allowed to dry before use. The PIT tag was checked for any breakages or cracks in the glass and the number was noted prior to implantation to ensure it was functioning correctly. On a few occasions the PIT tags were rejected and subsequently expelled from the body.

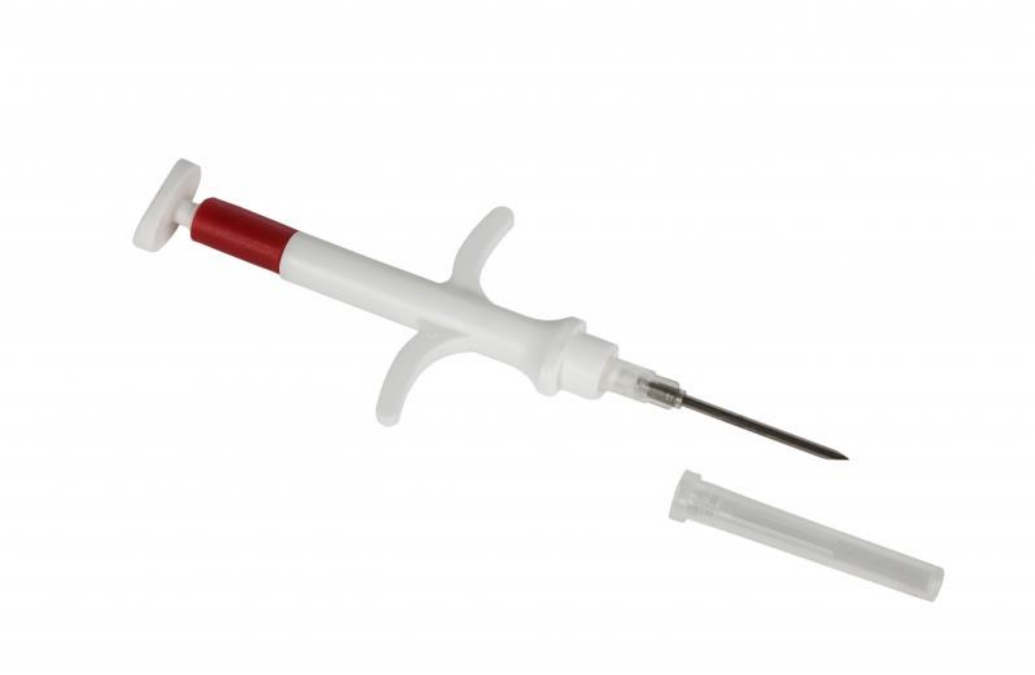

Figure 2.7 - Hypodermic needle and implanter for 7 x 1.35mm microchip (Loligo Systems, 2015).

2.5.4 Parasite control

Veterinary treatments were obtained from Abington Park Vets, Northamptonshire. Regular treatment with Xeno 450® (Dechra, Northwich) prevented fleas, mites and roundworm. The cages were cleaned periodically with hot water and Fam 30® (Evans Vandolin Int. PLC, Preston) disinfectant to ensure any remaining parasite eggs were unviable and to reduce bacteria within the enclosures.

2.5.5 Pre-release health testing

Release of disease free animals is essential for the maintenance of self-sustaining populations and for the longevity of existing and potentially naive

populations (IUCN, 1998). Based on the findings of Chester Zoo a sample of the release population was tested for *Campylobacter, Salmonella*, protozoans and endoparasites. Faecal samples were also provided for secondary parasitic analysis (Haverson, 2013). One *M. minutus* per population was euthanized by veterinarians from Abington Park Vets and the digestive system was removed by the researcher in lab conditions (Haverson, 2013). The samples were transported on ice to the Central Diagnostic Services of the University of Cambridge within two hours of removal. The results and treatments are detailed in Table 2.2. Physical health checks were carried out by the researcher before release; individuals with eye, tail or limb defects were not included in the release population.

Table 2.2 - Summary of samples provided for health testing in year one and two. Mice that had been integrated for over one month were considered the Moulton College population. × Denotes negative result.

Sample population	Year	Campylobacter	Salmonella	Endo-parasites	Protozoa
1. New Forest Wildlife Park	1	×	×	*Hymenolepsis* spp.	×
2. Moulton College	1	×	×	×	×
3. Battersea Park Zoo	2	×	×	×	*Trichomonas* spp. 2+
4. Moulton College	2	×	×	×	*Trichomonas* spp. 3+
5. Newquay Zoo	2	×	×	×	×
6. Moulton College	3	×	×	×	*Trichomonas* spp. 2+

2.5.6 Hymenolepsis spp.

As Table 2.2 shows, *Hymenolepsis* spp. were seen in the New Forest Wildlife Park's population. Profender® (Bayer, Kansas) was used to treat this infection (Peniche and Sainsbury, 2013). No mice were observed expressing an adverse reaction to the drug and all mice were treated as a precaution. Profender® became part of the prophylactic routine to prevent further infection. The results of year two health testing validated treatment as no further infestations were recorded.

2.5.7 Trichomonas spp.

Trichomonas spp. were found in years two and three. This protozoan is common in passerine birds. Thus, contamination is likely to have occurred when the mice were housed outside for their pre-release training. The initial treatment of *Trichomonas* spp. was carried out using liquid metronidazole, upon recommendation from Abington Park Vets. This was mixed with 1% sucrose solution to improve palatability and presented in small water bottles (Roach *et al.*, 1988). None of this solution was taken by the mice; alternative treatment was required. Following findings of Roach *et al.* (1988) metronidazole was provided in tablet form, 2.5 mg per ml in a 1% sucrose solution. Care was taken not to denature the active ingredient in the metronidazole when dissolving the crushed tablet in warm water.

2.5.8 Development of RFID technology

Bespoke RFID traps were commissioned for the purpose of post-release monitoring (Wallis, 2013). The function of these units was to autonomously collect individual microchip number, time, and location data when a mouse passed through the reader. A wireless radio within the unit sent data to a single data logger, recording all data from all units centrally (Wallis, 2013).

The autonomous RFID monitoring system is constructed around a wireless data logger that records data transmitted by RFID traps. The RFID trap

housing varied depending on the development stage. Being able to alter the housing meant it was highly versatile and adaptable during the prototype testing stages.

Each RFID trap is based on the same core hardware design. Table 2.3 outlines the components used for the RFID traps and data logger (Wallis, 2013). The bespoke RFID system allowed autonomous, continuous data collection, where instantaneous transmission and recording of PIT tag reads was achieved via an internal wireless radio linked to a central data logger.

Table 2.3 - Individual components required for reader and data logger wireless network configuration.

Reader Components	Data Logger Components
Electronic reader	Raspberry Pi® Model A and Model B for programming
Xbee radio ® (Digi International)	1 x 12 v battery
Internal Clock	Master Xbee radio ® (Digi International)
Microprocessor	Real time clock
Passive Infrared (PIR) sensor	SD card

2.5.9 Testing RFID equipment

This system was developed for the use in this research project and therefore underwent testing and adapting to maximise effectiveness during data collection. Thirteen RFID traps and one data logger featuring the master Xbee radio were constructed. However, the complex manufacturing process and sensitive electronic components resulted in

seven of these units not functioning effectively. Therefore, these were not used in any of the data collection. The remaining six RFID traps were attached over the entrance of 380ml plastic bottles (Figure 2.8). A small right-angled section of the bottle entrance was removed, this allowed the reader to sit over the entrance and ensured that it was within sufficient range to read the PIT tag as individuals entered. The combination of RFID readers, and their housing are referred to as RFID traps from this point onwards. Bottles ensured that the food provided remained dry in field conditions. High usage was expected in the captive environment as space was limited and food was provided within the bottles, making it difficult to identify how the system would react in field conditions. Thus, two pilot studies were undertaken at different stages for the RFID trap development.

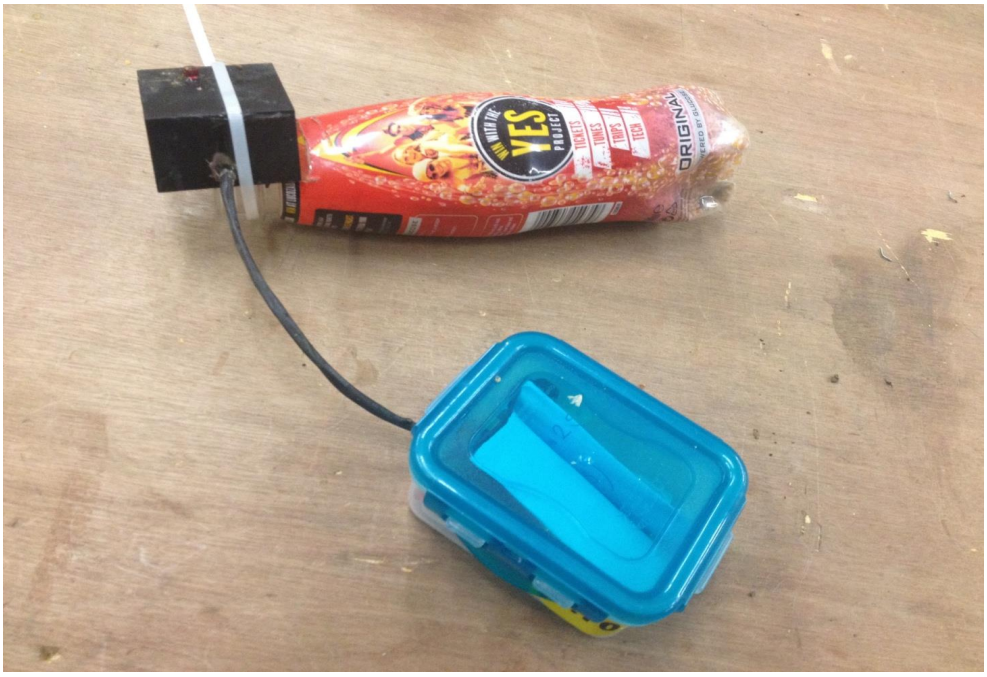

Figure 2.8 – Version 0.1 of the RFID trap. The reader is situated above the entrance of the bottle, the reader components were housed in the plastic water tight box. Traps were baited with Johnson and Jeff Cockatiel mix to encourage usage.

2.5.10 Pilot I

Pilot I tested the principle of using RFID on *M. minutus* in field conditions and allowed the functioning of the RFID traps and data logger to be assessed. A comprehensive description of Pilot I can be seen in Appendix A. This study identified several areas for improvements for the RFID traps to function efficiently.

Firstly, the energy consumption of the RFID traps was too high for the system to be efficient. This had a twofold impact, firstly when the RFID trap's batteries were exhausted it caused a loss of data and a total reset of the reader was required before the reader could function as normal.

This was a labour-intensive procedure, particularly when the battery needed replacing at least every 12hrs. Secondly when the batteries ran out, reads were not being recorded, resulting in a loss of data.

Furthermore, when processing the data collected during Pilot I it was apparent that the procedure for recording locations of the RFID traps was not effective. Location information was not recorded automatically, and extrapolation was required from the data string created for each read. Improving movement and range data collection is vital for this species. Therefore location information was paramount. Thus, a better system of recording needed to be implemented at the next stage.

Camera traps established in the release pen revealed individuals were experiencing high levels of disturbance by conspecifics. Therefore, future releases utilised a smaller release population to reduce this impact and to create a more realistic release scenario.

2.5.11 Pilot II

A comprehensive description is provided in Appendix B. In response to the high energy consumption of each reader, each RFID trap was fitted with Passive Infrared (PIR) (Adafruit, New York) motion sensors potted in epoxy resin to ensure they remained waterproof. The PIRs were positioned on the exterior of a 4.5 L plastic box allowing the reader to turn on only when required. This extended the battery life to 24 hours, although this was reduced if the RFID trap was heavily used throughout the monitoring

period, so was not an absolute fix. A second pilot study allowed the modified design to be tested in field conditions.

While the battery life was extended, during Pilot II, the real-time clock (RTC) malfunctioned irreparably and actual times of reads were not obtained. This malfunction was not noticed immediately and highlighted the intricate nature of this method. A system of resetting the data logger and immediately recording a read using a test chip and time of reset, allowed the time to be extrapolated when data were transferred to a spreadsheet. One caveat to this is, if the data logger turned off outside of the manual reset, the time was not recorded. During analysis if actual times were required and they could not be established, these data were omitted. All data from the pilot studies were omitted.

The Xbee radio (Digi International) was the fundamental element of the WSN and facilitated communication between the RFID traps and the data logger. Within a WSN a master Xbee radio was required and was situated within the data logger, this controlled the communication between the RFID traps and the data logger, allowing instantaneous transmission and storage of each PIT tag read recorded. However, the wireless range of the Xbee radio was limited to around 10m and the size and linear design of the release enclosure meant certain areas were out of range for recording reads, and thus, the consistency of the data collection was impacted. A booster Xbee radio could have been implemented to extend the range between an RFID trap and the data logger. However, this function could

not be established without additional expertise outside of the project team. Therefore, the length of the release pen was reduced for Release I and II to ensure a consistent approach to data collection.

The final version of the RFID trap was housed within a plastic box (Hobbylife – 26x17x18cm), which contained a 380 ml baited plastic bottle; this sat behind a circular entrance, which provided a barrier between the subjects and the electronic components of the RFID trap (Figure 2.9). As tagged individuals passed through the entrance (C) and into the bottle, the PIR motion sensor (B) was activated, thus powering the RFID reader (A), allowing the individual's microchip to be read and data transmitted instantaneously and wirelessly via the Master Xbee radio (J) to the data logger (F). A 13 mm gauge wire mesh, which was stretched slightly, was fitted to the entrance (C) to limit access by larger, non-target small mammal species. The data logger (F) was powered by a 12v battery and was ready to receive data from the RFID traps at any point. Data were stored on an SD card which was connected directly to the Raspberry Pi (G), allowing easy removal and rapid data downloads. The microchip could be read when an individual entered and left the RFID trap. Location data were extracted from the data string provided for each microchip read. There were occasions when the malfunctions in the system caused data to be lost, but details of these incidences were impossible to record.

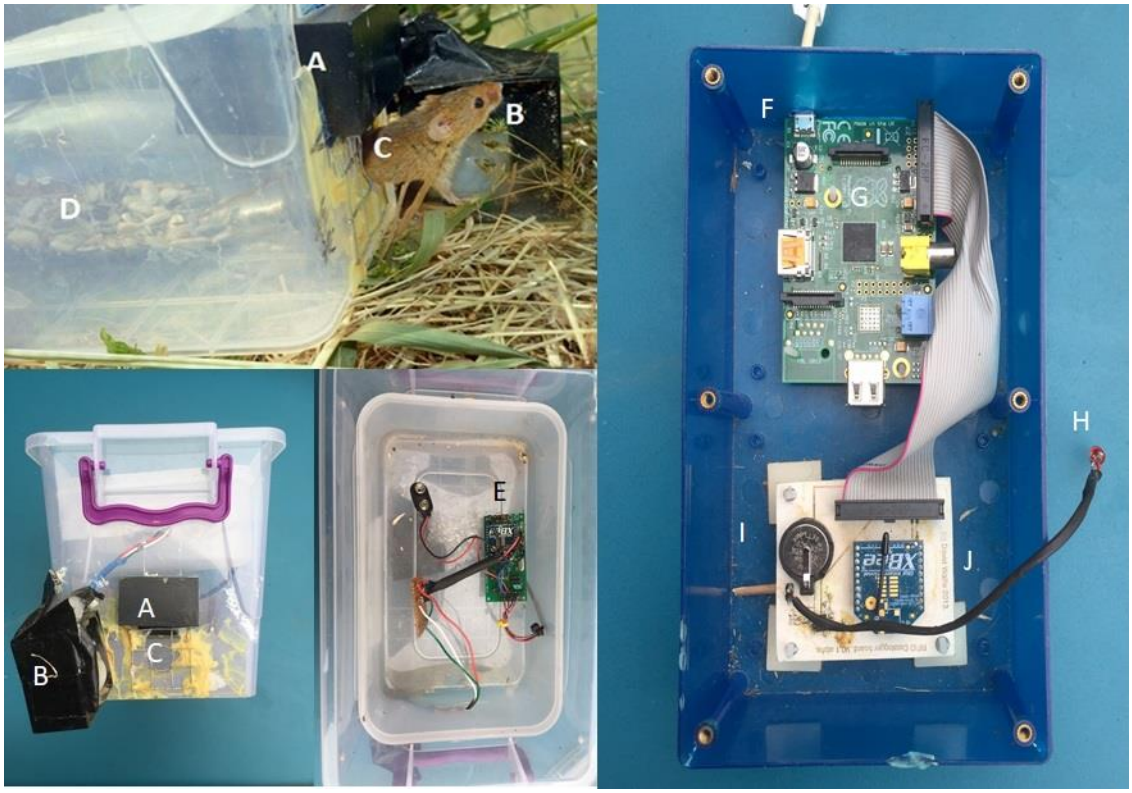

Figure 2.9 - RFID trap with *M. minutus* exiting, RFID trap and data logger. Components include: electronic reader (A), PIR motion sensor (B), trap entrance (C) and baited plastic drinks bottle (D), RFID trap printed circuit board components, this view shows the top of the trap, not accessible to the occupants (E), data logger (F), Raspberry Pi (model A) ® (G), LED data transmission indicator (H), real time clock (I) and Master Xbee radio (J) - (*M. minutus* picture courtesy of Upton (2015c)).

2.5.12 Pre-release data - open field test

To develop understanding of the links between behaviour, movement and survival of *M. minutus*, a method of assessing behaviour pre-release was implemented. Here an Open Field Test (OFT) was carried out for each individual.

This method was principally used to assess the effect of drugs on exploration, risk assessment and anxiety-like behaviours in rodents (Hall,

1936; Prut and Belzung, 2003; Schuster *et al.*, 2017a). Here there was no treatment. The OFT was simply utilised to quantify individual responses to a novel environment: firstly, to identify behavioural types and secondly to identify pre- and post-release correlated behaviours, providing a possible insight into the association between behavioural type, movement and survival.

2.5.13 OFT procedure

The OFT methods were adapted from Walsh and Cummins (1976). The mice were placed in the centre of a round white flexitub positioned on the ground (36cm diameter base and 47cm diameter top); the base of the tub was flat. Activity was recorded for 5 min using a Panasonic HC-V10 digital video camera. This was positioned above the tub using a Traveller compact pro tripod. Video footage was analysed after all individuals were tested. Each video was uploaded into Kinovea V.0.7.10 motion analysis video software. A standardised grid and centre circle template was applied to the computer screen to allow accurate data collection and analysis (Figure 2.10). The grid was transferred to clear acetate then manually applied to the computer screen and the Kinovea software window could be moved accordingly if required.

The video playback was slowed down to allow accurate recording of variables (frequencies or duration as per Table 2.4). The playback timer was used to calculate accurate durations to two decimal places. The initial

centre circle entry and centre circle duration was not included in the totals as this is where the individual was placed to begin the test. However, the line crossing upon exit of the centre circle was counted.

Between each test the flexitub was sanitised using Ark-Klens (VETARK, Winchester) germicide and dried before the next subject was placed inside. To ensure the light level during the OFT was available if required, Lux readings were taken using the Lux camera application for iPhones, but these readings were not utilised within the analysis.

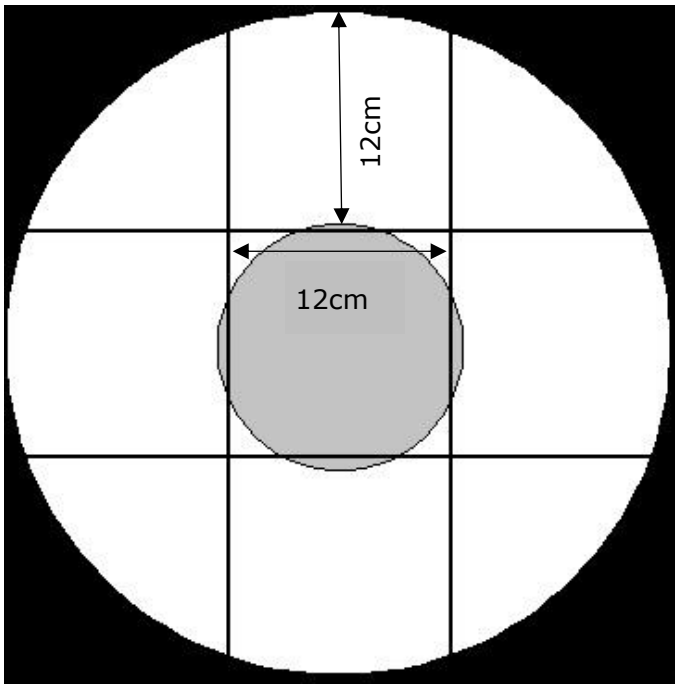

Figure 2.10 – Standardised grid applied to the computer screen when analysing open field test footage using Kinovea V.0.7.10. Light grey circle = centre circle; grid = lines used to measure the number of lines crossed, to estimate distance travelled; grey and white circle = the open field arena; solid black areas were outside of the open field arena. *M. minutus* were placed into grey circle at the beginning of the recording.

To ensure the area was quiet during the tests the observer left the room. A timer was set to measure the 5min recording interval. Mice were collected from their normal enclosure one at a time. To prevent any behavioural influences on the other mice, subjects were not returned to their normal enclosure until all the individuals were tested.

2.5.14 Open field test (OFT) dependent variables

There appears to be no straightforward meaning of activity in the OFT (Walsh and Cummins, 1976; Stanford, 2007), but locomotor activity has been used to define exploratory behaviours and subsequent responses to fragmented habitats (Cornelius *et al.*, 2017). Activity here has been measured by the number of lines crossed during the testing period (5 min) (Figure 2.10). In terms of measuring anxiety (boldness), the agreed measures include, the proportion of time spent in the centre circle of the open field arena and number of entries into the centre circle over the course of the test (Herde and Eccard, 2013); both "unsafe" areas. Individuals that feel higher levels of anxiety have fewer centre circle entries, spend less time in the centre circle and display higher activity level near to the walls (thigmotaxis) (Simon *et al.*, 1994; Stanford, 2007). Bourin *et al.* (2007) noted that individuals which were more negatively impacted by the open field arena express a decrease in the ratio of number

of squares visited in the centre and number of squares visited in the periphery.

Risk assessment behaviour which is traditionally recorded in the OFT, includes the frequency of stretch attend posture (SAP) which provides an insight into risk assessment and vigilance (Yang *et al*., 2004). However, SAP was not used here as the behaviour could not be accurately identified when analysing video footage. The alternative measure of risk assessment is rearing (individuals stood on hind limbs), an exploratory behaviour, which has an information gathering and risk assessment function (Brenes *et al.*, 2006; Brenes *et al.*, 2009). Therefore, rearing of duration >1s was used the measure risk assessment, which was easily identified during video analysis. Rearing was divided into <1s and ≥1s as the former was generally observed during ambulation and its similarity to 'scanning' described in Cornelius *et al.* (2017). Hence, it was therefore thought prudent to measure these behaviours independently, thus rearing <1s was referred to as scanning. Accordingly, rearing ≥1s was simply referred to as rearing. A comprehensive list and definitions of the pre-release dependent variables measured during the OFT are detailed in Table 2.4.

Table 2.4 – Dependent variables measured during the OFT, includes type of data collected description (adapted from Anon., 2005).

Dependent variables	Frequency (F) or Duration (D)	Description
Lines Crossed (TLC)	F	Mice crossed one of the grid lines with all four paws.
Centre Circle Entries	F	Mice crossed the centre circle line with all four paws.
Centre Circle Duration	D	Percentage of time spent in the central circle.
Scanning	F	Mice stood on their hind legs in any area of the arena which lasted <1s.
Rearing	D	Mice stood on their hind legs in any area of the arena which lasted ≥1s – may also be sniffing the air at the same time.
Grooming	D	Time spent licking or scratching while stationary.
Freezing	D	Mouse was completely stationary.
Defecation	F	Total faecal pellets produced.

Dependent variables	Variable calculation
Estimated distance travelled (cm)	Total lines crossed x 12 (straight line distance in cm of one square in the open field arena (see Figure 2.10)).
Centre lines crossed (CLC)	Centre circle entries x 2 (for each centre circle entry two line crossings were recorded)
Periphery lines crossed (PLC)	Total lines crossed – Centre lines crossed
CLC:PLC	Centre lines crossed ÷ Periphery lines crossed
$\overline{x}$ time per centre circle entry	Centre circle duration (s) ÷ Centre circle entries

2.5.15 Analysis of data

The results of the OFT were compared to the post-release dependent variables (last day recorded and movement between traps (Release I) and movement between patches (Release II)), firstly, to identify any behavioural types and, secondly, to establish if any significant correlations between weather, post-release behaviour, survival and movement could be detected. Where relevant, correlations between the OFT variables were undertaken to establish any patterns between behavioural type.

2.5.16 Pre-release selection of M. minutus

All individuals were over three months of age when released and as far as could be determined, all capable of reproduction. All individuals released were in optimal condition, determined by pre-release pathology and any individual with a visible physical defect (e.g., tail injuries, asymmetrical eyes) were removed from the release population. Individual animals released needed to be fitted with a functioning PIT tag for identification purposes. This subsequently allowed the autonomous collection of data for released individuals and manual identification when caught in a live trap using a hand-held reader. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

2.5.17 Release procedure (Release I)

Microchipped *M. minutus* were released into two outdoor enclosures which were modified from Pilot II (Appendix B). The enclosures were situated within Bennie's Quarry, Pitsford ($52^{0}17'40.68$ – $0^{0}53'29.50$) and adapted from Pilot II in May 2015. Ecofender newt barrier (Hy-Tex, Ashford) fencing was used to enclose the area. Wooden stakes (37x37x1200mm) were placed approx. every 1.5m to secure the newt barrier, fencing was secured to the wooden stakes using nails and staples, duct tape was used to secure the joins in the fencing materials. Netting was installed above the enclosures to reduce aerial predation, and to limit escape this was situated above the internal vegetation (Figure 2.11). Plots were accessible to non-target small mammal species as entry could be gained from outside the enclosures by climbing the wooden stakes. Natural dispersal was possible from the plot; thus, the actual mortality rate was not recorded, and true survival at the end of the experiment was not defined.

Figure 2.11 – An example of the enclosure construction and design, actual design differed slightly as the plot was smaller for Release I and II.

The semi-enclosed experimental plots were divided into two 2.4m x 14.8m (approx.) enclosures, which were positioned near to one another to ensure vegetative structure was replicated in each plot.

Native vegetation was dominated by *Dactylis glomerata* (cock's-foot) and *Arrhenatherum elatius* (false oat-grass) with occasional *Heracleum sphondylium* (hogweed) and *Urtica dioica* (common nettle) present. One enclosure was monitored using a line transect of Longworth live capture traps and will be referred to as LIVE patch (Flowerdew *et al*., 2004), whilst

the other used RFID traps, accordingly will be referred to as RFID patch from this point forwards (Wallis, 2013).

Seven microchipped *M. minutus* were released in each enclosure (LIVE ♂4 ♀3; RFID ♂3 ♀4). On the first night five Longworth traps were set at 2.5m spacing, and as all were occupied, seven were deployed from night two at 2m intervals and all traps were placed on the ground, whilst in the RFID plot there were between three to five RFID traps (Figure 2.9). However two of the traps failed to work after the first two nights so were removed. RFID traps were placed in a line on the ground with between 2.5m and 5m spacing. Each trap contained a cockatiel mix, firstly to supplement food and secondly as a bait. Water was provided in both plots. Fewer RFID traps were utilised compared to live traps as the access was not limited to one per capture period as found with live trapping. Live traps were not set between 08:00 and 18:00 as risk of hyperthermia was increased due to high ambient temperatures; traps were checked before 08:00 the following day. At the end of the experiment the plots were opened to allow remaining individuals to disperse.

2.5.18 Post-release data collection

Data collection began as soon as the individuals were released. Data were downloaded once a day from a central data logger and batteries were changed once every 24 hours. As per Pilot I and II Aerocell AA batteries (Lidl, Neckarsulm) were used as the battery life was superior to other brands. The monitoring period lasted for 14 days between 30/06/2015 and 13/07/2015. No replication of the experiment was undertaken.

2.5.19 Post-release dependent variables

The post-release dependent variables were defined as follows: survival was classified as the last day recorded, the day when an individual was last recorded, and this measure was consistent between the releases irrespective of monitoring method (LIVE or RFID). In contrast, movement variables were defined differently depending on experimental design. The movement variables were classified as follows: movement between traps for Release I and movement between patches for Release II. These were statistically analysed to identify significant correlations between the pre-release dependent variables described in Table 2.4.

2.5.20 Release II

Release II took place between the 17/08/2015 and 27/08/2015. The basic release pen structure remained the same. However three different sized gaps were created; 1m, 2m and 4.8m, resulting in four patches (1=4mx2.4m, 2=4mx2.4m, 3=4mx2.4m and 4=15mx2.4m). Gaps were created by cutting vegetation as close to the ground using a grass hook. As vegetation was not totally removed these gaps are referred to as 'soft gaps', rather than the 'hard gaps' a road or paved footpath may present. Plot 4 was the last available plot, and the size was larger compared to the other plots. Fourteen microchipped mice were released; N=♂5 and ♀9. It was possible that initial movement over the gaps could be a response to a novel environment rather than a natural motivation to move. Thus, to reduce the potential for this and to assess a more natural rate of movement over the

widest gap the access to the 4.8m gap was restricted until the 21/08/2015. One RFID reader was placed in the centre of each plot and the monitoring was carried out using only the RFID method described in section 2.5.8.

2.5.21 Data analysis (Release I and II)

The data were analysed as per section 2.4.7. When appropriate, regression analysis was undertaken, including cubic and quadratic regression if a non-linear relationship was suspected. Minitab v.17 and v.18 were used for all statistical analysis and significance level was set at 95%.

2.5.22 Comparing effectiveness of methods (Release I)

Equipment performance metrics were recorded and used to assess the effectiveness of each method. These metrics included capture probabilities calculated using the Cormack-Jolly-Seber (CJS) model in program MARK (White, 2016), the number of individuals encountered in 24h, the number of recorded reads per 24h and the temporal effectiveness of each method.

The use of inferential statistics to analyse these data were somewhat limited as direct comparison was difficult due to the difference in survey effort between the RFID and LIVE plots. RFID was capable of running continuously, while LIVE would only allow capture of one individual per trap per capture period, with further restrictions due to the daytime temperature. To provide capture probability for both methods which is comparable and to establish which factors influenced recapture rates, a population modelling approach was utilised.

Using program MARK (White, 2016), capture probability and survival was modelled using CJS, calculating parameter estimates for survival (Φ) and recapture (P). These parameters were not used to make biological inferences, but simply to provide apparent survival and recapture rates for each method utilised. Lebreton *et al.* (1992) noted that data sets used for modelling can be used as part of treatment and control experiments, which supports the use of the CJS model for this analysis.

2.5.23 Capture histories

Fourteen 24h capture periods were identified, and the capture histories of each individual were formulated (White, 2016). As the RFID could record more than one mouse per capture period, capture histories were created based on an individual's chip being read at least once in a capture period. However, they may have been recorded multiple times. Conversely, live traps could only record one animal per trap per capture period.

2.5.24 Modelling procedure

To identify if there was an even dispersion (variability) within the data, 200 bootstrapped data simulations were carried out on the global model Φ(g*t) P(g*t) (g = group (LIVE and RFID); time = (t)). As an over-dispersion was identified $\hat{c}$ was calculated (2.52) from the bootstrapped data and adjusted within MARK as per the method of Pryde (2003). The Quasi Akaike Information Criterion corrected for a small sample size (QAICc) was used for the model selection. U-CARE goodness-of-fit software identified the relevant assumptions of the CJS model were valid (Choquet *et al.* 2009). The recapture duration assumption associated with CJS was violated in this

study. However, O'Brien *et al.* (2005) found that violating this did not increase bias in survival estimates.

2.5.25 Comparison of activity between release I and II

The possibility that fragmented habitat caused a change in behaviour in *M. minutus* as described in the previous section was investigated further. Data from the release I and II were compared to identify any differences in activity depending on habitat category (fragmented or continuous). Ideally these would have considered an equal number of individuals and the patch would have equalled the same area.

To compare activity, data were divided in to reads within patches (no movement) and between patches (movement) for the release II and into reads at the same RFID trap (no movement) and different RFID traps (movement) for release I. Time between reads were then calculated to allow comparisons between the habitat categories

The reads from the same patch recorded during release II were compared to the reads occurring in the same RFID trap in the release I (no movement recorded). Similarly, reads from different RFID traps (release I) and different patches (release II) were also compared (movement recorded).

3 Chapter Three – The use of a detection dog to confirm the presence of *M. minutus* within suitable areas of habitat.

3.1 Introduction

The small size and cryptic nature of *M. minutus* have often meant that they are not regularly encountered during population studies (Poulton and Stone, 2008; Poulton and Turner, 2009; Riordan *et al.*, 2009) and their nests, which act as visual indicators of presence can often be overlooked when carrying out nest surveys (Harris, 1979a). An alternative non-invasive survey method, which does not require direct observation would prove beneficial for *M. minutus*. Olfactory indicators of presence could provide reliable presence data when compared to other standard survey methods commonly used, such as nest search surveys. Here it is proposed that a remote scent survey method is tested, which would not require direct observation or capture of *M. minutus*. A detection dog has the proven olfactory abilities to detect biological scents and with effective training could be taught to distinguish the target scent of *M. minutus* from other non-target small mammals. Thus could prove an effective non-invasive method of establishing presence. A *M. minutus* detection dog could provide a rapid and relatively cost-effective method to establish their presence, particularly in areas where *M. minutus* have been overlooked.

Browne *et al.* (2006 p. 101) summarises a detailed review of the varied uses of dogs by concluding, "*The major restriction to the use of trained scent-detection dogs appears to be human imagination*". This persuasive quote, in addition to the other presented evidence detailed in Chapter One

suggests that it is entirely possible that a dog could be used to detect *M. minutus*.

3.1.1 Aim

To establish whether a detection dog can be used to confirm *M. minutus* presence within areas of suitable habitat more effectively than nest searches.

3.1.2 Objectives:

Objective I: To evaluate the effectiveness of a detection dog at indicating *M. minutus* presence.

Objective II: To compare the effectiveness of scent surveys compared to standard nest searches at identifying *M. minutus* presence

3.2 Methods

The methods pertinent to this Chapter have been described in Chapter Two, Section 2.4 is most relevant to the results presented in the subsequent sections.

3.3 Validation of the remote scent surveys for *M. minutus*

This section considers three areas of validation for the proposed remote scent surveys and will act as a proof of principle study. These validation areas include, consistency during continuation training, discrimination testing and uncontrolled field testing. Each area will provide an indication of the potential for the proposed remote scent survey method to be used as a standardised monitoring tool for *M. minutus.*

3.3.1 Omitted Data

As continuation training pre-dated the publication of Porritt *et al.* (2015), recording the number of stands was not noted in each instance, accordingly any training session that missed this information was not included in the calculation of Eq. (1). Furthermore, the data gathered during the controlled field testing was not used within the formal analysis due to the limited scope of the data, however this has been included in Appendix C for reference.

3.4 Results

3.4.1 Temporal effectiveness of the detection dog.

The outcome of each run during the continuation training was classified as one of three detection categories (correct, missed and false). The analyses identified a significant difference between detection outcome categories per month ($F(2,14)=18.95$, $p<0.001$). Tukey's *post hoc* testing revealed a significant difference between all three categories (Figure 3.1).

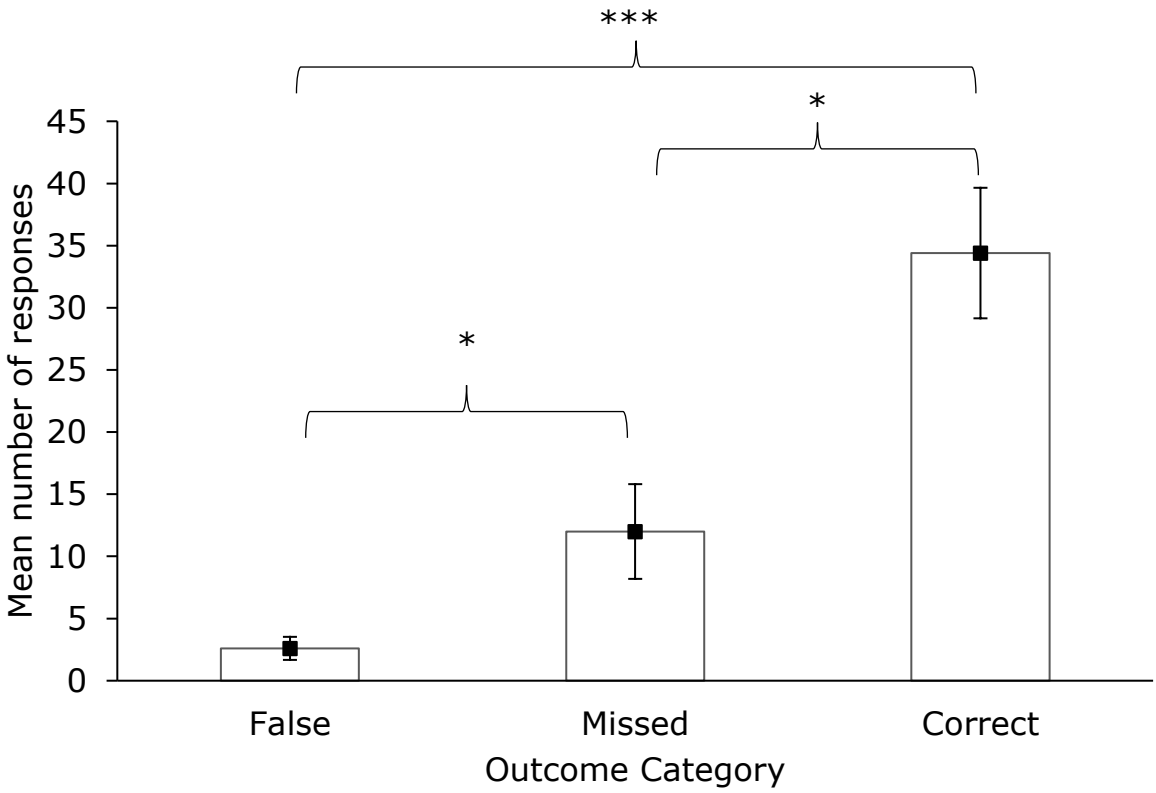

Figure 3.1 – Mean ± SEM of the observed outcomes for each of the categories (false, missed and correct) throughout continuation training. Significance levels - *=$p \leq 0.05$, ***=$p \leq 0.001$.

3.4.2 Temporal analysis of continuation training

A One-way ANOVA revealed no detectable significant differences in the detection score and month ($F(4,18)=0.02$, $p=0.999$) (Figure 3.2). Furthermore, these results show that 74% of the continuation training sessions resulted in a pass if the pass rate was *pa*60 (Porritt *et al.*, 2015). If the pass figure was increased to *pa*70 as recommended by Porritt *et al.* (2015) then the dog would have passed 70% of the sessions.

A Pearson correlation carried out on the detection score and training session showed there was no measurable significant association with time and the detection ability of the dog over the testing period (Rho=0.046, p=0.835).

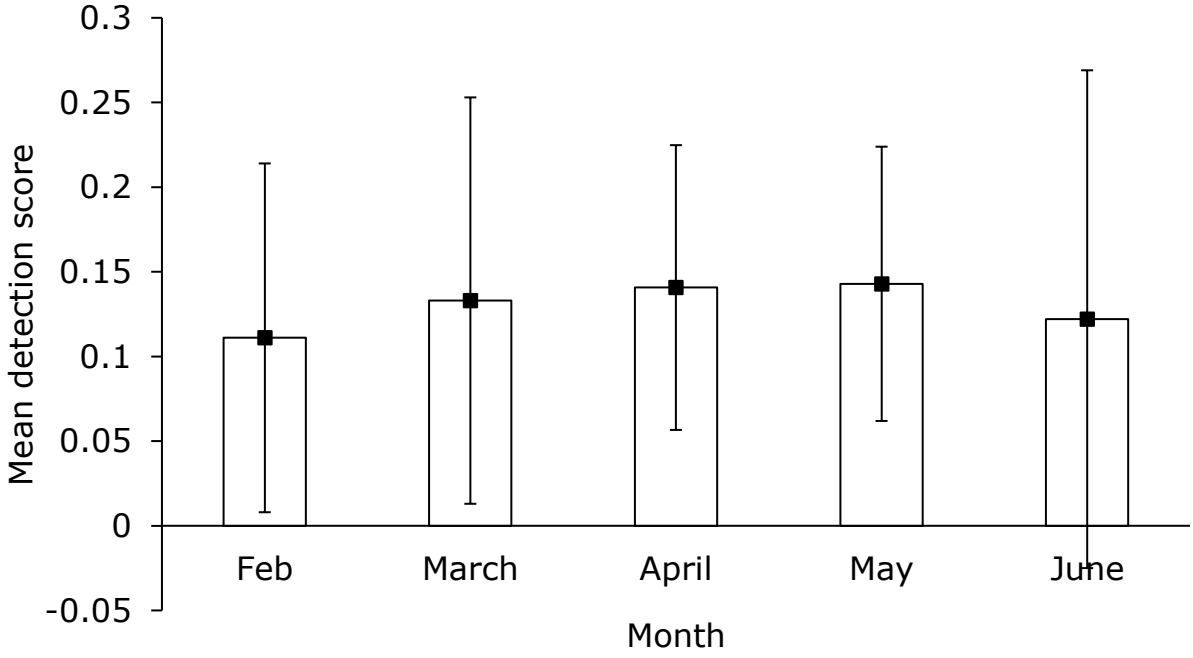

Figure 3.2 - Mean ± SEM of the detection score per month of detection dog continuation training, no significant differences were detected.

3.4.3 The effect of M. minutus sex on the detection rate

The results of a one-way ANOVA identified no detectable significant difference in the detection score between the sexes sample target scent ($F(1,13)=0.01$, $p=0.914$) (Figure 3.3). Thus, the detection dog did not display a sex bias during continuation training.

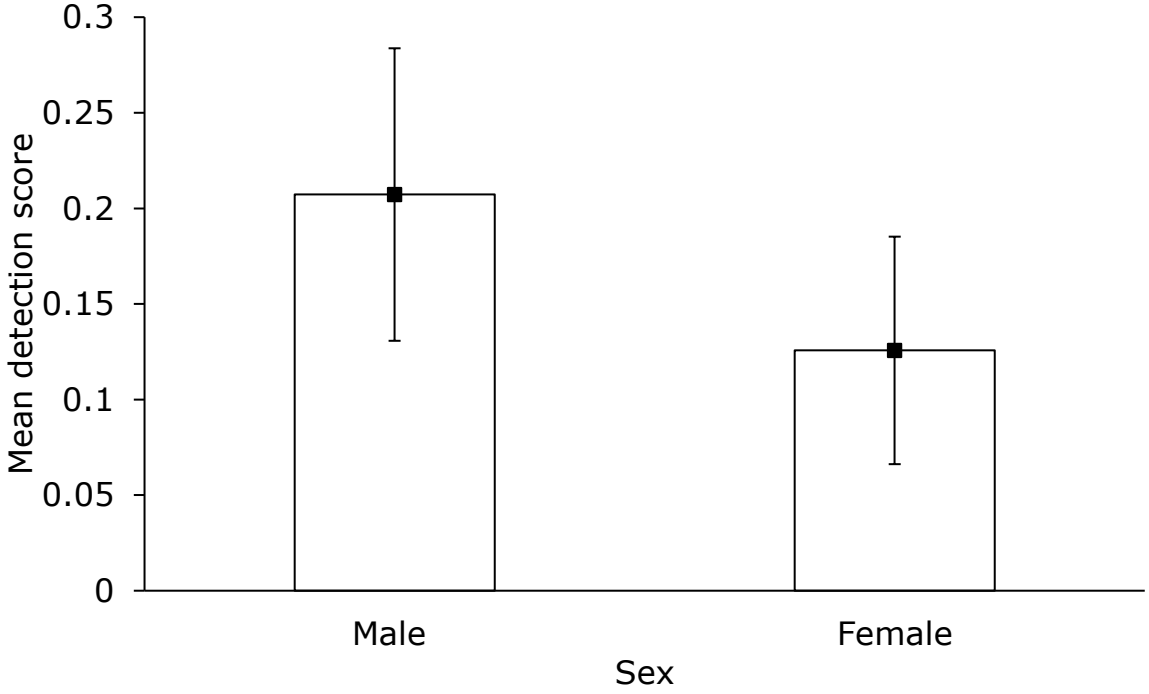

Figure 3.3 – Mean ± SEM of the detection score for continuation training when using male and female *M. minutus* scent as the target odour. No significant difference was detected.

3.4.4 Effect of abiotic factors on detection rate.

No significant correlations were detected between the detection score from continuation training sessions and the weather variables presented in Table 3.1.

Table 3.1 – Result from the correlation analysis between detection score and weather factor. *r*= Pearson correlation and Rho = Spearman's correlation

Weather Variables	Correlation	Statistic	*p*
Wind (knots)	*r*	0.314	0.145
Total rainfall (mm)	Rho	0.066	0.765
Duration of rainfall (hrs)	Rho	0.038	0.864
Cloud (otkas)	Rho	-0.001	0.998
Solar radiation (W/m^2)	*r*	-0.023	0.917
Sunshine (hrs)	*r*	-0.063	0.774
Relative Humidity %	*r*	-0.069	0.756
Temp (°C)	*r*	-0.188	0.391
Pressure (mb)	*r*	-0.194	0.376
Min temp (°C)	*r*	-0.343	0.110

3.4.5 Discrimination testing

The discrimination tests revealed 100% specificity (Eq. (2)) and 75% sensitivity (Eq. (3)) (Table 3.2) in the scent detection when tested using scent in a formal, fully randomised, single blind discrimination test (adapted from Porritt *et al.*, 2015).

$$\frac{29}{0+29} = 100\% \quad \ldots 2$$

$$\frac{6}{6+2} = 75\% \quad \ldots 3$$

Table 3.2 – Outcomes and formula components (sensitivity and specificity in **bold**; detection score in *italics*) for each run carried out in the formal discrimination test.

Run number	True positives (**a**) (*x*)	False negatives (**b**)	False positives (**c**) (*y*)	True negatives (**d**)	No. of targets in the test (*n*)	No. of blanks (*m*)
1	1	0	0	5	1	5
2	1	0	0	4	1	5
3	0	1	0	5	1	5
4	1	0	0	1	1	5
5	1	0	0	4	1	5
6	1	0	0	4	1	5
7	0	1	0	5	1	5
8	1	0	0	1	1	5
Total	6	2	0	29	8	40

The detection score was calculated by applying the results from the discrimination test to Eq. (1), with a pass rate of *pa*60 and *pa*70. A negative equation result would mean that the detection dog would have failed the

discrimination test and further training would be required. However, the results below indicate that the dog passed when the pass rate was *pa*60 and *pa*70. Therefore, the analyses of the discrimination element of the testing revealed that the dog performed to a sufficiently high standard and field testing of the method could begin.

$$ds = \left(\frac{6}{8} - 0.6\right) - \left(\frac{0}{42}\right) = 0.15 \quad \ldots 1$$

$$ds = \left(\frac{6}{8} - 0.70\right) - \left(\frac{0}{42}\right) = 0.05 \quad \ldots 1$$

3.4.6 Field testing

Of the eight remote scent surveys undertaken, seven were included in the analyses. The detection dog identified positive samples in three of these, with 11 indications in total, locations can be seen in Figure 3.4 and Figure 3.5. The detection percentage for each survey can be seen in Table 3.3, no detections were recorded for the remote scent surveys undertaken in winter.

Table 3.3 – Results of the field testing for the scent survey method

Survey number	Location	Detection %	Season
1	1	12	Autumn
2	1	27	Autumn
3	1	11	Autumn
4	2	31	Autumn
5	3	0	Winter
6	1	0	Winter
7	1	0	Winter

Figure 3.4– White markers depict locations (+/- 2m) (Location 1) of feeders with positive indications from the detection dog at Butcher's Lane, Boughton, Northamptonshire (approx. 52.290398, -0.890023) (picture adapted from Google EarthPro, 2016).

Figure 3.5– White markers depict locations (+/- 2m) (location 2) of feeders with positive indications from the detection dog at Cottage Field, Pitsford, Northamptonshire (approx. 52.293875, -0.890322) (picture adapted from Google Earth Pro, 2016).

3.4.7 Seasonal effectiveness of the remote scent surveys

A One-way ANOVA identified a significant difference between the detection percentage from the Autumn surveys and those carried out in the Winter ($F(1,5)=10.89$, $p=0.021$). The detection percentage was significantly higher for those carried out in the Autumn (Figure 3.6). Thus, a seasonal preference for this method was recorded.

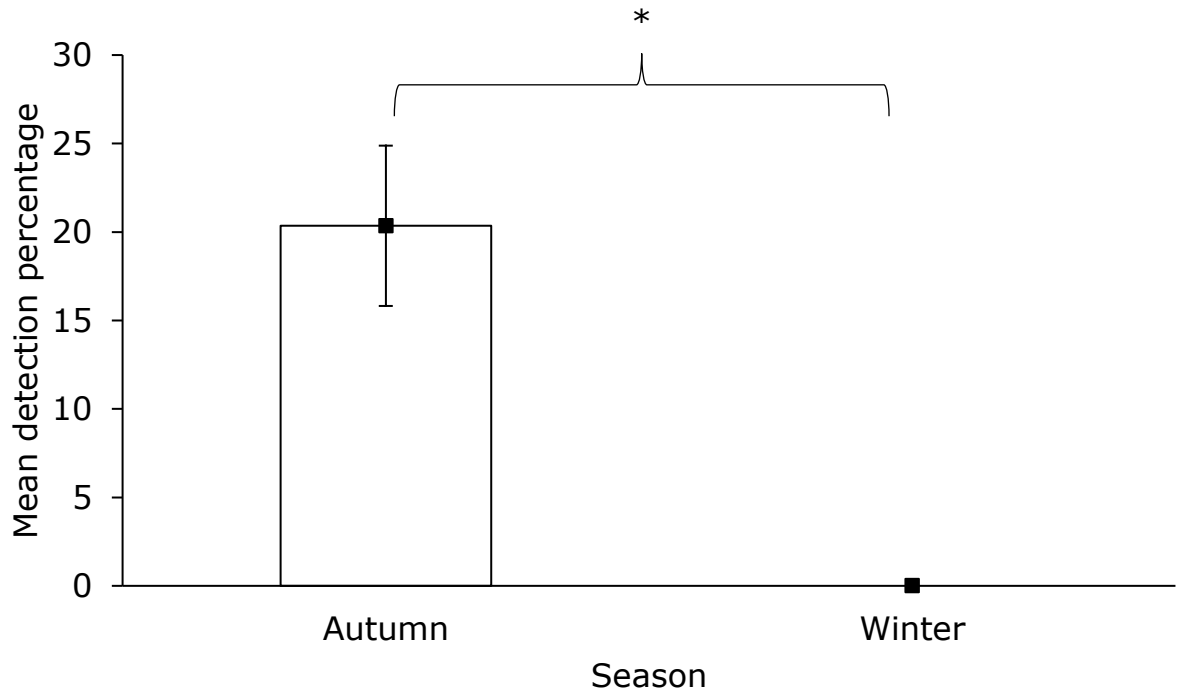

Figure 3.6 – Mean ± SEM of the detection percentage for remote scent surveys undertaken in Autumn and Winter. Significance level - *=$p \leq 0.05$.

3.4.8 Effect of weather on feeder usage

Correlation analyses were carried out to assess whether the seasonal differences reported in detection percentage were related to weather variables, and as the results in Table 3.4 indicate, no significant correlations were detected. Changes in the recording of weather variables by Pitsford

weather station meant that the variables considered in section 3.4.4 could not be replicated here thus, analyses were limited to the five variables described in Table 3.4.

Table 3.4 – Result of the Pearson correlation analyses between detection percentage and weather variables.

Weather variables	*r*	*p*
Max temp (°C)	0.138	0.768
Min temp (°C)	0.231	0.618
Average rainfall (mm)	0.563	0.188
Sunshine hours	0.063	0.892
Max atmospheric pressure (mb)	0.106	0.821

3.4.9 Nest search survey results

Nest searches were carried out by volunteers in two of the areas, which covered three remote scent surveys (locations 1 (Figure 3.4) and 2 (Figure 3.5)). As Table 3.5 shows, nests were located by volunteers in one survey. There were also positive indications from the detection dog at this location.

Table 3.5 – Results of the nest search surveys carried out by volunteers.

Location	Nest Height (cm)	Nest Category	Grass Species
1	19	Shelter	Cocks foot
1	40	Shelter	Mixed
1	70	Breeding	Mixed
2	-	-	-
3	Survey not possible due to hedges being cut		

3.4.10 Comparison of methods by presence measure

The result from the occupancy estimation modelling in Program MARK shows, assuming presence of the species, the detection probability for the remote scent survey method was P=0.57 and the nest search method was P=0.50 (Table 3.6). However, when the Autumn data were considered

independently, the detection probability increased to P=1.00; all surveys undertaken in the Autumn resulted in an indication. The raw detection rate (Eq.(4)) corresponds with the occupancy modelling results, with a higher raw detection rate recorded for the remote scent survey (0.66) than the nest searches (0.5).

Table 3.6 – Detection probability measures for nest searchs and remote scent survey. * indicates result of occupancy modelling in program MARK.

Presence measure by method		
Raw detection rate	**No. of detections**	**Rate**
Detection dog	11	0.66
Nest searches	3	0.5
Probability of detection (Autumn and Winter)	**P***	**SE**
Detection dog	0.57	0.19
Nest searches	0.5	0.35
Probability of detection (Autumn)	**P***	**SE**
Detection dog	1.00	
Nest searches	0.5	0.35

3.5 Discussion

The three elements of scent data considered in the results section (continuation training, discrimination testing and uncontrolled field testing) all indicate that a detection dog can be used to detect the presence of *M. minutus*, both during training and in field conditions, albeit with additional validation needed for the latter.

3.5.1 Continuation training outcome analysis

The results of the detection category (false, missed, correct) analysis revealed that the detection dog correctly identified the target scent at a

significantly higher rate than missed targets or false indications. Furthermore, false indications were significantly fewer than missed targets (Figure 3.1). These results show that the detection dog is reliable at detecting *M. minutus* correctly and the training methods utilised were effective. Thus, from this simple preliminary analysis it appears the method is valid and reliable. Were the false indication or missed target rate to prevail over correct outcomes, appropriate corrective training would have been required, and the validity and reliability of the remote scenting method would need to be questioned. These results provide evidence that the detection dog achieved a high level of specificity and sensitivity during training. Actual calculations of specificity and sensitivity were not possible as recording the position of the target scent in the run was overlooked at this stage. The importance of reporting these measures when assessing the effectiveness of detection dogs was discussed by Johnen *et al.* (2017) and omitting these calculations may increase potential bias when reporting the results. However, the additional analyses where the detection score was calculated for each continuation training session, provided additional supporting evidence for the reliability of the detection dog, as the position of target scent within the run was not required.

The detection score analyses revealed a consistent pass rate of *pa*60, which corresponds to the results discussed above, and supports the preliminary findings. Furthermore, no significant differences or correlations were detected in the detection score over the five months of continuation training. The detection dog passed 74% of sessions at *pa*60 and if the pass criteria were increased to *pa*70 this figure would have reduced to 70%. Both

are highly encouraging results, and demonstrate the validity of the proposed method and the reliability of the detection dog during training. These results are more favourable than reported by Willis *et al.* (2004) who employed similar methods and a 41% detection rate in bladder cancers were described. While other studies have achieved better detection results, for example Cablk and Heaton (2006) recorded over 91% accuracy when locating *Gopherus agassizii* (desert tortoise), Waters *et al.* (2010) and O'Connor *et al.* (2012) found results varied between 100% and 62% respectively, when using dogs to locate bumble bee nests (*Bombus* spp.). Lastly, Reindl *et al.* (2004) reported 86% accuracy when using dogs to locate *Mustela nigripes* (black-footed ferrets). The methods vary depending on the aim of the study and the target species' ecology, making direct comparisons of detection results difficult. However, the *M. minutus* detection dog tested here has worked reliably and consistently, which was demonstrated by consistently passing training sessions according to the detection score criteria.

These consistent results during continuation show that the dog's motivation to work was not unduly hampered by the repetitive and potentially tedious task as described in studies where similar, remote scenting methods were utilised (Kerley and Salkina, 2007; Wasser *et al.*, 2009; DeShon *et al.*, 2016). However, there were occasions during uncontrolled field testing where the dog became tired, so this cannot be fully ruled out at this stage. However, the data presented in Figure 3.2 identified a greater variability in the detection score during June 2015 when compared to the other months. It is possible that external, unrecorded variables were affecting detection

rates, which may have included the internal state of the dog. Even highly motivated dogs will not work effectively 100% of the time (Settle *et al.*, 1994 in: Wasser *et al.*, 2009), but importantly this variability did not translate into a significant reduction in reliability.

3.5.2 The effect of target sex on detection rates

The target scent was freshly collected for the majority of training sessions and as scent was linked to sexual selection in *M. minutus* (Roberts and Gosling, 2004), it was possible a detectable difference in the scent of male and female *M. minutus* was present, with a potential impact of the detection and discrimination ability of the dog. The results presented in Section 3.4.4 indicate that the dog's ability was not impacted by sex of *M. minutus* since no significant differences between the detection score and sex were observed. These results correspond with the findings of Cablk and Heaton (2006) who suggest that the dog would be equally effective at detecting and discriminating males and females during uncontrolled field conditions.

While the sex of individuals utilised for target scent varied throughout the training, other variants within the target scent profile were not accounted for, which included diet and age, which could have impacted the dog's ability to generalise target scent (Oldenburg *et al.*, 2016).

3.5.3 Effect of abiotic factors on detection rates

The impact of abiotic factors as described in other studies were not detected in the results here (Wasser *et al.*, 2004; Reed *et al.*, 2011). No significant correlations between any of the weather variables and detection score

calculated from the continuation training sessions were detected. These findings may be attributed to the scent collection method, which afforded the scent some protection from the elements, subsequently minimising degradation and provided a concentrated area for the dog to "check". Furthermore, the continuation training was undertaken outdoors in a consistent environment, where the impact of some of the measured weather variables could be reduced, for example the direction the bottles faced when the dog was working were altered to minimise the effect of wind. Anecdotally, there was some evidence abiotic factors affected the behaviour of the detection dog. For example, if the ground was wet after rain, the dog was more reluctant to provide a full sitting indication and would hover slightly over the ground.

After the continuation training data were collected, all training and testing was undertaken within a controlled indoor environment, where the impact of abiotic variables were reduced, firstly to minimise distractions from interesting scents outside, and secondly to provide a consistent environment for testing, which was important when validating this method.

3.5.4 Discrimination testing

The results of the single blind discrimination testing revealed a highly encouraging result for the validation of this method, with 100% specificity (true negatives) and 75% sensitivity (true positives) observed. The results of the detection score concluded that the dog passed the discrimination test at *pa*60 and if the pass rate was increased to *pa*70, a pass would still have been achieved. The *pa*70 pass criteria proposed by Porritt *et al.* (2015)

would equate *pa*70 to a 75% detection rate, plus two false indications, based on eight runs, with one target scent per run. Here it is important to note that there were no false indications, but there were two missed targets. These occurred when the target scent was located in the same position. Therefore these results could have been attributed to another unrecorded factor which had caused confusion to the dog.

The specificity result of 100% is key for validation of the method. The dog ignored the non-target scent 100% of the time, strongly indicating that the dog was discriminating and selecting the correct scent because of learned behaviour and concept formation, rather than guessing (Oldenburg *et al.*, 2016). Having fewer, but more confident indications on target scent and ignoring true negatives is more desirable. False indications would have serious implications for validity and reliability and would result in misreporting range and distribution data which is counter-productive in terms of developing ecological knowledge of *M. minutus.*

While the specificity, sensitivity and detection scores were providing similar calculations, Johnen *et al.* (2017) noted that documenting the sensitivity and specificity is vital for accuracy when reporting detection ability and systematically quantifying the outcome in controlled conditions, allowing an unbiased decision on the detection dog progressing to uncontrolled field testing.

Based on the detection score and the sensitivity and specificity analysis, the results discussed here show that the detection dog can reliably discriminate between *M. minutus* scent and the scent of another sympatric species when

in a controlled situation, and therefore justified undertaking uncontrolled field testing. Furthermore, when assessing the validity of the methods utilised, many of the requirements for reporting the dog's ability and methods to minimise bias described by Johnen *et al.* (2017), were met within this Chapter, despite data collection pre-dating this paper.

3.5.5 Uncontrolled field testing

Of the seven surveys carried out, the detection dog identified positive samples in three of these, with 11 indications in total. The detection percentages reported in Table 3.3 ranged from 0-31%. These results indicate that Autumn was the most effective time to undertake remote scent surveys as the detection percentage ranged from 12-31%, compared to Winter 0%. These results are not as high as comparative studies on cryptic rodents. For example Duggan *et al.* (2011) reported a 44% detection rate with one dog and 67% when two dog teams were utilised. The results of detection percentages reported here do not imply that targets were missed, but simply that targets were detected, which is a measure of feeder usage by *M. minutus* and in theory maximising feeder usage should increase the detection percentage.

As discussed in Section 3.4.4, the dog did not appear to be impacted by weather variables. Therefore, the detection rate in winter is likely to be due to a reduction in feeder usage by *M. minutus* caused by another variable. The correlation analyses indicated this reduction did not appear to be related to the weather variables analysed. Therefore, it is likely that another, unrecorded factor was impacting feeder usage during the winter

months; it is likely to be related to a reduction in population size due to higher mortality over winter (Kettel *et al.*, 2016). Alternatively, seasonal changes in internal or external factors, such as, activity, foraging behaviour or predator density may reduce the home range of individuals (Borowski and Owadowska, 2010; Schuster *et al.*, 2017b), and it is possible during winter *M. minutus* simply did not encounter a feeder; thus, the optimal feeder spacing may need adjusting.

While there is no way of being 100% positive that the detection dog is indicating *M. minutus* scent, examination of the faecal pellets within the samples were of the correct size for *M. minutus*. The supporting evidence from the discrimination testing and continuation training, in addition to the nests located within the survey area, mean there is confidence in the results, but it is not absolute. This is an issue for many detection dog validation studies, where the target odour cannot be fully defined (Gadbois and Reeve, 2014; Johnen *et al.*, 2017) and where sample identification during uncontrolled elements cannot be immediately achieved (Duggan *et al.*, 2011). However, where possible, carrying out cross-checks and testing the dog in a controlled environment where all the locations of the target scent is known can be used as a reliability indicator (Cablk and Heaton, 2006; Wasser *et al.*, 2009). It is likely that collecting scent samples within a feeder reduces the impact of degradation by abiotic factors. However, there is no way to control the potential impact of individual behavioural type (Schuster *et al.*, 2017b) and seasonal behavioural variations affecting the use of the feeders by *M. minutus.*

3.5.5.1 Comparison with nest searches

Nest searches were carried out by volunteers in two of the areas, which covered three surveys. As Table 3.5 shows, nests were located by volunteers in only one survey (location 1). There were also positive indications from the detection dog in this area. However, the researcher encountered at least five nests within this area. At location 2 where the volunteers did not encounter nests, the researcher encountered two nests while setting up the surveys. Furthermore, there were positive indications from the detection dog in this area. Even though the results here are limited, they correspond with other studies, in that nests are easily overlooked (Harris, 1979a; Riordan *et al.*, 2009). Meek (2011) reported favourable results when using nest searches, with nests normally located within 10 min if *M. minutus* were present. The number of nests encountered by the researcher in this study corresponds with the findings of Meek (2011) who noted that effectiveness of nest searches maybe dependent on the experience of the surveyor. From the results of Poulton and Turner (2009), Riordan *et al.* (2009) and Meek (2011), the results from nest searches are far from consistent.

The probability measures identified that the remote scenting method was marginally more effective than nest searches. This corresponds with the results reported by O'Connor *et al.* (2012), where in certain situations humans and dogs located bumble bee nests at a similar rate. However the dog was more effective in certain habitats. The aforementioned study noted that the cost of training the dog was prohibitive and the human surveyors proved to be a useful tool and could achieve similar results. Conversely,

Long *et al.* (2007) compared other non-invasive methods (hair snares and camera trapping) to the results of detection dog surveys for forest carnivores. The detection dog proved substantially more effective than hair snares or camera trapping. They also noted that the cost of implementing a detection dog survey was higher, but only one visit was required to determine presence; the terrain made repeated visits challenging, therefore the detection dog proved more efficient. The balance between cost and efficiency of using detection dogs varies, and species ecology, project aims and habitat type can be critical deciding factors when employing a potentially expensive, yet more efficient method (Long *et al.*, 2007; Duggan *et al.*, 2011; Orkin *et al.*, 2016).

These results indicate that a detection dog may be more effective than nest searches at determining presence, but not significantly so, and more data would be required to draw conclusions about each method's effectiveness. The main benefit of using the remote scent surveys over nest searches is the provision of a finer scale indication of *M. minutus* presence. If the results of the uncontrolled field testing can be relied upon, then the remote scenting method indicated that *M. minutus* were present over the course of the survey period, whereas nest surveys can only identify presence at some point over the previous breeding season. The proposed method has great potential for gathering real time range and distribution data for *M. minutus*, which has never been achieved before. The only similar method that could be located within the literature was to use tennis balls as feeders. However this method relied on direct observation of *M. minutus* using the feeder,

which is hugely labour intensive and unobserved activity could not be conclusively attributed to *M. minutus* (Warner and Batt, 1976).

3.5.6 Remote scent survey results in comparison with faecal DNA analysis

When comparing the results to that of Morris *et al*. (2013), who used DNA analysis to identify presence of *M. minutus*, 10 samples were positively identified, of a possible 40 (25%). The detection dog method identified 11 samples out of a possible 104, with a detection probability of 11.6%. However, if the percentage during the Autumn is considered independently then this nearly doubles to 20.4%. This is still less than when using DNA to confirm presence, but the high cost associated with the DNA analysis could prove unsustainable if applied on a larger scale.

There is great potential for the use of genetic censusing in *M. minutus*, whereby DNA extracted from *M. minutus* faeces could be used to formulate individual capture histories (Kohn and Wayne, 1997), without the need to observe, trap or physically mark an individual, which eliminates the most challenging aspects of collecting data on *M. minutus.* Genetic censusing can prove particularly beneficial when assessing the impact of fragmentation on geneflow and dispersal (Arandjelovic and Vigilant, 2018) and would achieve this at a larger scale than was attempted in this study. Wasser *et al.* (2009) found the use of sample matching dogs can make population biology more accessible to wildlife scientists. Combining the methods for *M. minutus* (genotyping and remote surveys) has the potential to improve the efficiency of sample handling, with a further benefit of addressing the detection dog

validation issue. Thus, problems relating to population biology and assessment of population declines could be addressed (Wasser *et al.*, 2009; Arandjelovic and Vigilant, 2018).

3.5.7 Limitations and recommendations

The overall purpose of this proposed method was to replace nest searches with the remote scent survey method to allow finer scale presence data to be obtained. However, the key caveat of the method was a lack of validation for the detection dog's accuracy when checking scent samples collected in field conditions. This is a standard limitation in detection dog studies when samples are collected in uncontrolled conditions. Wasser *et al.* (2009) noted that cross-checking was vital when the samples were blinded to the handler, where other trained dogs were utilised to cross-check, a procedure which has not been possible in this preliminary study but could be implemented in the future. Cablk and Heaton (2006) noted that the only way to test a dog's accuracy is experimentally, when all the target scents are known. Thus, further refinement of the method is required, which includes additional validation measures. For example, camera traps would allow identification of species using the feeders, although there would be significant cost involved to obtain the number of cameras needed to provide sufficient data for validation.

The overarching recommendation is that further uncontrolled field testing is required, which employs a systematic approach to establish optimum survey length. This would facilitate further assessment of the reliability and validity of the remote scenting method, with a stronger focus on the

comparisons with nest searches. Here, not all the scent survey locations had nest searches carried out. The surveys undertaken in February were the wrong time of year for formal nest searching to be undertaken. Further logistical challenges were encountered at the final survey in location 3. The hedges and field margins were cut prior to the nest searches being undertaken. For a more robust analysis, nest searches should be completed at all sites where the remote scenting method has been tested and if possible, the same volunteers should be used at all sites, although this is a somewhat unrealistic scenario.

Depending on the scale of operation the cost of training and maintaining a detection dog is high, particularly when considering the resources required compared to carrying out nest searches. A dedicated handler is required, and with only one dog available for the current study, the risk of failure during each stage of training was significant. Funding for the training was provided by the PTES (£1020.00) and Moulton College (approx. £700). However, this does not include the time dedicated to training the dog outside of formal training sessions. No specific finance was provided to the researcher for training the dog which would be expected in other situations. Furthermore, the cost of purchasing the dog, insurance, food, prophylactic treatments, veterinary care and all other husbandry costs were absorbed by the researcher (approx. £1400 per annum). While the cost was lower than other similar studies, this was a unique situation where the cost of training was minimised, which is unlikely to be applicable to other scenarios and would need to be considered before undertaking a study based on this model. The timeframe to complete the detection dog's training should also

be assessed carefully, as this was underestimated here. Several factors contributed to the delay in the detection dog being fully trained and discrimination tested. If this method were to be pursued and a dog trained for this purpose within a similar framework, it would be advisable to allow more time for the initial training.

Whilst the potential variation in target scent caused by sex and reproductive state was considered during training, other potential variants of target scent, caused by differences in diet or age were not considered in this study, but are important when retaining specificity and to ensure that the context is accounted for (Oldenburg *et al.*, 2016). The recommendations by Oldenburg *et al.* (2016) were published after the training had been implemented. Therefore any repetition should consider training the detection dog to generalise between target scents. These limitations highlight the difficulties of testing a method where the variables and target odour cannot be entirely defined (Gadbois and Reeve, 2014; Johnen *et al.*, 2017), which is particularly challenging when applying this method to field conditions. However, this does not mean that the remote scent survey for *M. minutus* is not valid or reliable, as demonstrated by other studies with similar methodology and by the favourable results of the continuation training and discrimination testing (Willis *et al.*, 2004; Wasser *et al.*, 2009).

During the uncontrolled field surveys, there were many samples where it was apparent that non-target small mammals had either occupied the feeder or were active on the exterior. These were identified by large faecal pellets, urine, or nesting material being within the feeder and in some circumstances, there was a total depletion of seed. Anecdotally, *M. minutus*

characteristically would not normally remove all the food provided or move nesting material into the feeders. However the researcher has observed this behaviour within other species of mice and voles. As mentioned previously, high levels of contamination during Survey Five, resulted in the survey being discounted. This contamination could potentially cause confusion for the detection dog and reinforce indications on non-target scent. Consequently, it would be recommended that the survey duration would be less than six days. It is possible that the optimum survey duration and spacing for the feeders are linked to optimum foraging theory, behavioural type, cyclical population changes and predator density. Therefore values may vary between season, habitat type and year (Korpimaki *et al.*, 2005; Borowski and Owadowska, 2010; Schuster *et al.*, 2017b). As far as can be determined, optimum foraging behaviour has yet to be assessed in *M. minutus*. Therefore, further research needs to be carried out in this area before conclusive recommendations based on empirical studies can be made. Some standardised testing of scent persistence would also be beneficial; establishing how long the scent remains detectable by the dog would be useful and could benefit the assessment and validation of this method over the different seasons.

There were other occasions where the feeders had these characteristics and were not discounted as the contamination appeared to be at lower levels, yet they did not elicit any indications by the detection dog. There are two explanations for this. Either, the presence of non-target small mammal scent was stronger and therefore masking *M. minutus* scent or the presence of non-target species prevented *M. minutus* using the feeders. In either of

these scenarios, the detection dog did not provide an indication and thus, provides further evidence of the discrimination ability of the dog, as no indication is better than a false indication. Realistically some degree of contamination should be expected in a survey of this type and it is possible that with refinement of the scent collection method this effect can be minimised. Some minor adjustments to the feeder design would be beneficial, for example Yamao *et al.* (2016) have proposed a baited feeder designed by Ishawaka (2013) which may offer some improvements. Testing this method during the summer months should reduce the effect of non-target small mammal species as there is more opportunity to place the feeders within the stalk zone of suitable habitat.

3.6 Conclusion

It is evident from the results of the continuation training and discrimination testing that a dog can be trained to reliably indicate the scent of *M. minutus*. Here, the detection dog passed 74% of the continuation training sessions that were undertaken, with correct outcomes significantly higher than missed and false indications.

The analysis of the discrimination testing data has also provided strong evidence that a dog can discriminate between target and non-target small mammal species, and 100% specificity can be achieved in the training environment when all targets are in a known location. As far as can be determined from the literature, training a dog to detect *M. minutus* has never been undertaken. This preliminary study has shown that this can be achieved in the training environment.

However, further research needs to be undertaken to validate these methods when scent samples are collected in uncontrolled field conditions, where the target scent cannot be immediately identified. There is evidence that the remote scenting method undertaken in Autumn had a higher detection probability than nest searches undertaken by volunteers. Therefore, the detection probability is strongly in favour of the remote scenting method. While the application of the remote scenting method to field conditions and comparison with nest searches cannot be validated in this study, it can be determined with high confidence that a dog can discriminate and accurately detect *M. minutus*, and there are several realistic scenarios for the application of this method.

Firstly, a conservation detection dog organisation could continue the validation. A dedicated organisation can facilitate training, testing and cross-checking at a larger scale, with wider expertise and resources than was possible for this preliminary study. However, this would entirely depend on external funding as there would be no commercial application at this stage.

Secondly, the proposed remote scent survey method could be used to screen faecal samples prior to expensive genotyping for "genetic censusing" (Kohn and Wayne, 1997). Screening would reduce cost of blanket analysis of all samples as per Morris *et al.* (2013), by pinpointing those likely to contain *M. minutus* faeces, and thus genetic material. As described previously non-invasive marking methods are preferred, to reduce the impact on the animals both physically and physiologically.

It is evident that further progress needs to be made with *M. minutus*, while using a detection dog could answer some population questions, other methods are likely to be effective and perhaps have wider application in the field. Developments in technology such as RFID are likely to provide alternative novel methods that can be more easily validated in a systematic and quantifiable manner.

4 Chapter Four - The effectiveness of radio frequency identification at monitoring *Micromys minutus*

The findings from Chapter Three revealed that it is possible to train a dog to detect *M. minutus* in a controlled situation, though some further validation is required before it can be reliably applied to an uncontrolled field situation. As discussed in Chapter Three, the remote scenting method could answer some population questions, but other methods are likely to be effective and perhaps have wider application in the field, RFID for example. This chapter focuses on validating RFID for monitoring *M. minutus*; the validation process will consider raw trapping rates, sex, abiotic variables and behaviour.

M. minutus present many challenges in describing their movement and behavioural ecology since they are not commonly encountered during surveys (Poulton and Stone, 2008; Poulton and Turner, 2009; Riordan *et al.*,2009) and there has been a significant lack of published literature when compared to other small mammal species (Kettel *et al.*, 2016; Robertson and McKenzie, 2015). To progress the understanding of *M. minutus* advances in species-specific monitoring methods are required. As discussed in Chapter One, *M. minutus* behaviour, size and habitat preferences mean that this species is difficult to trap, mark and subsequently recapture. Therefore, undertaking population modelling and assessing individual behaviour has been challenging and somewhat unsuccessful thus far. Here it is proposed that Radio Frequency Identification (RFID) offers a viable and

effective alternative to traditional methods, with the potential to collect detailed movement data and apply Lagrangian modelling techniques to better understand their motion and navigational capacity and movement propensity (Baratchi *et al.*, 2013).

4.1 Benefits of RFID

In general, the stress response when being trapped and handled cannot be accurately measured, especially in species where sampling stress hormones is not always possible (Touma and Palme, 2005; Sheriff *et al.*, 2011). In *M. minutus* the stress response may be compounded for wild individuals as they have not been habituated to being captured and handled (Gannon and Sikes, 2007). The use of RFID can reduce stress exposure by allowing free moving access to traps, which means they are not handled after the initial trapping and microchipping.

Recording RFID traps is almost entirely computer-based hence the possibility of human error is reduced. However, reliance on technology is not without risk of data loss since it is entirely possible that files may be lost, damaged or corrupted; so, whilst not error-proof, this approach offers some benefits over human recording for data collection. Further benefit can be seen in the autonomous and instantaneous data collection. When compared to live trapping this method facilitates data collection 24 hours a day if the battery is sufficient to support the usage of the RFID system (Baratchi *et al.*, 2013) and can accurately time stamp events which facilitates robust analysis.

4.2 Rationale

There is evidence that an autonomous RFID system could be pivotal in developing ecological knowledge of *M. minutus*, as similar systems have been successful in monitoring other challenging species (Becker and Wendeln, 1997; Charney *et al.*, 2009). Accordingly, a Wireless Sensor Network (WSN) incorporating an autonomous RFID system was developed and tested, and the method's effectiveness at trapping *M. minutus* in comparison to live trapping methods is assessed. To account for the impact of extrinsic influences on trapping rates, biotic and abiotic factors which could influence live trapping and RFID methods' longevity in monitoring *M. minutus* were analysed. With historical trapping rates of *M. minutus* being low in comparison to other species, it was also important to assess the individual effects of trapping on the released population.

Aim: To assess the effectiveness of RFID in comparison with live trapping at monitoring *M. minutus* in a semi-natural environment.

Objectives:

Objective III: To estimate capture probability of monitoring *M. minutus* using Radio Frequency Identification (RFID) compared to live trapping to assess the effectiveness of both.

Objective IV: To investigate the inter-individual behavioural differences on the survival and movement propensity of *M. minutus*.

4.3 Methods

The methods pertinent to this Chapter have been described in Chapter Two, section 2.5 is most relevant to the results presented in the subsequent sections.

4.4 Results

This results section considers the variables that have impacted trapping rates of RFID and live traps. The overall aim was to compare the effectiveness of RFID and live trapping to validate the use of RFID trapping for monitoring *M. minutus*. Furthermore, the use of the RFID traps allowed the pre-release behaviour of *M. minutus* to be assessed and compared to individual post-release responses.

4.4.1 Individuals recorded per day (raw trapping rates)

The results from statistical analyses showed that there were significantly more individuals recorded per day in the RFID plot compared to the LIVE plot (df=1, W=148, p=0.01) (Figure 4.1). However, Figure 4.1 also reveals that more individuals were unrecorded than recorded in both plots. Therefore, the data were broken down into individuals recorded per week. This reveals that the number of individuals recorded in the first week in both plots were significantly higher than recorded in the second (RFID: F(1,12)=21.13, p=0.001; LIVE : df=1, H=6.78, p=0.009).

No significant difference was detected in the number of reads recorded between the first and second week of monitoring in the RFID plot (F(1,12)=0.80, p=0.389) (Figure 4.2). Conversely, a significant difference

was detected in the trap occupancy rate in the LIVE plot between week one and week two (*df*=1, H=6.87, *p*=0.009) (Figure 4.3).

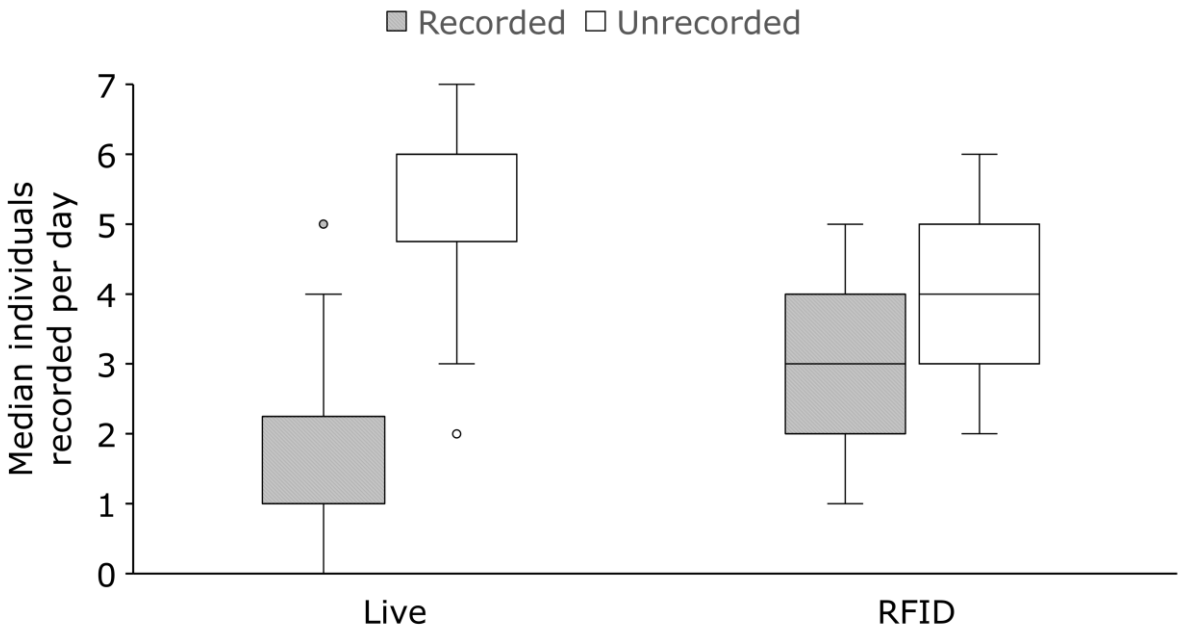

Figure 4.1 –The median ± CI of the number of individuals recorded per monitoring method over the 14-day monitoring period; (N=14; LIVE n=7: RFID n=7). Centre line indicates medians with box equalling median ± 1 quartile. Whiskers extend to maximum and minimum values for each trapping method.

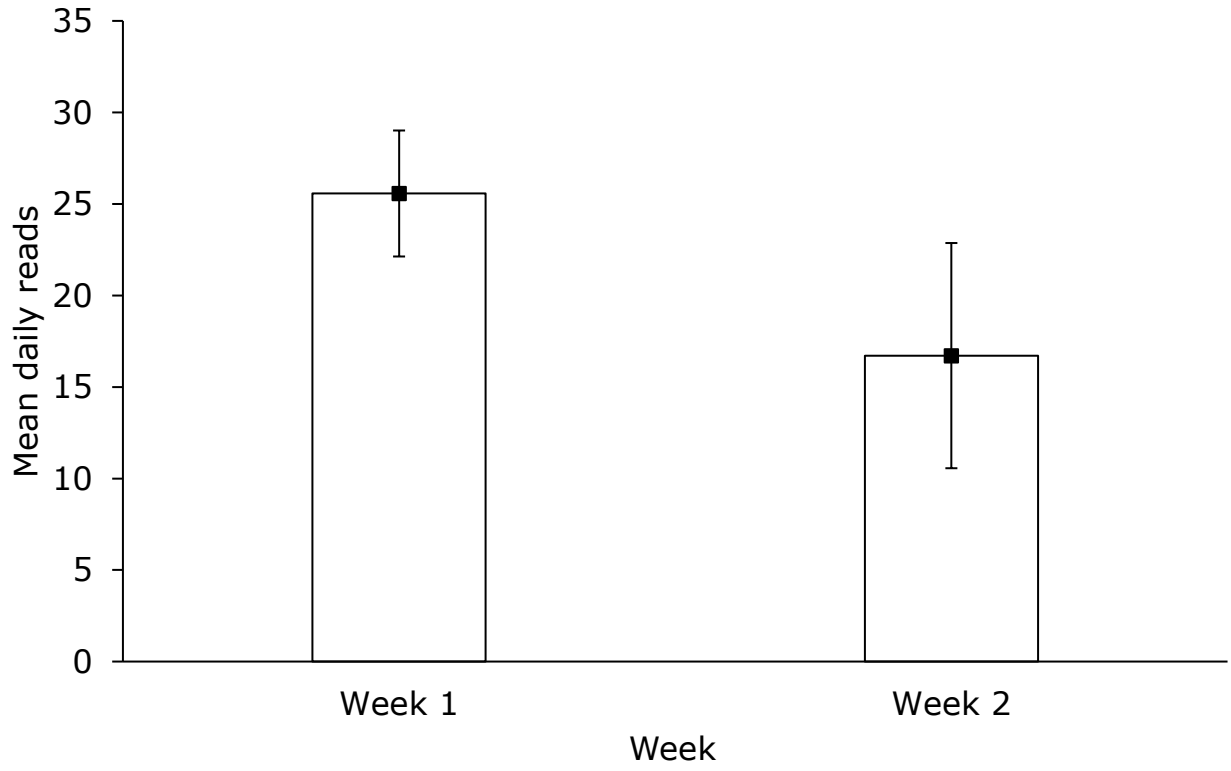

Figure 4.2 – Mean ± SEM of RFID reads recorded in week one and week two of the 14-day monitoring period. No significant differences were detected between weeks.

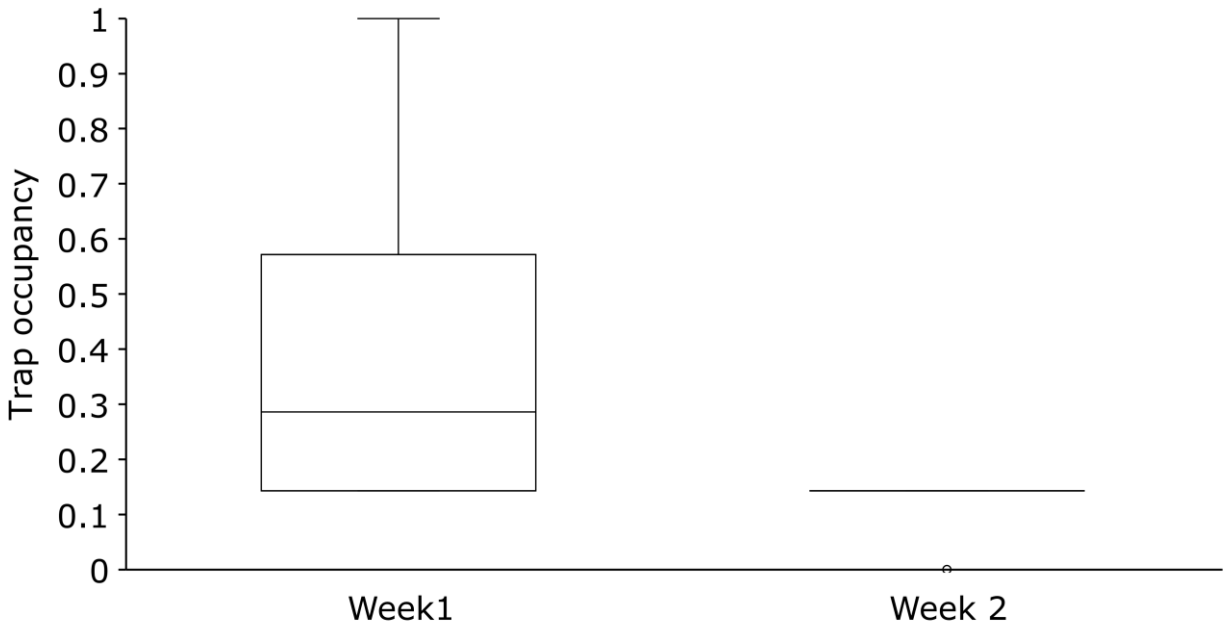

Figure 4.3- Median of Live traps recorded in Week One and Week Two of the 14-day monitoring period. Centre line indicates medians with box equalling median ± 1 quartile. Whiskers extend to maximum and minimum values for each week.

4.4.2 Survival and recapture probability

The results of the parameter estimation undertaken in program MARK (Figure 4.4) revealed a marginal difference between (survival) in the RFID and Live plots (0.91 and 0.84 respectively). However the error bars indicate that the difference would not be significant. The parameter estimates for recapture probability revealed a greater difference between the RFID and live trapping methods (0.91 and 0.65 respectively). Therefore, recapture rates were estimated to be 27% higher in the RFID plot.

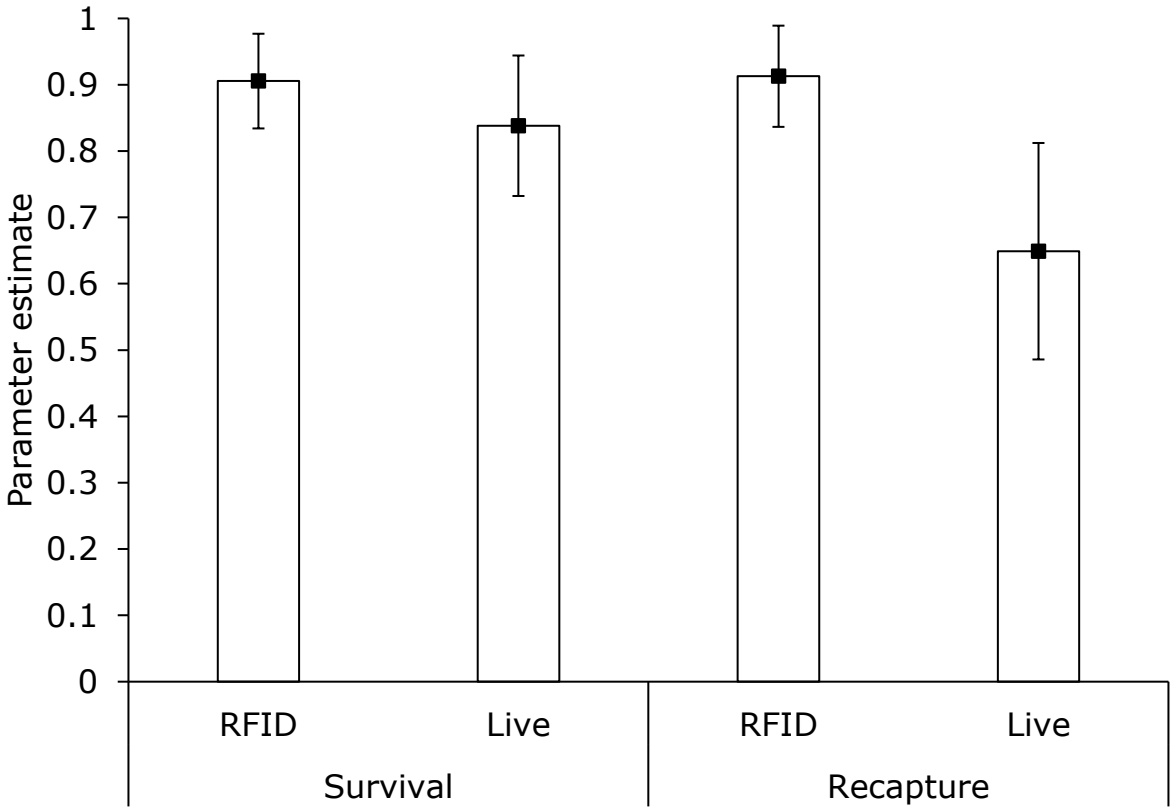

Figure 4.4 - Parameter estimates for survival and recapture ± SE for the RFID and Live trapping methods calculated using the CJS function in program MARK.

4.4.3 The effect of sex on trapping success

For either monitoring method to be successful across a population, equal trapping probability would need to be observed between sexes. The results from the analysis of these data reveal no significant differences in the number of traps in either the LIVE or RFID plots between the sexes ($F(1,5)=0.43$, $p=0.541$; $df=1$, $F(1,5)=1.02$, $p=0.359$, respectively).

4.4.4 The effect of weather variables on RFID trap usage.

As the results displayed in Table 4.1 indicate, weather variables did not appear to significantly influence the effectiveness of recording *M. minutus* in the RFID traps. Conversely there was a potential effect of relative humidity on trap occupancy in the LIVE plot as a significant negative correlation was identified (Table 4.2 and Figure 4.5). In addition, there was a strong negative correlation between day and occupancy rates, with the occupancy reducing over the 14-day monitoring period (Rho=-0.782, $p=0.001$ Figure 4.5) and a further significant correlation between day of experiment and relative humidity (Rho=0.584, $p=0.028$). No other significant correlations between live trapping and weather variables were recorded.

Table 4.1 – Summary of the parametric (Pearson's test) and non-parametric (Spearman's) correlation results between the RFID traps per day and the listed weather variables.

Weather variable	*r*	*p*
Max. temperature (ºC)	0.108	0.714
Min. temp (ºC)	0.024	0.935
Average wind speed (mph)	0.112	0.703
Sunshine hours (hrs)	0.149	0.610
Relative humidity (%)	0.021	0.943
Pressure (mb)	0.103	0.725
Solar radiation (W/m^2)	0.313	0.276
Cloud (oktas)	0.120	0.682
	Rho	*p*
Daily rainfall (mm)	0.311	0.278
Daily rainfall Duration (hrs)	0.348	0.223
Rainfall intensity ($\bar{x}$ per hour)	0.080	0.786

Table 4.2 – Summary of the correlation results between the listed weather variables and live traps per day. Significant values appear in **bold**.

Weather variables	Rho	*p*
Max. temperature (ºC)	0.481	0.082
Min. temp (ºC)	0.108	0.713
Average wind speed (mph)	0.172	0.556
Sunshine hours (hrs)	0.214	0.462
Relative humidity (%)	**-0.626**	**0.017**
Atmospheric pressure (mb)	0.171	0.560
Solar radiation (W/m^2)	0.344	0.229
Cloud (oktas)	-0.254	0.382
Daily rainfall (mm)	-0.410	0.145
Daily rainfall duration (hrs)	-0.433	0.122
Rainfall intensity ($\bar{x}$ per hour)	-0.374	0.188

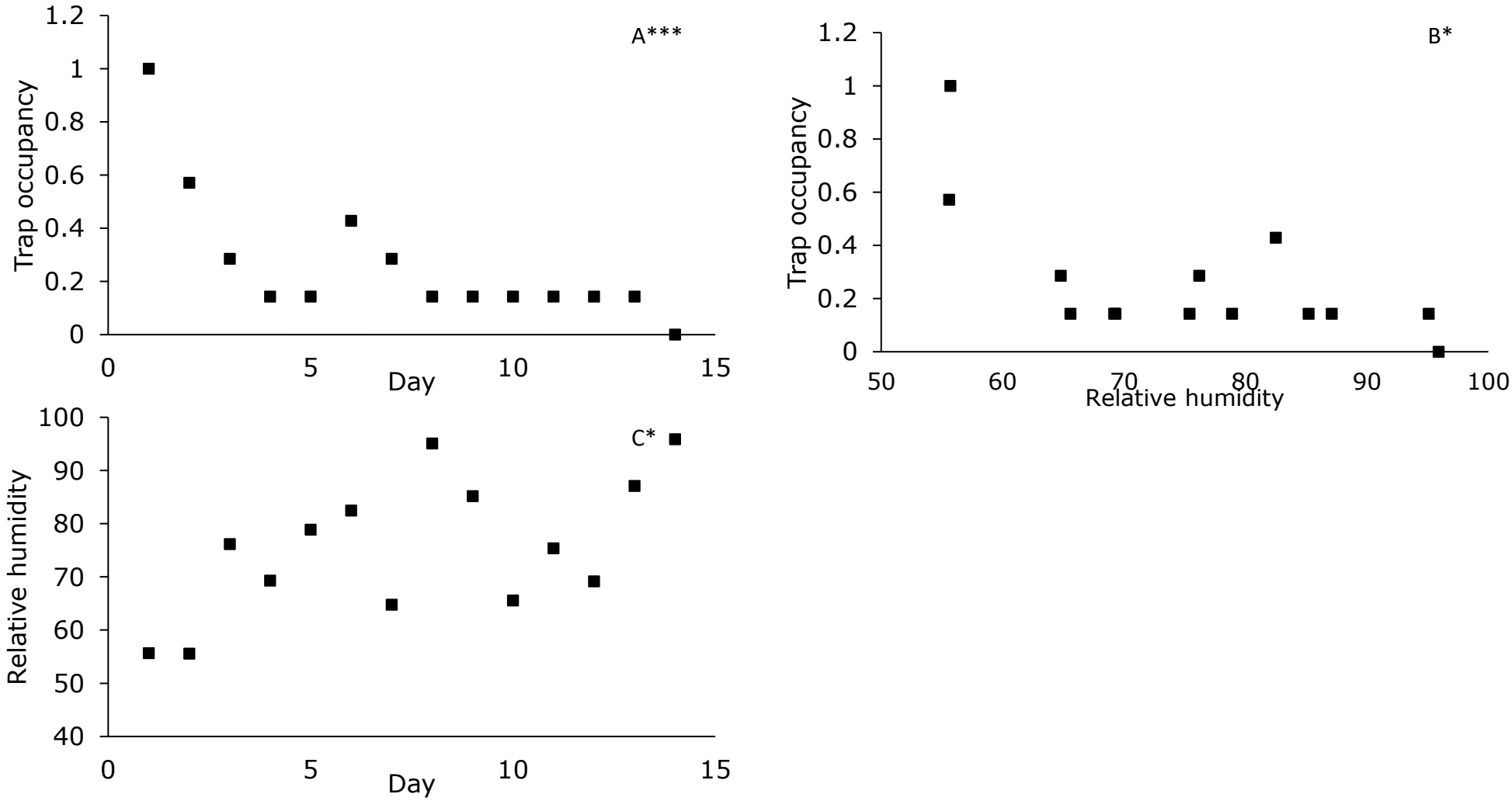

Figure 4.5 – Plots of correlations between day and live trap occupancy (A), relative humidity and live trap occupancy (B) and day and relative humidity (C) over the 14-day monitoring period. Plot identifier is followed by significance levels - *=p=≤0.05, ***=p≤0.001.

4.4.5 M. minutus circadian activity patterns.

Regression analyses revealed significant quadratic and linear relationships between hour and number of reads (Rsq adj.=18.28%, p=0.046; Rsq adj.=17.44%, p=0.037 respectively). As the quadratic regression model had a more favourable Rsq value and would be more applicable to a circadian cycle, this was considered more relevant, despite the weaker p value, albeit still significant (Figure 4.6). A comparative analysis was not possible on the LIVE plot as there was no facility to time stamp trapping events.

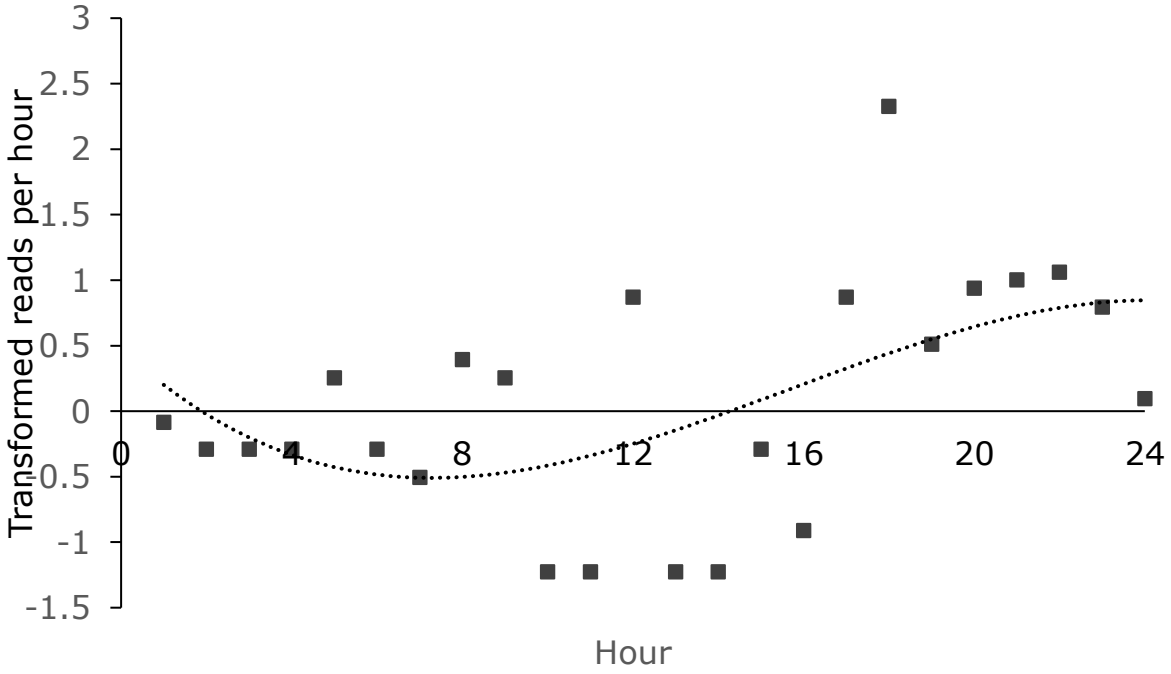

Figure 4.6 – Significant quadratic relationship between the traps per hour using the RFID method (transformed using Johnson transformation) and hour of the day.

A paired t-test revealed no significant differences in RFID trap usage between light and dark conditions in individual *M. minutus* (*df*=1, t=0.76,

p=0.476) (Figure 4.7). Thus there was no apparent difference in activity between these conditions. However, the light:dark ratio in June/July when the study took place was proportionally in favour of light hours compared to dark (approx. 17:7), accordingly, it was prudent to assess the data on a finer scale.

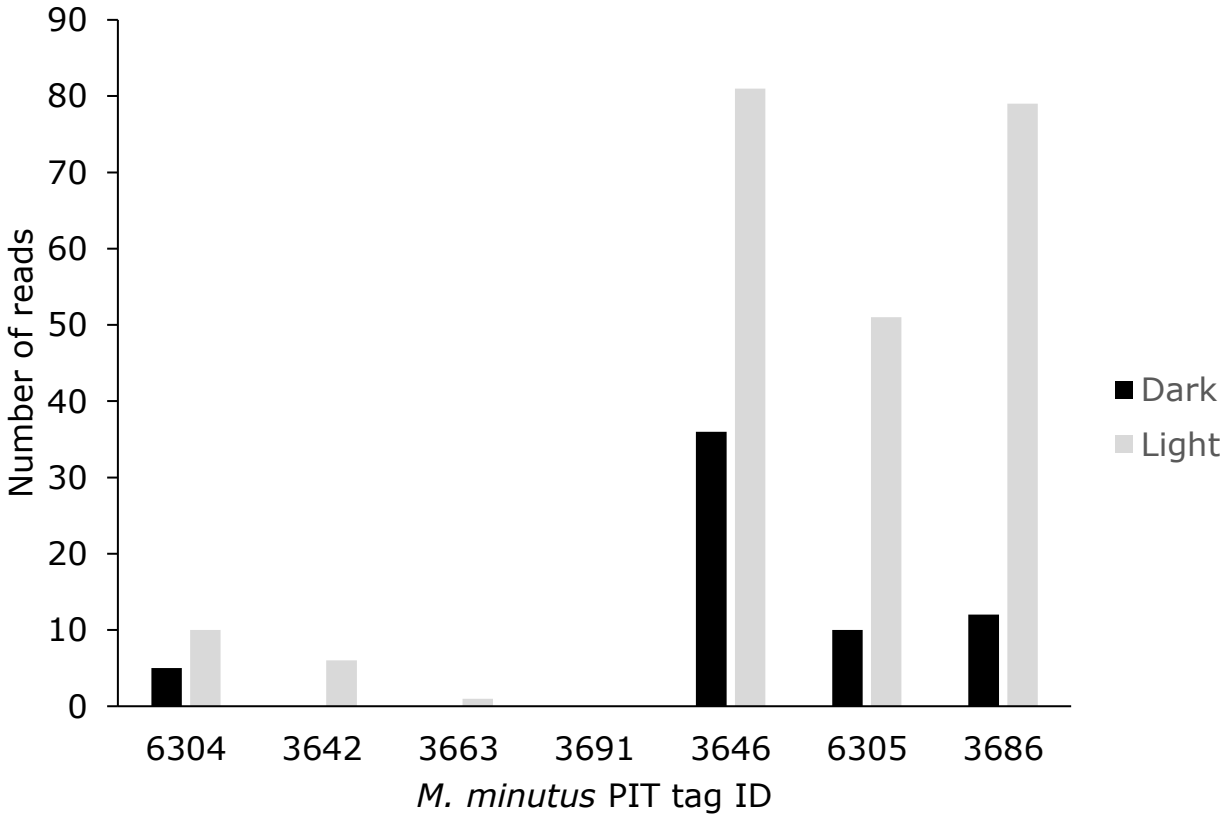

Figure 4.7 – The total number of reads for individuals released in the RFID plot over the 14-day monitoring period during light and dark ambient conditions (Light: 04:47:00 - 21:27:59; Dark: 21:28:00 - 04:46:59).

To account for this disproportionate ratio of dark light hours, 24hrs were divided into three time-blocks, one for the dark and two for the light hours, Time-block 1=(Dark) 21:00:00 - 04:59:00, 2=(Light) 05:00:00 - 12:59:59 and 3=(Light) 13:00:00 - 20:59:59. A series of Mann-Whitney difference

tests did not detect a significant difference in the number of reads between the three time-blocks (**1-2**: W=41.00, *p*=0.806; **1-3**: W=34.00, *p*=0.470; **2-3**: W=32.00, *p*=0.294).

Lastly, the comparative activity at dusk and dawn compared to the rest of the day were assessed. The RFID trapping data were categorised into dusk, dawn and rest of the day (ROD). No significant difference was detected between these categories ($F(2,10)=0.49$ $p=0.627$).

4.4.6 Anxiety score and post-release behaviour

There was a strong correlation between the documented indicators of anxiety centre circle duration and the centre lines crossed:periphery lines crossed ratio (CLC:PLC) and last day recorded (post-release variable) (Table 4.3), with centre circle duration providing the strongest correlation. Regression analysis revealed a significant linear relationship between centre circle duration and last day recorded (Rsq=39.7%, *p*=0.011) (Figure 4.8).

Table 4.3 – Results of the correlation between the OFT behaviour post-release variables N=14, ♂=7, ♀=7. Significant values appear in **bold**. LDR=Last day recorded, EDT=Estimated distance travelled, CLC:PLC ratio (centre lines crossed:periphery lines crossed).

Open Field Variable	LDR		EDT (exploratory score)	
	r	*p*	*r*	*p*
CLC:PLC	**-0.590**	**0.026**	-0.231	0.427
Centre circle entries	-0.194	0.506	**0.783**	**0.001**
Centre circle duration	**-0.653**	**0.011**	-0.090	0.759
$\bar{x}$ Centre circle duration	-0.436	0.119	**-0.784**	**0.001**
Grooming	-0.228	0.433	-0.330	0.250
Defecation	-0.019	0.950	0.266	0.357
Climbing sides	-0.025	0.933	**0.784**	**0.001**
Rearing	0.108	0.713	**-0.860**	**<0.001**
Scanning	0.167	0.569	**0.761**	**0.002**

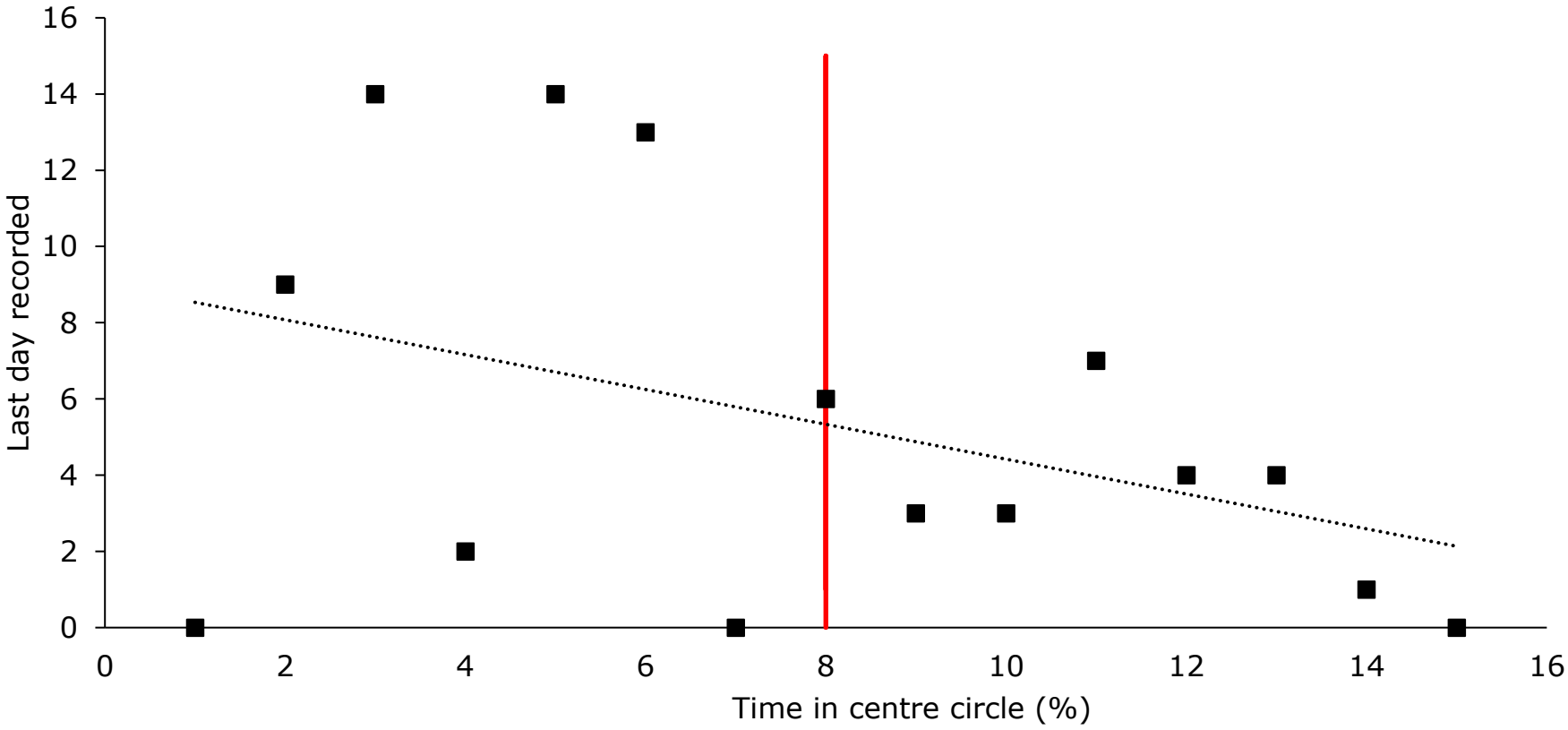

Figure 4.8 –The percentage of time spent in the centre circle (centre circle duration) during the open field test and last day recorded during the 14-day monitoring period. The dotted line shows the line of best-fit; the solid red line, the division between clusters. Classification was as follows <8 % ($\bar{x}$) time in centre circle results in individuals being classified as Anxious (**A**), consequently ≥8% would be classified Non-Anxious (**NA**).

4.4.7 Anxiety category and post-release behaviour

To assess the anxiety as categorical variables (anxiety category), it was proposed that individuals spending <8% ($< \bar{x}$) of the time in the centre circle were classified as anxious (**A**). Conversely individuals spending ≥8% ($\geq \bar{x}$) were classified as non-anxious (**NA**) as per Figure 4.8, these categories were applied to the pre- and post-release variables (Table 4.4).

A significant difference was detected in the last day recorded between the anxiety categories (**A** and **NA**) ($F(1,12)=13.30$, $p=0.003$), while a borderline significant difference was detected in the CLC:PLC between the anxiety categories ($F(1,12)=4.49$, $p=0.056$). No further significant differences were found in the OFT variables between the anxiety categories. However, when the number of traps were analysed per trapping method and per anxiety category, a significant difference was detected in the RFID plot ($F(1,5)=34.44$, $p=0.002$), with **NA** individuals using the RFID traps significantly less than **A** individuals. While in the Live trapping plot, no significant difference was observed in the trapping rates between the categories ($F(1,5)=2.48$, $p=0.176$) (Figure 4.9).

Table 4.4 – Summary of results between the anxiety categories and OFT dependent variables, significant values appear in **bold** and $^{+}$ indicates a borderline result. CLC:PLC ratio (centre lines crossed:periphery lines crossed).

OFT behaviour and anxiety categories (A and NA)	Test statistic *df*=*F*(1,12), H(1)	*p* value
Rearing	*F*= 3.15	0.101
Scanning	*F*= 0.75	0.403
Mean **centre circle duration**	*F*=0.76	0.400
Centre circle entries	***F*=5.85**	**0.032**
Est. distance travelled	*F*=1.32	0.273
Grooming	H=0.22	0.641
Defecation	H=0.08	0.744
Climbing sides	*F*=0.45	0.514
CLC:PLC	*F*=4.49	0.056^{+}
Last day recorded	***F*=13.30**	**0.003**
Movement between traps	H=0.162	0.121

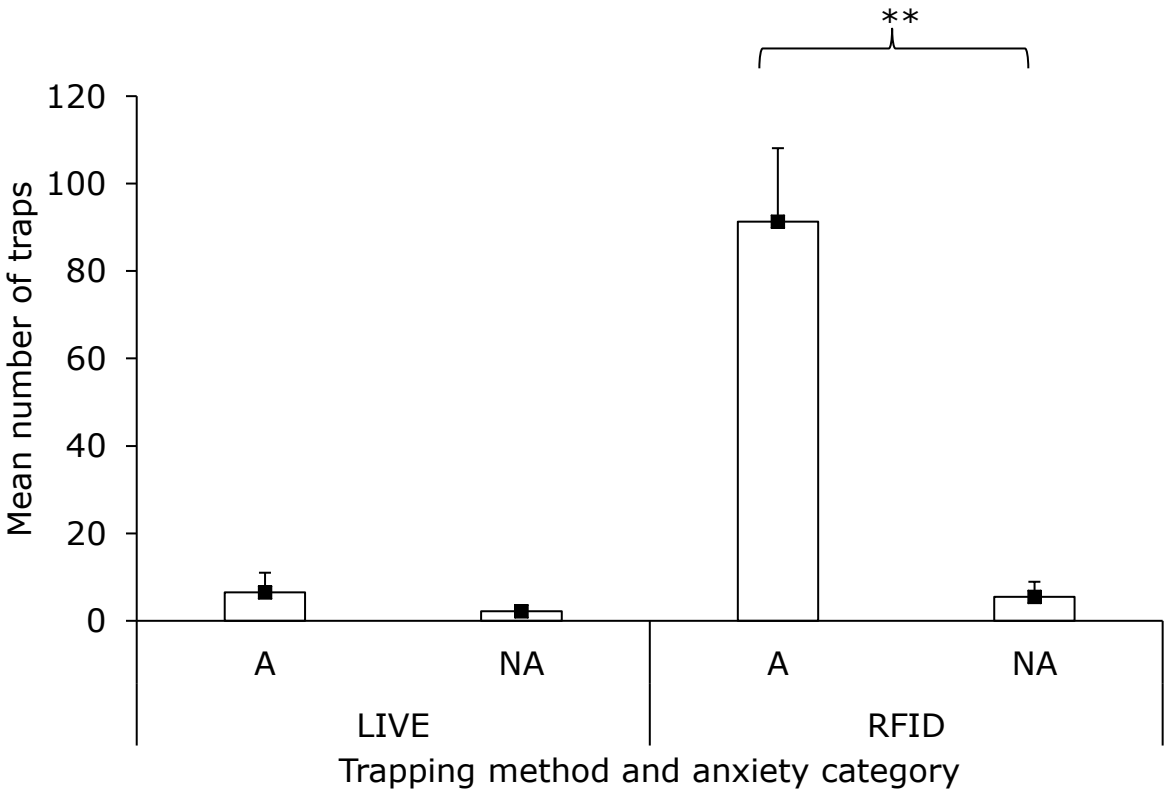

Figure 4.9 – Mean ± SEM of the traps recorded using live and RFID trapping for the anxiety categories (**A** and **NA**) over the course of the experiment. Significance levels - **=$p \leq 0.01$.

4.4.8 CJS modelling on anxiety categories

The program MARK analysis undertaken in Section 4.4.2 were rerun with the data grouped into **A** and **NA** rather than trapping method. These results (Figure 4.10) revealed that the **A** individuals had a 21% higher survival estimate compared to the **NA** (0.95 and 0.74 respectively). Similarly the recapture estimate for **A** individuals was 40% higher than **NA** categorised individuals (0.90 and 0.50).

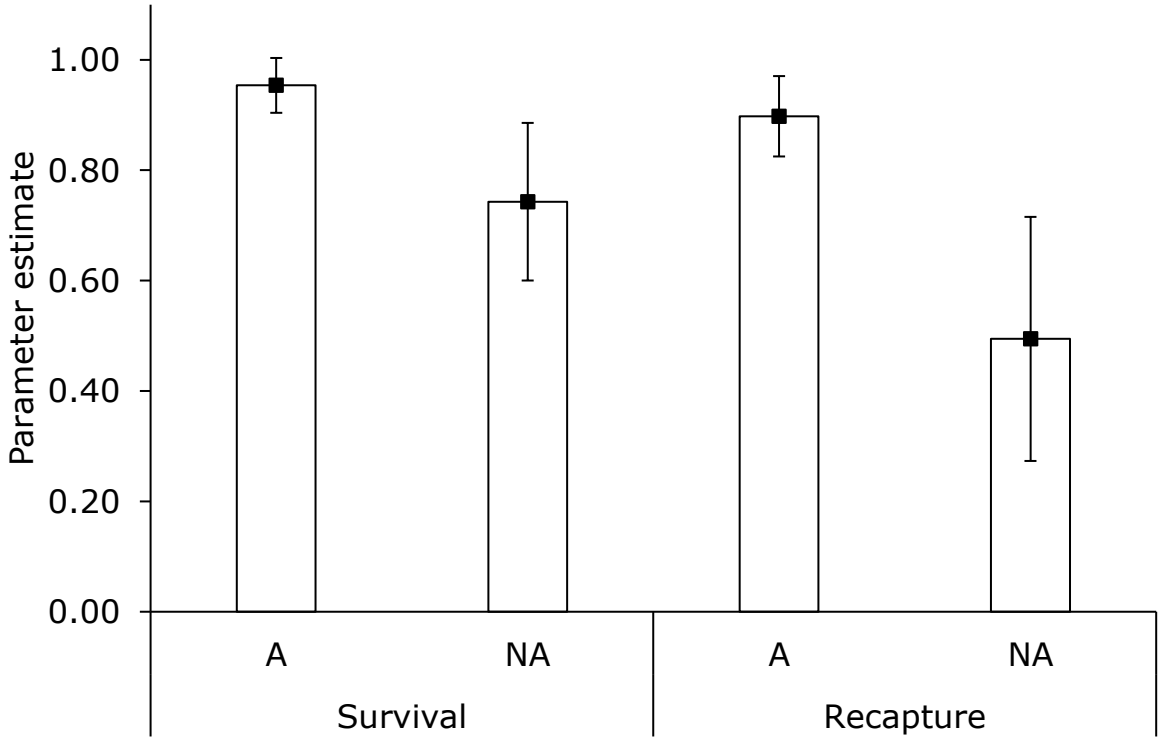

Figure 4.10 - Parameter estimates for survival and recapture ± SE for the **A** and **NA** anxiety categories (anxious and non-anxious respectively) calculated using the CJS function in MARK.

4.4.9 Exploratory score and post-release behaviour

The estimated distance travelled was used as a measure of exploration and will be referred to hereafter as exploratory score. As Table 4.3 indicates

there were no significant correlations between the exploratory score and either of the indicators of anxiety (centre circle duration and CLC:PLC). However, significant positive correlations were observed between the exploratory score and centre circle entries (r=0.783, p=0.001), climbing sides (r=0.784, p=0.001) and scanning (r=0.761, p=0.002). Negative correlations were observed between the exploratory score and rearing (r=-0.680, p<0.001) and exploratory score and the mean centre circle duration (r=-0.784, p=0.001). Significant correlations are presented in Figure 4.11. A one-way ANOVA revealed no significant differences in the exploratory score between the release plots (RFID and LIVE) (F(1,12)=0.09, p=0.771).

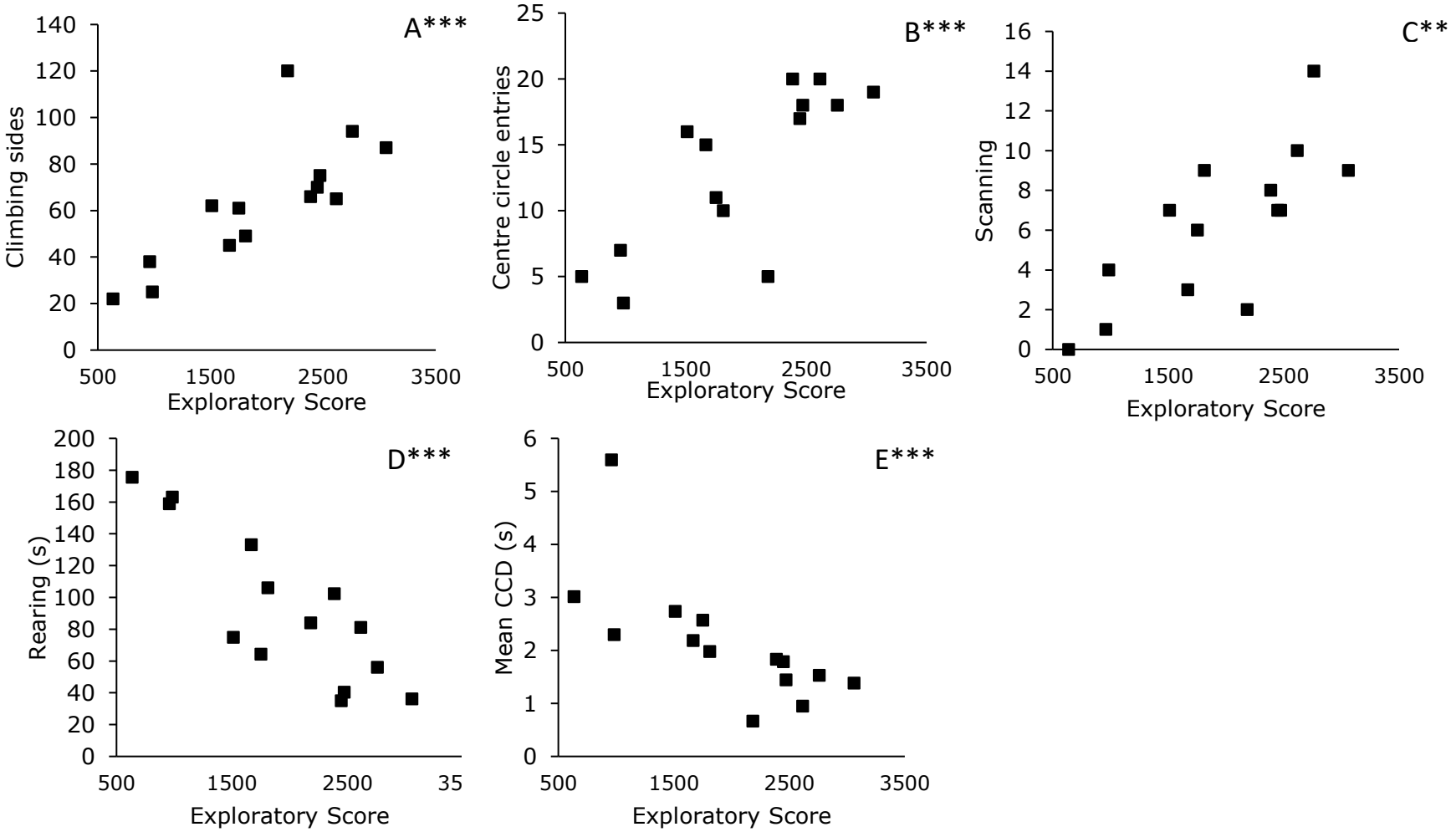

Figure 4.11 – Plots between the measures of vertical exploration and exploratory score, positive correlations between the horizontal exploration and the exploratory score (A, B and C) and negative correlation between vertical exploration D and E. Exploratory score was classified as estimated distance travelled in the OFT. Plot identifier is followed by significance levels - **=$p \leq 0.01$, ***=$p \leq 0.001$.

4.4.10 Sex and OFT variables

Table 4.5 indicates there were no significant differences between males and females and their behavioural responses to the exposed environment during the OFT.

Table 4.5 – Summary of results relating the OFT behaviours and sex N=14, ♂=7, ♀=7.

OFT Variable	Test Statistic df=*F*(1,13), H(1)	*p* value
% time in centre circle	*F*=0.23	0.638
Sniffing	*F*=0.68	0.424
Rearing	*F*=0.56	0.469
Centre circle entries	*F*=0.74	0.405
Grooming	H=2.16	0.142
Climbing sides	*F*=0.14	0.713
Defecation	H=0.23	0.630
Lines Crossed	H=0.49	0.482

4.5 Discussion

To summarise the findings in this chapter, the percent effectiveness of each method was estimated by allocating a point to the method which is least impacted by tested variables (Table 4.6). As this table shows, the RFID method had a 71% effectiveness, and was impacted by internal factors (anxiety). The results for the live trapping indicated that it was 43% effective and was most influenced by external factors (time and weather). While this approach allocates simple arbitrary values to the results, the points reveal that differing internal and external factors can account for the variability in recapture rates.

Table 4.6 – Summary of findings in relation to the variables tested. Points were applied to each method if analysis did not detect a detrimental impact from the independent variables listed. CJS = Cormack Jolly Seber.

Variable	RFID	LIVE
Raw detection rates	1	0
Sex	1	1
Temporal effectiveness	0	0
Weather variable	1	0
Anxiety	0	1
CJS survival	1	1
CJS recapture	1	0
% effectiveness	71	43

4.5.1 Raw trapping rate

The analysis of the individuals recorded per day revealed significantly higher frequencies for the RFID traps compared to live traps, and as seen in Figure 4.1, there was a greater difference between the recorded and unrecorded in the LIVE plot compared to the RFID. However, both methods saw a significant reduction of individuals in the second week of monitoring although no significant difference was detected in the number of traps in the RFID plot between weeks one and two. Conversely, a significant drop in trap occupancy was detected in the second week of monitoring in the LIVE plot. Therefore, in the RFID plot in week two, fewer individuals were using the RFID traps at a higher frequency compared to week one. Considering the functionality of the RFID, whereby there is no physical capture (after initial tagging) and an autonomous and continuous ability to record traps, it is no surprise that the RFID system performed better than

live trapping, albeit at a basic level. These findings reveal that RFID traps were used more frequently by a higher number of individuals over the monitoring period, yet, the temporal effectiveness of both methods was inconsistent. As Table 4.6 summarises, the trapping rate in the LIVE plot was largely impacted by abiotic variables (extrinsic factors), whereas, RFID trapping was apparently impacted by anxiety (intrinsic factors), which, directly or indirectly may account for the reduction in temporal effectiveness in the second week. This will be discussed in more detail in section 4.5.2.

A similar pattern of trap occupancy was detected between anxious and non-anxious individuals within the live trapping enclosure, but not significantly so. Although, as an individual could only be trapped once per day in the LIVE plot and the data collected were limited when compared to the RFID trapping data, a similar significant result may have been detected if additional data were collected.

4.5.2 Anxiety and trapping rates

The strong correlation between centre circle duration and last day recorded reveals that individuals displaying higher anxiety (based in time spent in the centre circle of the OFT) were recorded for longer over the course of the experiment when compared to those displaying lower anxiety. These findings support the presence of a behavioural type and additional data may also support the presence of a behavioural syndrome. When these data were considered within categories (anxious and non- anxious), the number of reads recorded in the RFID plot were significantly higher for the anxious individuals than the non-anxious.

There are several potential explanations for this, one being the non-anxious individuals simply did not survive, perhaps engaging in higher risk-taking behaviours or less effective predator avoidance strategies (Sih *et al.*, 2003). Thus, the significant difference reported between the categories may be a consequence of population size. The MARK estimations for survival in anxious and non-anxious revealed the survival estimation for the anxious was 21% higher than the non-anxious. Therefore, there is evidence to support this scenario. However more data would be required to draw definitive conclusions. Nonetheless, these findings correspond with the results of Bremner-Harrison *et al.* (2004) who reported that more cautious (anxious) individuals had a higher survival rate post-release than their bolder counterparts. Yet, the results of correlations between the risk assessment behaviours in the open field (rearing) (Brenes *et al.*, 2006; Brenes *et al.*, 2009) do not support this as there was no detectable significant correlation between anxiety and risk assessment.

Interestingly, Sih *et al.* (2012) noted that if behavioural correlations exist between situations (behavioural syndrome) this can limit a species' ability to cope with changing environments. Individuals transfer behavioural responses to a new environment but these maybe sub-optimal, thereby reducing their survival. For example, individuals with higher activity are at a higher risk of predation simply because they move more, which results in a higher chance of being detected. Hence, when exposed to a novel environment with high predation pressure they do not survive (Sih *et al.*, 2012). Yet, these behavioural types persist in wild populations. Therefore

there must be survival advantages of acting apparently sub-optimally (Luttbeg and Sih, 2010). Clark's (1994) theory is that behaviour is determined by state, whereby high state individuals (larger resources or energy reserves) can avoid predators more effectively than low state individuals, by fleeing faster or defending themselves more effectively. Thus, it is possible that displaying sub-optimal behaviour may be mitigated by high state. It is possible that individuals that were recorded more were high state and thus able to survive better, and the significant results relating to anxiety is a type I error. While the current study did not assess behavioural syndromes *per se*, there were correlated behaviours which indicated inter-individual behavioural differences (a behavioural type). This, coupled with the evidence from Schuster *et al.* (2017a) and Schuster *et al.* (2017b), supports Sih *et al.*'s (2012) theory that limited behavioural plasticity and sub-optimal responses may have resulted in non-anxious individuals displaying lower survival rates. Interestingly, these sub-optimal responses maybe an artefact of captive breeding, as Luttbeg and Sih (2010) hypothesised differences in initial state can determine the long-term trajectory of behavioural types. Similarly, Bremner-Harrison *et al.* (2004) noted that individuals bred for several generations in captivity could be at a disadvantage as they have habituated to stimuli that would normally provoke fear, reinforcing the need to carry out behavioural assessment of wild *M. minutus*, which itself presents many challenges, including the potential bias caused by behavioural plasticity (Dingemanse *et al.*, 2010).

The second scenario is that the non-anxious individuals avoided the traps and utilised natural resources within the enclosure. Such behaviour could be due to an increased willingness to travel further, thereby encountering more resources (Schuster *et al.*, 2017b). Highlighting a possible link between behavioural type and optimal foraging strategies. Equally, anxious individuals could have missed foraging and breeding opportunities as their movement was limited, as per the findings of Sih *et al.* (2012). Alternatively, non-anxious individuals may have dispersed more readily than the anxious individuals and thus were no longer present within the experimental plot. Lastly, the non-anxious individuals may have been discouraged by trap design with a direct impact on trapping rate. This is an unknown variable, which could easily be empirically assessed in captivity. Yet, if this was the case, then a consistent number of individuals should have been recorded per day from the beginning of the experiment. However, the results do not support this, as trapping rates in non-anxious significantly reduced between week one and two, suggesting that, for some reason, non-anxious individuals stopped using the traps rather than showing a reluctance to do so from the outset.

However, these results could simply be a consequence of experimental design, whereby the provision of food, protection from aerial predators and opportunities to shelter within traps (to a lesser extent in the RFID due to size restriction), minimised predation in small mammal species already resident within the enclosure, in turn, increasing mortality in *M. minutus* (Wróbel and Bogdziewicz, 2015).

These findings indicate that there is an indirect impact of anxiety on trapping rates, which is not related to trap design. Reduced trap usage is likely to be a consequence of either utilisation of natural resources or lower survival because of behaving sub-optimally in a 'dangerous environment'. Burger *et al.* (2009) discussed the importance of using optimal trapping methods which are species-specific, as sub-optimal methods may lead to conservation decisions based on misleading information. Therefore, utilising a method that provides the most reliable information is essential. However, these findings may indicate that trapping methods for *M. minutus* not only need to be species-specific, but, perhaps they need to be behaviourally-specific as well.

These interpretations are by no means conclusive or comprehensive, and further data would be required to draw conclusions on the impact of behaviour on survival and trap usage. These results highlight the importance of considering behaviour when undertaking releases, re-introductions and translocations. Pre-release selection of individuals may prove beneficial for improving post-release survival. Equally, species which do not display behavioural plasticity may not be suitable for release/reintroduction at all, as they may not be capable of adapting to the pressures a new environment presents.

4.5.3 Weather and trapping rates (activity)

Intrinsic limitations of live trapping caused a direct impact of weather on trap occupancy, as live traps could not be set during the day due to high

temperatures and risk of hyperthermia. This limitation emphasises the restrictions of live trapping, thus, ecological inferences are also limited.

A significant correlation was detected between relative humidity and live trap occupancy, which also corresponded with a significant negative correlation between day of experiment and trap occupancy; conversely, relative humidity was positively correlated with day of experiment. Therefore, apparent significant correlations presented here may be misleading; relative humidity increased consistently over the experiment while trap occupancy declined. Therefore the direct effect of relative humidity is questionable, and it may simply be a coincidence. Additional data are required to establish any associations between these variables.

Since RFID trapping rates are a direct measure of activity, these results also provided additional insight into how weather influences activity. As described above there were no conclusive effects of weather. However, there were periods of heavy rainfall which directly corresponded with individuals using the traps in higher frequencies, although not significantly so. Wróbel and Bogdziewicz (2015) found that *A. flavicollis* had higher capture rates during rainfall, which was linked to higher activity at times when predators were less active (offsetting cost of thermoregulation when wet) (Brandt and Lambin, 2005) and the masking effect of rainfall from sounds of movements (Vickery and Bider, 1981). Higher activity in the rain appears to be counter intuitive as being wet causes heat loss (Vickery and Bider, 1981), but this behaviour must have positive feedback for it to persist

within populations. However, here it is possible that the *M. minutus* were attempting to stay dry.

4.5.4 Circadian activity and RFID trapping rates

The time stamped RFID trapping records facilitated assessment of circadian activity. Initially, analysis was undertaken on the activity recorded during light and dark conditions which revealed no significant preferences for activity or trap usage in either conditions. However, as Figure 4.7 shows there was a general preference for activity during light conditions; one caveat to this measurement is that these traps could be indicating feeding activity only, and there are likely to be periods of activity that were unrecorded (although this is true of many activity recording methods).

The finer scale analysis also revealed no significant differences in the number of traps between the three time-blocks. Wróbel and Bogdziewicz (2015) noted that rodents display plasticity in daily activity to minimise the impact from changing predation risk, and species may rely on external indications of increased risk. Upham and Hafner (2013) proposed that one of these clues maybe moonlight, since lower cloud cover and increased moonlight facilitated detection from predators. However, moonlight and phase of the moon were not measured here, therefore it would be recommended for future studies.

Additional analysis revealed a significant quadratic relationship between hour and number of traps, indicating a polyphasic activity pattern in *M. minutus*, which is similar to other rodents (Lövy *et al.*, 2013), with peak

activity in the latter part of the day. These results correspond with the findings of Cross (1970) and Warner and Batt (1976) who also found *M. minutus* to be active throughout the day and night. However, the Cross (1970) study focused on *M. minutus* in captivity and the only comparable data on wild *M. minutus* was presented by Warner and Batt (1976), who noted that the highest activity occurred around dawn and three hours after sunset. While this corresponds with Cross (1970), these data cannot fully be attributed to *M. minutus*. Whilst not fully comparable with the subjects' wild counterparts, these results offer a different perspective into *M. minutus* activity patterns. The development of RFID has facilitated the collection of these data, yet, as discussed earlier, a bias in the RFID data in favour of anxious individuals may exist. Consequently, the non-anxious individuals' circadian activity may not have been accurately recorded within these data; these individuals may express a different activity pattern entirely. Alternatively, the apparent bias could have occurred by chance as the sample size is relatively small. Accordingly, additional data would be useful for determining circadian activity.

4.5.5 Sex-dependent behaviour

An even trapping rate was important when modelling population demographics as suggested by Schaub *et al.* (2010). The findings related to sex bias and trapping method indicate there were no detectable differences in trapping rates between the sexes in either of the tested methods. Furthermore, the results of the OFT did not reveal a significant difference in the behavioural responses to the open field arena between

male and female *M. minutus*. Therefore, as far as can be determined here, variability in trapping rates between the methods do not appear to be associated with sex, which corresponds with the findings of Burger *et al.* (2009) and Torre *et al.* (2016).

4.5.6 Exploratory variables

The exploratory score calculated as per Dingemanse *et al.* (2002) was not correlated with any of the post-release variables (last day recorded, movement between traps and reads), suggesting that the exploratory propensity of an individual did not impact on the trapping rates. Interestingly, there were significant correlations between the exploratory score and pre-release variables (centre circle entries, mean centre circle duration, climbing sides, rearing and scanning), but none which are indicative of anxiety. These findings correspond to the findings of Schuster *et al.* (2017b), who noted that boldness (anxiety) and activity were not correlated in the OFT. However, these variables did correlate during other behavioural tests. As the exploratory score is based on distance travelled in the open field, some of the correlations are to be expected, whereby vertical exploration is negatively correlated with the exploratory score and horizontal exploration is positively correlated with exploratory score, due to a function of time, yet these correlations support the presences of behavioural type within the release population. Huang *et al.* (2016) and Cornelius *et al.* (2017) found fast explorers travelled further and scanned more frequently than slow explorers. However this exploration maybe more superficial than slow explorers (van Oers *et al.*, 2004). Here, individuals

that spent longer assessing risk (rearing) and stayed in the centre circle longer per visit, ultimately spent less time engaging in horizontal activity and thus was classed as slow explorers, conversely fast exploring individual's time was spent climbing the sides of the arena, engaging in horizontal locomotion (including scanning) and, consequently entered the centre circle more often than slow explorers (Cornelius *et al.*, 2017). These findings also correspond with Schuster *et al.* (2017b) proposed responses, whereby individuals in the fast-behavioural type move further and exploit more resources than the slow explorers. Additional data are required in this area which would allow conclusions to be drawn about the associations between exploration and movement, and to investigate the interaction between anxiety, exploration and movement propensity.

4.5.7 Recommendation and limitations

The key considerations when employing RFID and a WSN are cost and system reliability, as discussed by Baratchi *et al.* (2013). This system was developed in 2012 - 2013, in terms of technology this time frame is likely to mean the hardware is outdated. Today there are expected to be cheaper alternatives which can be utilised to simplify the system and minimise system failures experienced during this study. The benefit of the RFID trap was the adaptable housing, which can facilitate rapid species-specific modifications when required, and thus, could be used to monitor a wide variety of species; particularly those that present monitoring challenges. Furthermore, as the PIT tags required no external power and are comparatively small compared to other tag types, RFID could present

opportunities for the study of smaller taxa, where the use of radio telemetry is unsuitable.

The findings of Chapter Three indicated that age of *M. minutus* needed to be considered when undertaking detection dog training. Here, it is also apparent that the omission of age data has proven somewhat limiting. Schuster *et al.* (2017a) found that behaviour was consistent between age classes in captivity, it is possible that behaviour in semi-natural/natural situations may change with time, potentially leading to differences in exploration and risk assessment, with knock-on effects on trapping rates. Therefore, it is recommended that age is recorded in any future research of this kind. Although cost would limit this somewhat, individual housing of the *M. minutus* whilst in captivity would be required, substantially increasing the cost of housing and husbandry, and thus it is likely that it would need to either be absorbed by an existing organisation or be dependent on suitable funding.

Mortality is a potential issue when undertaking live trapping studies, particularly so when trapping an endangered or threatened species. Burger *et al.* (2009) and Torre *et al.* (2016) proposed that mortality can be reduced by focusing trapping when the species is most active. However, *M. minutus* peak activity in the wild has yet to be accurately measured. The RFID traps reduce the impact of mortality, as after the initial trapping and fitting a PIT tag there is no requirement to capture individuals, which allows more individuals to be recorded at a higher frequency than live trapping, as per the finding of this Chapter. Furthermore, it is thought that *M. minutus*

mortality during winter is high, which is mostly based on live trapping results (Trout, 1976 in Trout, 1978b). However, the evidence of behavioural types presented here, coupled with Schuster *et al.*'s (2017b) suggestion that slow behavioural types restrict their range, may mean that trapping should be carried out on a much finer scale. It is possible the historically poor winter trapping rates are a result of individuals simply not encountering traps rather than the effects of mortality *per se*. Thus, this should be investigated further.

The findings also suggest that the individuals recorded using both methods were behaviourally distinct from those that were unrecorded, either because of trap design, lower survival or higher natural resource utilisation. Accordingly, there is a possibility that pre-release selection may favour a behavioural trait or help to avoid undesirable traits. There is a need for further research in this area, to investigate the behaviour of wild *M. minutus* in order to develop the understanding of species demographics and behavioural differences occurring in wild populations.

It would also be recommended for a battery of pre-release behavioural tests to be undertaken, as per Schuster *et al.* (2017b), as here only the OFT was utilised. However, there are alternatives which when used in conjunction with the OFT can be used to identify the existence of personality and behavioural syndromes (Schuster *et al.*, 2017a; Schuster *et al.*, 2017b).

Réale *et al.*'s (2007) perspective on categorical analysis of temperament traits implied that this form of analysis was misleading. Yet, here temperament analyses were undertaken on categorical and continuous

data, predominantly as the categorical data facilitated CJS modelling and independent parameter estimation undertaken in program MARK. Furthermore, the categorical analysis undertaken revealed a significant difference between the number of traps in the anxious and non-anxious categories when using RFID trapping (Figure 4.9 and Figure 4.11); it is possible that the significance of this would not have been identified if categorical analyses were excluded.

While live traps can determine whether an individual was active over a coarse temporal scale (Wróbel and Bogdziewicz, 2015), RFID trapping refined this scale greatly, firstly by having time-stamped trapping events and secondly by allowing free movement in and out of the traps. Therefore, it would be useful to record activity data in conjunction with finer scale weather data, so the impact of abiotic variables can be evaluated in greater detail. This would not only prove useful for *M. minutus,* but these improvements to data collection would be beneficial for extending the ecological knowledge of other sympatric small mammal species. Furthermore, while there were no detectable significant effects of weather on RFID trapping, indirect effects cannot be excluded, such as system failure during rain or high temperatures, additional testing of the hardware would therefore be recommended. These data can only be compared against weather variables that occurred during the experiment, accordingly, the limited window of data collection needs to be considered when drawing conclusions on the impact of abiotic factors such as weather.

A key limitation of this Chapter is the small sample size, results are based on data collected from 14 individuals, with seven representing each group. Therefore the scope is somewhat limited and further investigation is needed. The methodological challenges and complexities involved in the experimental design, coupled with the ecological complexities of the species, provided some mitigation for the small sample size. It is also important to note that as far as can be determined from the literature, comparisons of trapping rates between RFID and live traps in *M. minutus* has yet to be attempted. Therefore, these results are entirely novel and offer a baseline for the use of RFID trapping in *M. minutus*.

4.6 Conclusion

In terms of validating the use of RFID for monitoring *M. minutus*, the raw trapping rates, impact of weather and CJS recapture probabilities support the use of RFID over Live trapping. However, a potential bias exists between behavioural types, which is potentially linked to reduced survival in non-anxious individuals meaning that behaviour may have impacted trapping, either, directly through a reluctance to use the traps or indirectly through reduced survival in non-anxious individuals. The findings presented here support the hypothesis that behaviour indirectly affected trapping rates as non-anxious individuals were using the traps at a higher rate in the first week compare to the second.

The preliminary findings relating to behaviour indicate that no single monitoring method is conclusively more effective at monitoring *M. minutus* and it is possible that a suite of methods and trap designs are required to

gather sufficient data across behavioural types, seasons and habitat. Yet, the relative effectiveness of RFID should not be underestimated as the detailed behavioural and ecological data that were collected using the RFID are entirely novel and have provided direction and vision for future research.

There are a number of scenarios where the RFID method could be applied in the future. Firstly, as little is known about the overwinter behaviour of *M. minutus*, PIT tags that record internal temperature could be utilised which provide an understanding of activity over the seasons and the impact of external temperature on behaviour (Roark and Dorcas, 2000). This could also provide an insight into survival strategies during the winter. Alternatively, Hou *et al.* (2015) utilised automated RFID traps fitted with scales to allow weight of hummingbirds to be recorded. A similar system could be utilised for wild *M. minutus*, which may reveal details about breeding condition, or data could be linked to behavioural types, optimal foraging strategies and survival. The greatest potential benefit of utilising the RFID trapping method is the potential to collect fine scale spatial and temporal data which are of sufficient quality and quantity to develop knowledge of the movement ecology of *M. minutus*. When compared to the data collected using live trapping methods RFID offers great potential.

Crucially, there is a caveat to utilising PIT tags, in that individuals need to be caught at least once to fit the PIT tag, and as discussed throughout this section, there is individual variability in trapability. Thus, a potential bias is present from the outset, a paradox which will need further attention to

overcome. As suggested earlier, the trapping method may need to be behaviourally-specific as well as species-specific. Thus, either a suite of monitoring methods may be required, or if a direct effect of behaviour was found, improvements could be made to the housing of the RFID trap which would accommodate different behavioural types.

At an autecological level, the findings presented in this Chapter support the activity patterns indicated by Cross (1970), whereby the animals are active throughout the 24hr period, and these data have provided evidence of a polyphasic pattern to activity. These findings also support the existence of inter-individual differences in behaviour within the release population, which corresponds with the findings of Schuster *et al.* (2017b), and there is also evidence of fast and slow behavioural types which may impact movement, particularly in fragmented habitats (Cornelius *et al.*, 2017; Schuster *et al.*, 2017b).

With the RFID trapping proving more effective in most categories considered in this chapter, this method was utilised to facilitate the collection of *M. minutus* movement data. Knowledge of movement and dispersal in this species is limited, particularly the impact of fragmentation on movement propensity. As presented in Chapter One, habitat fragmentation is an ever-increasing issue and the effects may be exacerbated by climate change. A stronger understanding of *M. minutus* movement propensity and the impact of fragmentation would aid future conservation of the species, by defining habitat management strategies and by creating new habitats which maximise movement opportunities. In the

following chapter *M. minutus* movement over differing gaps sizes were quantified, and analysis in relation to anxiety and exploration was undertaken to identify any behavioural associations with movement propensity.

5 Chapter Five - *M. minutus* movement in fragmented habitats

5.1 Introduction

The findings of the previous chapter revealed that Radio Frequency Identification was effective at monitoring *M. minutus* in a semi-natural environment. However, the inter-individual behavioural differences (behavioural types) appeared to impact RFID trapping, and thus indicated that individuals which were recorded were behaviourally distinct from those that were unrecorded. Therefore behavioural types may also influence responses to fragmentation. Fragmented habitats are an ever-increasing cause of species decline, resulting in increased edge habitat and the restrictions of anthropogenic barriers can result in source-sink population dynamics (Remeš, 2000), limit dispersal and alter the behaviour of some species, *Vulpes vulpes* and *Canis aureus* (golden jackal) for example (Shamoon *et al.*, 2018). Understanding the impact of these factors is essential for effective conservation of species. However, the impact of fragmentation and physical barriers has yet to be studied in *M. minutus,* consequently their movement ecology is little understood.

5.2 Rationale

This chapter focuses on *M. minutus* movement between patches within a semi-natural enclosure with integrated gaps of differing sizes (1m, 2m and 4.8m). Gap widths were chosen to represent fragmentation typically occurring in agricultural habitats, such as farm tracks, gateways and footpaths. Pre-release dependent variables were analysed in relation to *M.*

minutus movement and behaviour. Other biotic and abiotic factors were assessed in relation to movement propensity.

The findings of Chapter Four indicated that RFID is a more effective method for monitoring *M. minutus* than live trapping and with the instantaneous method of data collection. The RFID traps can be employed to answer key questions relating to *M. minutus*, in particular their movement ecology. This is vital for understanding how the species responds to fragmented habitats and the scope of movement attempts. The RFID is particularly useful as it offers reliable data that can be gathered with minimal human interference. Furthermore, the functionality of the RFID traps would allow behavioural type to be assessed in relation to movement propensity. While there are shortfalls in the RFID method and further validation is still required, when compared to the live trapping results, the RFID was more favourable. Hence the RFID traps were utilised here to quantify *M. minutus* movement over fragmented habitats.

5.2.1 Aim:

To establish if *M. minutus* have sufficient motion capacity and perceptual range to make successful movements over different sized gaps.

5.2.2 Objectives:

Objective IV: To investigate the inter-individual behavioural differences on the survival and movement propensity of *M. minutus.*

Objective V: To investigate the effects of fragmentation at different scales on *M. minutus* movement.

5.3 Methods

The methods pertinent to this Chapter have been described in Chapter Two. Section 2.5 is most relevant to the results presented in the subsequent sections of this Chapter.

5.4 Results

This section considers the variables that have impacted *M. minutus* movements over a variety of gap widths between patches of habitat. The overall aim was to establish if *M. minutus* have sufficient motion and navigational capacity to make successful movements over different sized gaps, and to establish if behaviour influenced their ability to make these movements. The findings from the analyses undertaken are presented in the following sections.

5.4.1 Movement between patches

The number of recorded reads in the RFID traps per patch are as follows; patch 1 (268), 2 (362), 3 (36) and 4 (0); as Figure 5.1 indicates the patches were not utilised equally and, of the patches that were used, the lowest number of traps was seen in patch 3. There were no recorded movements over the 4.8m gap, all the recorded movements occurring over the 1m (40 recorded crossings by 10 individuals), and 2m gap (17 recorded crossings by six individuals crossed). A significant difference was detected in the number of recorded movements over the different width gaps per individual (df=2, H=15.24, p<0.001) (Figure 5.2). *Post-hoc* testing using a Mann-Whitney test revealed that there was no significant difference detected

between the movements over 1m and 2m gap widths. However, as no movements were recorded over the 4.8m gap, it was not possible to run *post-hoc* testing on these data. Thus the difference detected by the Kruskal-Wallis would likely be between the 1m and 4.8m and the 2m and 4.8m width gaps. The number of movements decreased as gap width increased. When the number of individuals recorded per day were analysed, there was a significant reduction in the number of individuals recorded per day after the 4.8m gap was opened to allow movement over this gap to patch 4 (df=1, F(1,8)=27.31, p=0.001).

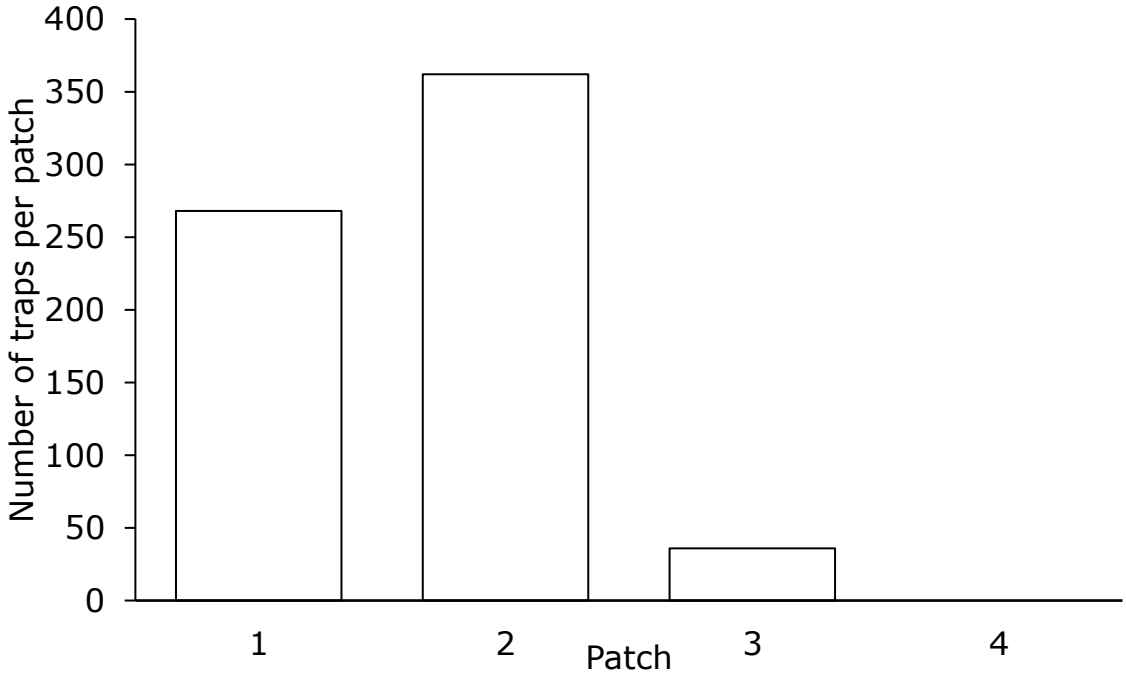

Figure 5.1 – Total number of RFID traps recorded within a semi-natural enclosure in each of the patches (1,2,3 and 4) over the course of the experiment.

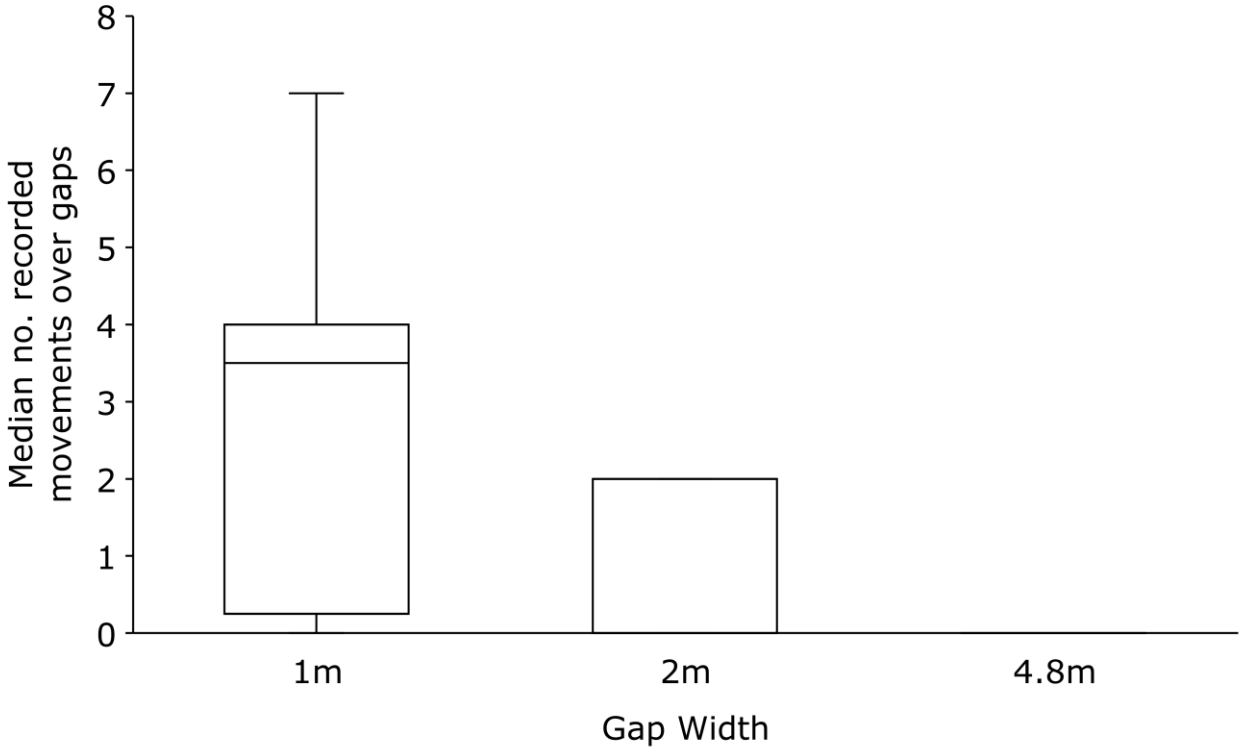

Figure 5.2 – The median number of recorded *M. minutus* movements over Three gap widths (1m, 2m and 4.8m) within a semi-natural enclosure. Centre line indicates medians with box equalling median ± 1 quartile. Whiskers extend to maximum and minimum values for each gap size.

5.4.2 Post-release dependent variables and movement over gaps

A Pearson's correlation detected no significant correlation between time to first crossing and the total number of crossings recorded for each individual (r=0.237, p=0.404). The effect of sex on movement was also investigated to established if a sex bias existed and no significant difference was detected using a one-way ANOVA (F(1,12)=0.08, p=0.787).

5.4.3 Effect of OFT-dependent variables on movement

As Table 5.1 shows there were no significant correlations detected between the centre circle duration (hereafter referred to as anxiety score) and

number of 1m gap crossings per unit time (r=-0.101, p=0.731) or the anxiety score and 2m gap crossings per unit time (Rho=-0.220, p=0.451) (Table 5.2). Furthermore, no significant correlation between last day recorded and anxiety score was detected (r=-0.156, p=0.595) as per Chapter Four.

A significant negative correlation was detected for estimated distance travelled - hereafter referred to as exploratory score (r=-0.568, p=0.034) and scanning (r=-0.614, p=0.019) (Figure 5.3 D and B respectively and Table 5.1). A significant positive correlation between grooming and number of 1m gap crossings was detected (r=0.601, p=0.023) (Figure 5.3 A). Borderline results were detected between rearing and 1m gap crossing (r= 0.517, p=0.058) (Figure 5.3 C) and between 2m gap crossing and the number of faecal boli produced during the OFT (Rho=-0.52, p=0.054). No other significant results were detected.

Table 5.1 – Results from the parametric (Pearson's test) and non-parametric (Spearman's test) correlation analyses between number of recorded movements over 1m width gaps and OFT variables. Pearson's statistics = *r* and Spearman's = Rho. Significant values appear in **BOLD** and $^{+}$ indicates borderline values. (CLC:PLC=centre lines crossed:periphery lines crossed ratio, EDT=estimated distance travelled).

OFT variables	Test Statistic	
	r or Rho	*p*
CLC:PLC	-0.002	0.993
Centre circle duration (anxiety score)	-0.101	0.731
$\bar{x}$ time per centre circle entry	0.279	0.334
Climbing sides	0.088	0.764
Faecal boli produced (per s)	0.087	0.767
EDT (exploratory score)	**-0.568**	**0.034**
Scanning	**-0.614**	**0.019**
Grooming	**0.601**	**0.023**
Rearing	0.517^{+}	0.058^{+}

Table 5.2 - Results from the Spearman's Correlation analyses between number of recorded movements over 2m width gaps and OFT variables. Significant values appear in **BOLD** and $^{+}$ indicates borderline values. (CLC:PLC=centre lines crossed:periphery lines crossed ratio, EDT=estimated distance travelled).

OFT variables	Test Statistic	
	Rho	*p*
CLC:PLC	-0.225	0.440
Centre circle duration (anxiety score)	-0.220	0.451
$\bar{x}$ time per centre circle entry	-0.044	0.880
Climbing sides	0.030	0.920
Faecal boli produced (per s)	-0.52	0.054^{+}
EDT (exploratory score)	-0.299	0.300
Scanning	-0.390	0.168
Grooming	0.200	0.493
Rearing	0.030	0.920

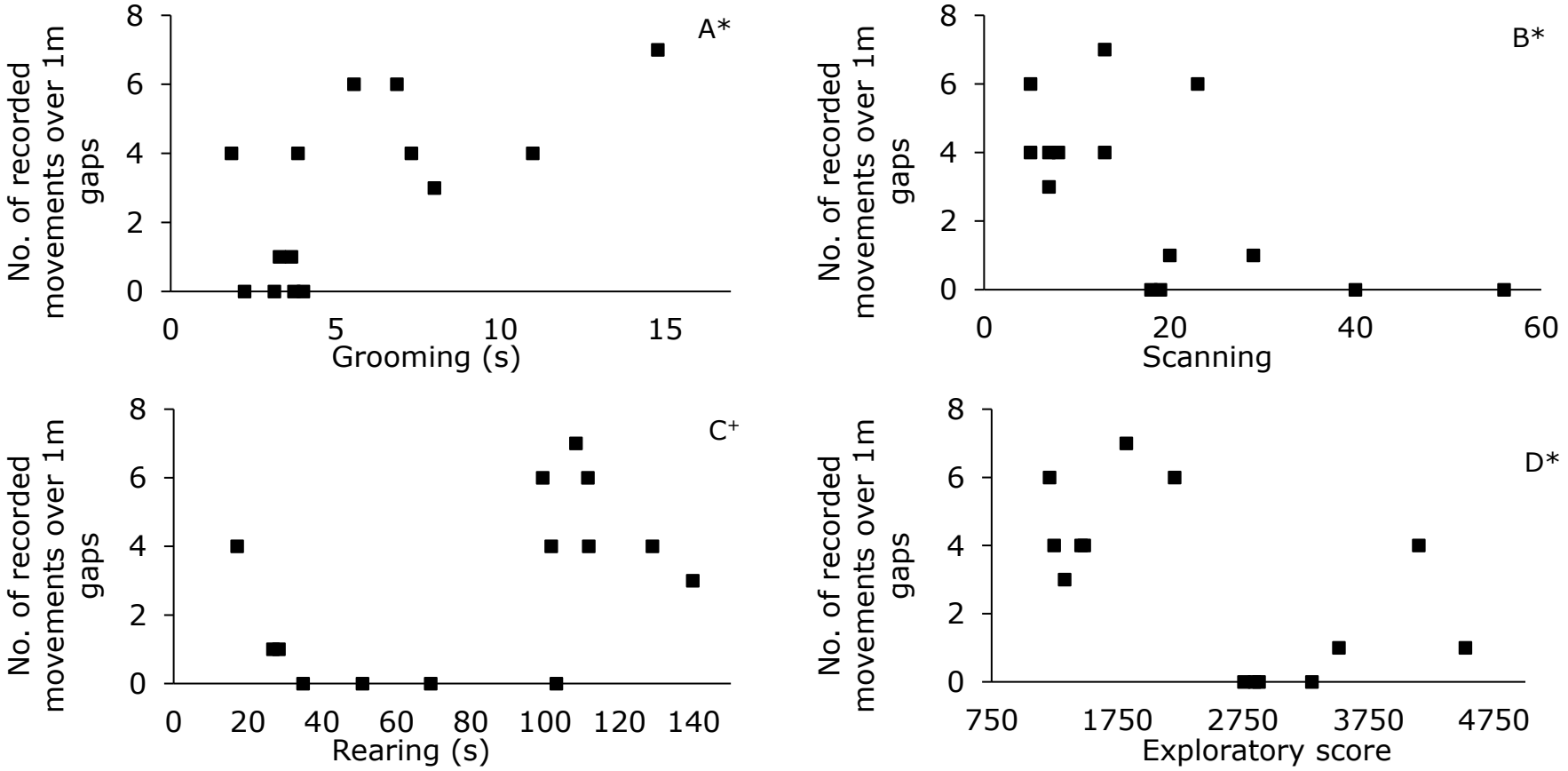

Figure 5.3 –Plots of correlations between OFT variables and number of 1m gaps crossed per unit time post-release. A and C Positive correlations between grooming and rearing and 1m movements respectively; B and D negative correlations between scanning and exploratory score and 1m movements respectively. Plot identifier is followed by significant levels - *=$p \leq 0.05$, +=borderline result.

5.4.4 The effect of exploration score and category on movement between patches

As mentioned in section 5.4.3, a significant correlation was detected between the exploratory score and the number of 1m crossings per unit time (r=-0.891, p=0.034) (Figure 5.3 D). These data were compared as categories, as per Chapter 4 these were categorised as fast (**FE**) and slow explorers (**SE**). These tests revealed a strong significant difference in the number of recorded movements between the exploratory categories (**FE** and **SE**) ($F(1,12)$=26.13, p<0.001). Figure 5.4 shows that **SE** individuals were recorded making a higher number of successful movements between patches when compared to **FE** individuals. However, no significant correlation between number of 2m movements and exploratory score were detected (Rho=-0.299, p=0.300). Further analysis revealed that the **SE** individuals on average spent longer (but not significantly) in a patch before moving than the **FE** individuals ($F(1,8)$=1.62, p=0.238) (Figure 5.5). There was a highly significant correlation between the exploratory score and time spent assessing risk (rearing) (r=-0.911, p<0.001) (Figure 5.6 (B)), as well as exploratory score and Grooming (r=-0.754, p=0.002 Figure 5.6 (A)), and rearing and grooming (r=0.732, p=0.003 Figure 5.6 (C)).

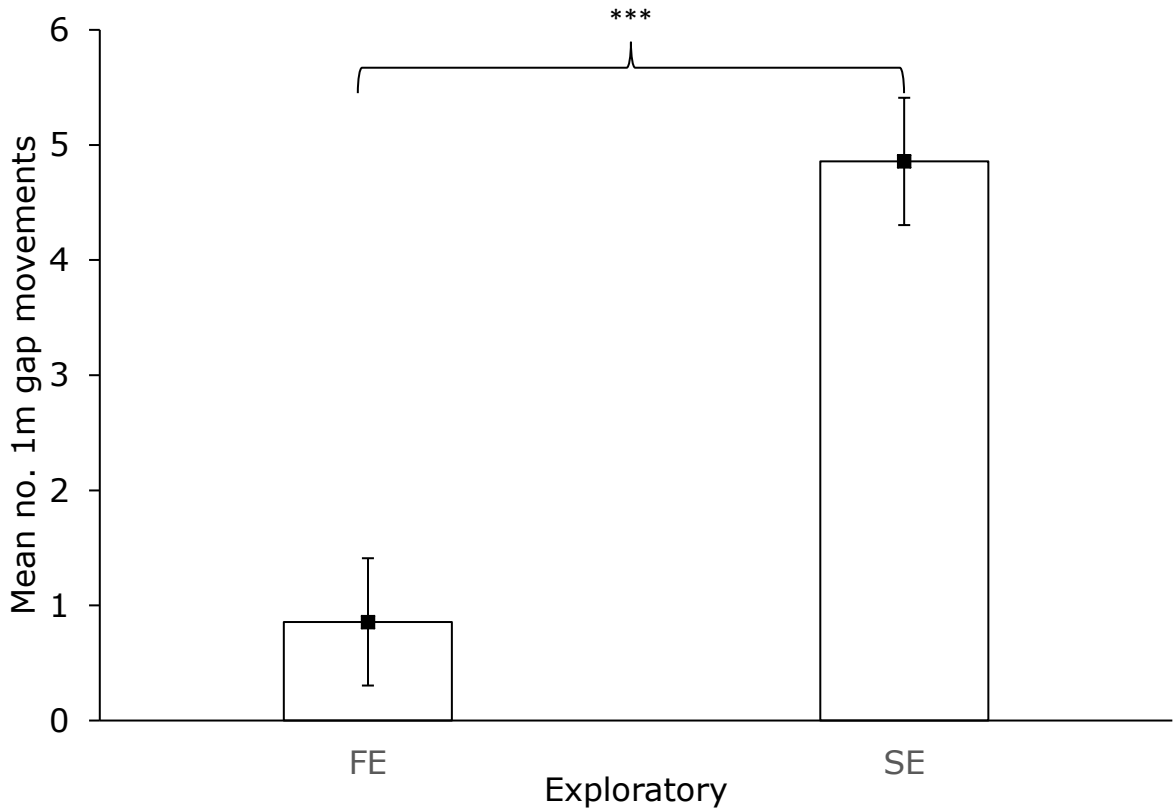

Figure 5.4 –The mean ± SEM of the number of 1m gaps crossed between the exploratory categories (**SE=**slow explorers and **FE=**fast explorers) over the 11-day monitoring period. Significance levels ***=p<0.001.

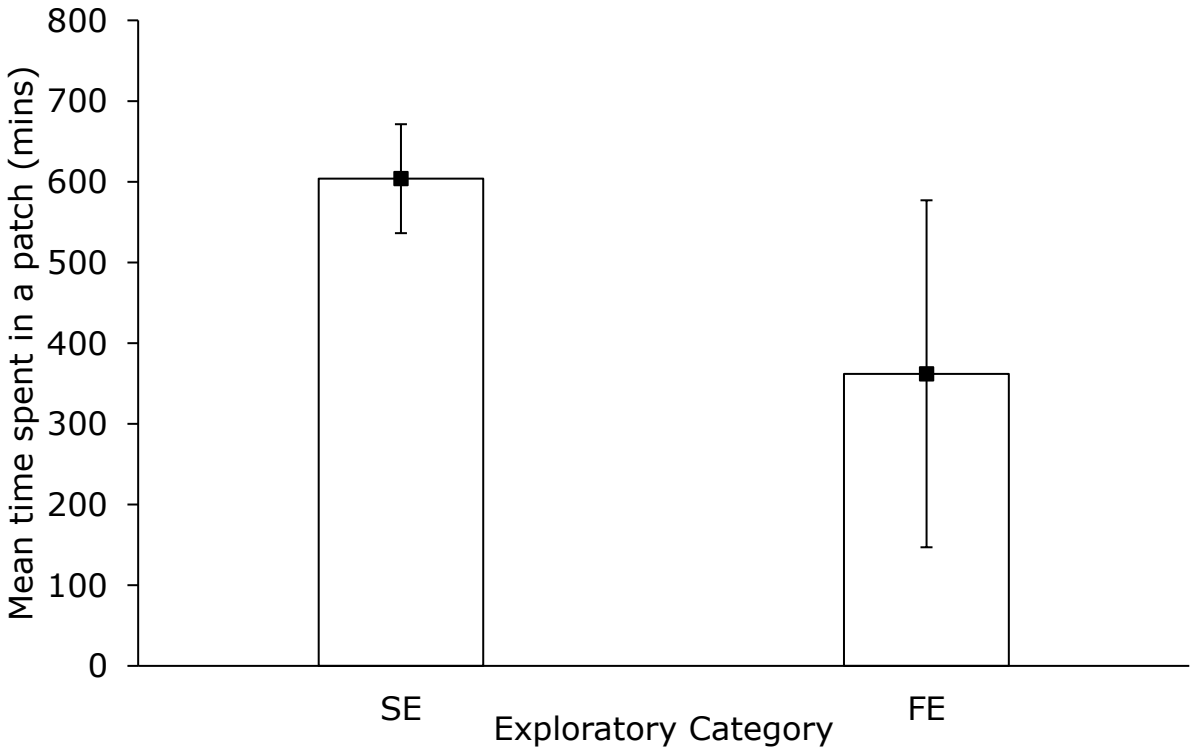

Figure 5.5 – The mean ± SEM of the time spent in a patch before moving according to the exploratory category over the 11-day monitoring period.

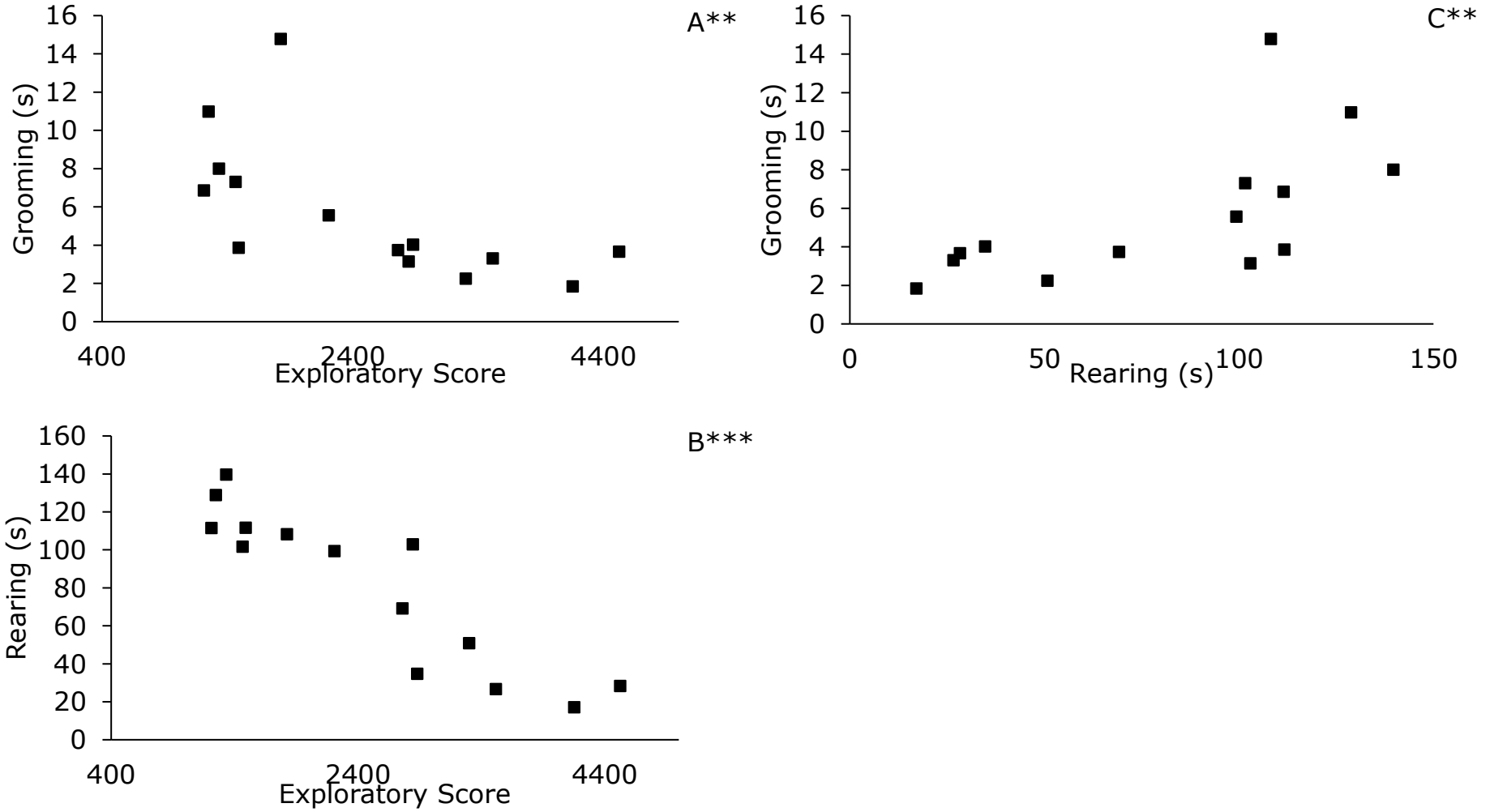

Figure 5.6 – Plots of correlation between the OFT (Open Field Test) variables - A=Negative correlation between the exploratory score and grooming, B=Negative correlation between the exploratory score and Rearing and C=positive correlation between Rearing and Grooming. Plot identifier is followed by significance levels - **=$p \leq 0.01$ ***=$p < 0.001$.

There was a possibility that an interaction between the behavioural types also impacted the recorded movements. Therefore the behavioural types were combined to investigate this (**ASE**=Anxious Slow Explorer, **AFE**=Anxious Fast Explorer, **NAFE**= Non-Anxious Fast Explorer and **NASE**=Non-Anxious Slow Explorer). A one-way ANOVA revealed a significant difference between the combined behavioural types and number of 1m gap crossings ($F(3,10)=9.85$, $p=0.002$). Tukey's *post-hoc* testing revealed that these differences were between the **NAFE** and **NASE**, **NAFE** and **ASE** and **ASE** and **AFE** (Figure 5.7). When the same analysis was undertaken for the combined behavioural types and 2m crossing, no significant difference was recorded ($df=3$, $H=3.37$, $p=0.338$).

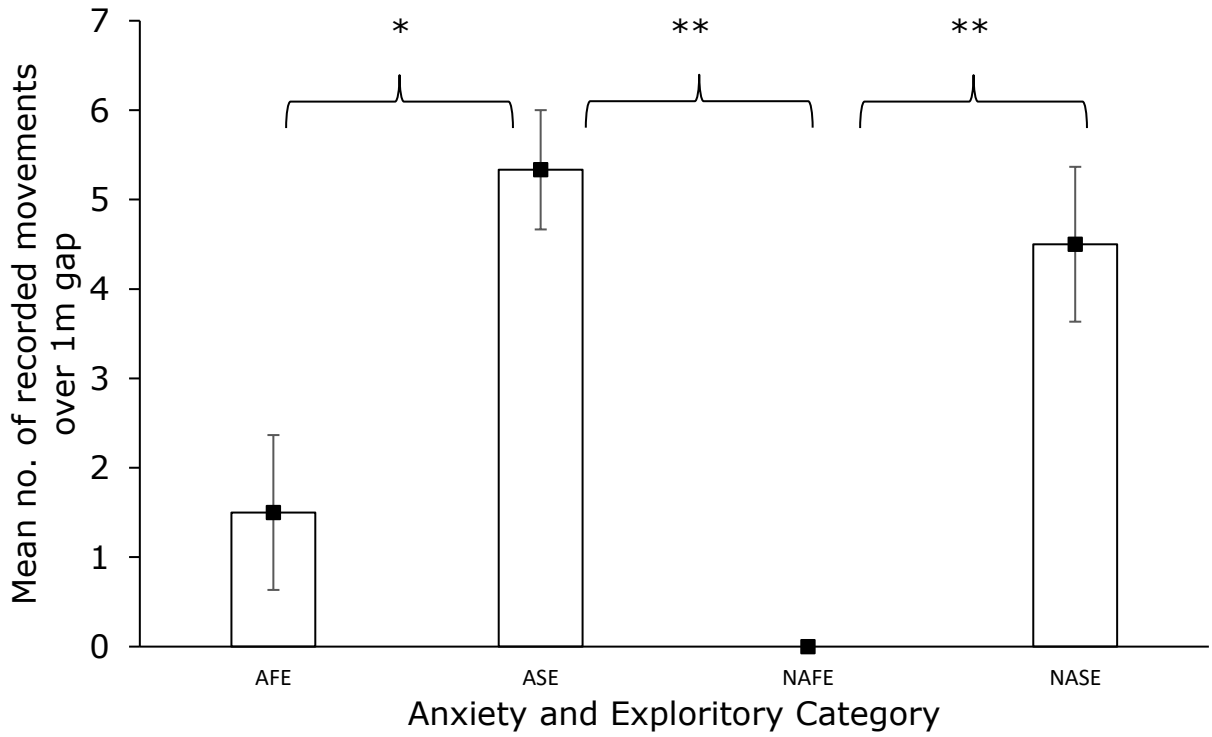

Figure 5.7 – The mean ± SEM of the number of recorded movements for the combined behavioural types. Significance levels - *=<0.05; **=≤0.01.

5.4.5 Comparison between anxiety categories and exploratory category in releases I and II

The data from release I were revisited to determine if anxiety category and exploratory category were linked to movement within a continuous habitat, measured as movement between traps. No significant difference was detected in the number of recorded movements between traps and the exploratory categories ($F(1,5)=2.25$, $p=0.194$). However there was a significant difference in the number of movements between the anxiety categories ($F(1,5)=16.62$, $p=0.010$) (Figure 5.8), with **A** individuals making a higher number of recorded movements than **NA** individuals.

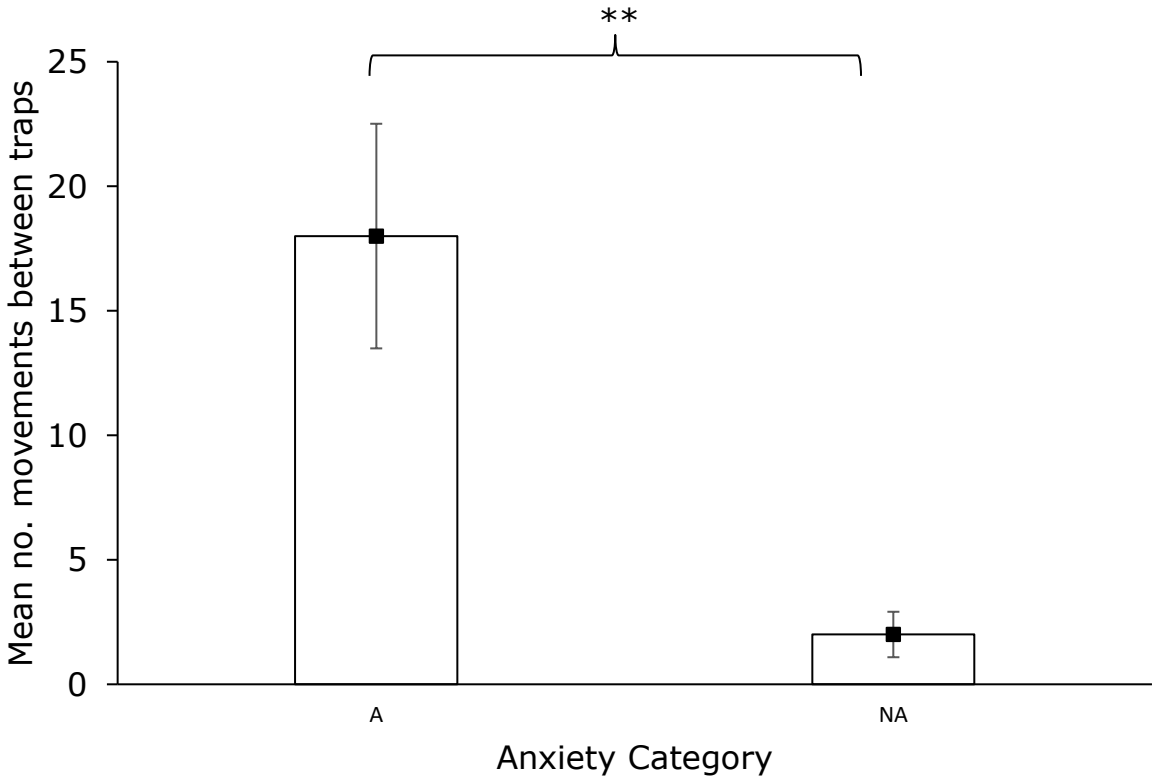

Figure 5.8 – Mean ± SEM of the number of movements between traps recorded for individuals released into a continuous habitat (release I) according to the anxiety category A=anxious and NA=non-anxious. Significance levels - **=≤0.01.

5.4.6 M. minutus activity in fragmented habitats

A one-way ANOVA carried out on normalised data detected a significant difference between releases in the time between traps where no recorded movement occurred in fragmented and continuous habitat ($F(1,13)=4.93$, $p=0.045$) (Figure 5.9), with the time between reads in continuous habitat being longer than in a fragmented habitat. The same significant pattern was not detected when comparing time between reads in individuals visiting different patches/RFID traps (movement recorded) in fragmented and continuous habitats using a one-way ANOVA ($F(1,13)=2.92$, $p=0.111$).

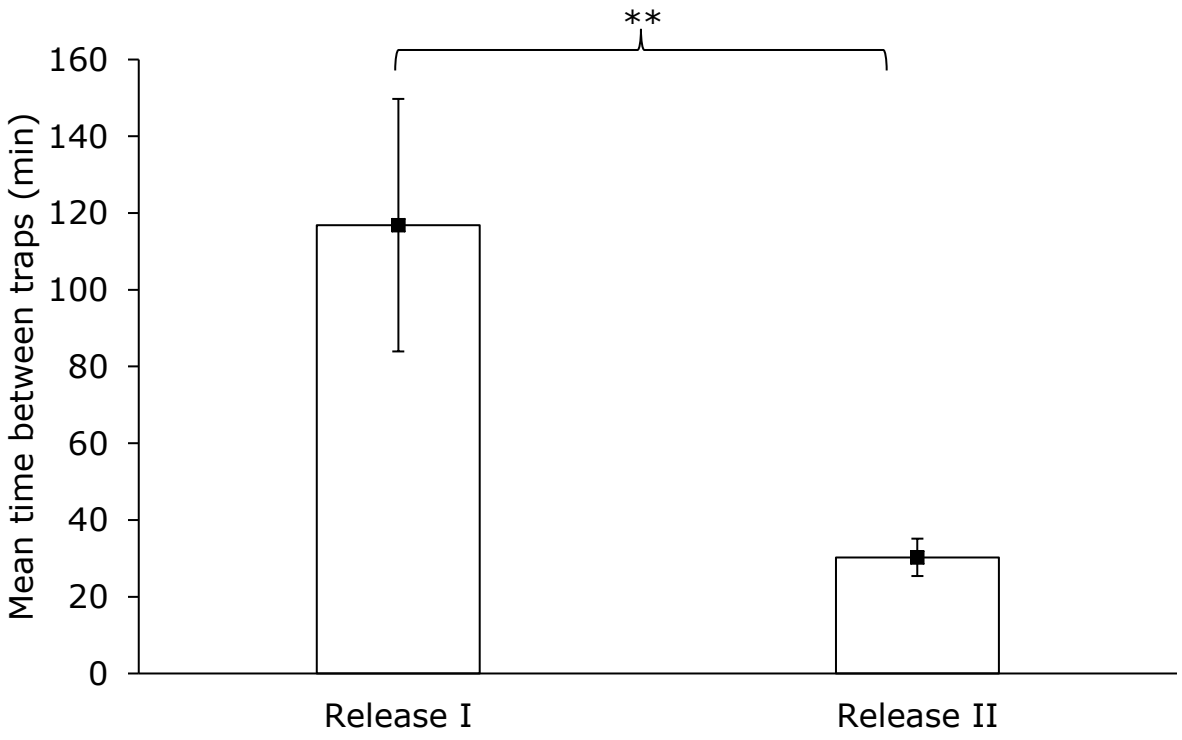

Figure 5.9 – The mean ± SEM for the time between traps for individuals recorded at a single RFID where no recorded movement occurred in continuous (release I; n=7) compared to fragmented habitat (release II; n=14). Significance levels - **=≤0.01.

5.5 Discussion

The utilisation of RFID technology enabled novel movement and activity data to be collected in previously unobtainable detail; the findings are discussed in the following sections.

5.5.1 M. minutus movement in fragmented habitat

The findings presented in this chapter provide evidence that *M. minutus* have the perceptual range, motion and navigational capacity to make successful movements over 1m and 2m gaps in habitat. Hence, it is apparent that they have sufficient capacity and perceptual range to undertake these movements if required to in natural conditions. However, the movement data indicates that as gap width increases, successful movements decrease, which is consistent with Oxley *et al.* (1974) and Grilo *et al.* (2018). Several possible explanations may account for this finding, firstly, the individuals were not attempting to cross the 4.8m gap at all, which could be a result of internal factors, such as the high perceived risk of movement, sufficient resource availability in their current patch, a lack of navigational or motion capacity, or perhaps, reduced perceptual range due to fragmentation and gap size (Nathan 2008). Due to their asociality a less likely possibility is that individuals chose to remain in patches which were occupied by their conspecifics (Doherty and Driscoll, 2018). Grilo *et al.* (2018) found that movement over roads was linked to exploration propensity, and described this as a species trait, where higher movements were undertaken by generalist species. This finding is potentially

inconsistent with the evidence thus far presented within this thesis, whereby exploration is an individual trait as described in Chapter 4. The associations between exploration and movement are discussed in more detail in section 5.5.2.

It is also possible that individuals crossed the 4.8m gap but were not recorded in patch 4 after movement. The area of patch 4 was larger than 1, 2 and 3, thus, the probability of encountering the RFID trap was reduced. This, coupled with the significant reduction in individuals recorded per day after the 4.8m gap was opened, provides evidence which at a basic level may suggest that movement occurred, but was unrecorded. Yet, the patch utilisation shows that patch 3, which adjoined patch 4 was not utilised to the same degree as patches 1 and 2, with the greatest number of traps recorded in patch 2. Accordingly, individuals remained in patches 1 and 2, with only six successfully moving over the 2m gap to reach patch 3, and therefore the likelihood of attempted movements over the 4.8m gap were reduced. This is consistent with Grilo *et al.* (2018) who found that when roads acted as an artificial home range boundary the number of crossings were reduced as the edges were used less.

This finding could also be a consequence of mortality over the course of the experiment, as the findings presented in Chapter Four indicate a reduction in individuals was recorded over the duration of the experiment, with a possible cause attributed to an individuals' behavioural type, specifically non-anxious. Therefore, a similar pattern could have occurred during this experiment and the reduction in individuals recorded after the gap was

opened was merely coincidental. Yet, if this was the case then the non-anxious individuals released as part of the current experiment would have been recorded for a shorter duration over the course of the experiment compared to anxious, yet this was not observed here. In fact, the slow explorers appear to have more favourable survival out of all the behavioural types as these individuals were recorded for significantly longer over the experiment. The impact of behaviour on movement will be discussed in more detail in section 5.5.2.

During pilot testing, two individuals were known to have moved over a 4.8m gap, although the gap was classed as "hard" as the ground cover imitated the hostile surface of a road (see Appendix B). While these data are not included in analyses, it provides evidence that *M. minutus* will attempt to move over a 4.8m gap, even when no vegetative cover is present. It is possible that the hard road surface may have improved perceptual range, as the low vegetative cover present during release II may have inhibited the individuals' ability to navigate successfully over the gap, and rather than providing a less hostile surface to cross. Potentially this created an additional barrier to movement. McLaren *et al.* (2011) found that the provision of trees in the middle of the gap did not prove advantageous for small mammal crossings. Furthermore, Prevedello *et al.* (2011) found that fragmented habitats improved perceptual range as vegetation obstructed visual range. Doherty and Driscoll, (2018) pointed out that while fragmentation can increase the need for an animal to move, it can also increase the chances of successfully locating a new patch.

To summarise, the initial findings indicate that, while small gaps in habitat do not appear to prevent the movement of some individual *M. minutus* and are not proving a total barrier to dispersal, gaps greater than 2m could restrict movement and isolate populations. This may mean that the source-sink habitats which can result within agricultural landscapes, could become isolated with even a 4.8m gap restricting *M. minutus* emigration and immigration from source to sink, and vice versa, which has implications for population sustainability. This would be in line with the findings of Meek (2011), who noted that in areas where suitable habitat was available but lacked connectivity *M. minutus* were not present.

5.5.2 The impact of behaviour on M. minutus movement in fragmented habitats

As described by Spiegel *et al.* (2017), linking behaviour to movement can be helpful when understanding and identifying how species cope with changes in land use and structure as a result of human activities. Here, there were no significant correlations between anxiety indicators and movement between the patches. Therefore at a basic level, successful movement does not appear to be linked to anxiety in fragmented habitats. However, when movement between traps for release I (continuous habitat) were analysed, there were significantly more movements recorded for anxious individuals. The correlation discussed in section 4.4.6 of Chapter Four indicated that anxiety could be used as a predictor of the last day recorded and thus suggested that anxious individuals survived longer. However, the findings presented here do not correspond with this, as the

last day recorded was not correlated in these data. Therefore, it is possible these differences may be related to the divergent responses of behavioural type in continuous and fragmented habitats (Cornelius *et al.*, 2017).

Further analyses indicated that exploration was impacting movement rather than anxiety. A significant correlation between number of recorded movements over 1m gaps and the exploratory score indicated that *M. minutus* had a similar response to fragmented habitat as found in *Pyriglena leucoptera* (White-shouldered Fire-eye) (Cornelius *et al.*, 2017) whereby individuals categorised as slow explorers made a higher number of successful movements between patches and spent longer in a patch before moving. However, Cornelius *et al.* (2017) utilised radio tracking technology to locate the individuals, whereas, in this study individuals could only be located once they visited an RFID trap and therefore, their fate could not be conclusively determined. Accordingly, it is not possible to provide definitive conclusions as to whether the fast exploring individuals, made fewer movement attempts, or were taking higher risks during movement, which resulted in predation. Nevertheless, these findings are consistent with Guillette *et al.* (2011) who found that the slow behavioural type would have an advantage in unstable environments, in this case fragmented habitats, which may be related to this behavioural type making slower but more accurate decisions (Koolhaas *et al.*, 1999; Guillette *et al.*, 2011). During the OFT the slow explorers spent longer engaging in risk assessment, and post-release these individuals spent longer in each patch before moving, suggesting that they were taking more time to learn about their

environment and when they did move they made a higher number of successful movements as per Cornelius *et al.* (2017). A Fast behavioural type would be beneficial in stable environments, where faster learning and higher risks would result in quicker payoffs (Koolhaas *et al.*, 1999; Guillette *et al.*, 2011; Sih *et al.*, 2012).

Memory-based movement is a fundamental element of navigational capacity, and it is thought that fitness benefits of memory-based movements are higher in habitats with intermediate levels of fragmentation and lower in continuous and highly fragmented habitats (Fagan *et al.*, 2013). When this is applied to the results presented in this chapter, coupled with what is known about the fast and slow behavioural types, whereby they tend to spend longer surveying their surroundings, it is possible that slow explorers have a better spatial understanding of their environment and are more likely to take memory-based movements compared to fast explorers, consequently slow explorers may have a better navigational capacity than fast explorers.

There appear to be a series of correlated OFT behaviours which also correlate with movements over 1m gaps post-release. These correlations provide support for Schuster *et al.*'s (2017b) findings that fast/slow behavioural syndromes occur in *M. minutus*. Interestingly, grooming was one of these behaviours, and strongly correlated with 1m movements. Grooming is believed to play a key role in behavioural adaptation to stress (Kalueff and Tuohimaa, 2005), it is a complex behaviour and in rodents can be displayed in both high and low stress situations. There is evidence to

suggest that grooming is displayed as a dearousal from stress, whereby an individual has undergone a stressful event, but has overcome the effects (Rojas-Carvajal *et al.*, 2018). Previously, grooming was thought to indicate anxiety (Kametani, 1988 in Shaw *et al.*, 2007), and while this may be the case in some situations, certain types of grooming may indicate the contrary (Rojas-Carvajal *et al.*, 2018). Here, grooming and rearing (risk assessment) were positively correlated, and both these variables were negatively correlated with the exploratory score and scanning, which could indicate that individuals that expressed higher levels of grooming and rearing were slow explorers and engaged in effective risk assessment, which is typical of this behavioural type. When coupled with the findings of Estanislau (2012) and Rojas-Carvajal *et al.* (2018), these results suggest that individuals that were classified as slow explorers, engaged in effective risk assessment and overcame the stress of the open field arena more rapidly than fast exploring individuals, who were recorded engaging in more superficial risk assessment behaviours (scanning), spent less time grooming and were recorded crossing fewer gaps. Therefore, it is possible that risk assessment may have eased stress levels as effective assessment of threat, or perceived threats was undertaken. When combined with what is known about slow explorers in other species, namely *P. leucoptera* (Cornelius *et al.*, 2017); and *Parus major* (great tit) (van Oers *et al.*, 2004) and when compared to fast explorers , slow explorers make more accurate decisions, spend longer assessing risk and gather more information about their surroundings, there is support for this theory.

The movement recorded in release I and II provides preliminary evidence that *M. minutus* respond to habitat fragmentation according to their behavioural type, with anxiety driving movement in continuous habitat and exploration in fragmented habitats. The combined findings of Chapters Four and Five, where non-anxious seemingly survived less well than anxious (Chapter Four), and where fast explorers did not appear to survive as well as slow explorers (Chapter Five), it is possible that the combined effects of non-anxious and fast explorers behavioural types may have resulted in individuals being maladapted to both continuous and fragmented habitats, and as there were no recorded movements for these non-anxious and fast explorers (Figure 5.7) there is some support for this hypothesis. Further investigation would be beneficial. As previously discussed, early experience can determine behavioural type (Luttbeg and Sih, 2010), and behavioural type is thought to be consistent across age class (Schuster *et al.*, 2017a). It is therefore possible that this potential maladaptation occurred as a result of captivity and persisted as individuals matured; potentially resulting in these individuals seemingly not adapting their behaviour appropriately (Schuster *et al.*, 2017b). These findings, coupled with the findings of Chapter Four pertaining to last day recorded and anxiety category, indicate that preselection of individuals for release maybe important as some traits maybe incompatible with survival in the wild, yet, identifying traits that are beneficial still requires further investigation, these data indicate that the non-anxious and fast explorers combination may be detrimental in terms of

survival and fitness, and it is possible a that this behavioural type would not occur in wild populations.

Maladaptation can be problematic for species, particularly if it leads to overcrowding within a habitat. Interestingly, if source-sink dynamics are applied to the findings of Bence *et al.* (2003), whose conclusions implied that beetle banks provided a higher quality habitat for *M. minutus* as they supported higher densities compared to field margins. However, higher density may actually be a result of overcrowding; as discussed by Remeš (2000), and as Hall *et al.* (1997) noted density may not be the best indicator of habitat quality. These results may suggest that this type of habitat is actually a pseudo-sink, whereby high immigration rates take a source habitat over carrying capacity, therefore appearing as sinks, and thus, could be providing misleading data and could be counterproductive in terms of conservation (Gates and Gysel, 1978; Watkinson and Sutherland, 1995; Dias, 1996; Hall *et al.*, 1997; Remeš, 2000; Bence *et al.*, 2003; Nathan *et al.*, 2008; Meek, 2011). It is important to note that the harsh environment of sink habitats have a role in facilitating adaptation to human induced habitat changes (Dias, 1996; Holt *et al.*, 2004). Thus, the spatiotemporal variability that *M. minutus* experiences each year, may mean that selection pressures are strong for behavioural plasticity and these results may demonstrate that they can adapt their behaviour according to immediate environmental pressures (Gabriel *et al.*, 2005). The habitat patches utilised for data collection within this chapter, were in essence a series of edge habitats, which also may have impacted the results, and it is possible that

predation and parasitism within the release cohort was increased (McCollin, 1998; Krawczyk *et al.*, 2015).

5.5.3 Activity in fragmented habitats

The comparative results of chapters Four and Five could indicate a potential difference in activity when released into continuous compare to fragmented habitat, with time between reads significantly shorter in the fragmented habitat compared to continuous. Therefore, if feeding was occurring whilst using the RFID trap, this may provide preliminary evidence that *M. minutus* adapt their consumer/resource behaviour when exposed to different environmental pressures. There are a number of possible explanations for this. Firstly, in the fragmented habitat, individuals needed to spend longer foraging for food, either as a result of fewer resources due to reduced habitat quality or as a result of increased competition, both resulting in more frequent visits to the RFID traps (Macdonald *et al.*, 2004; Beasley and Rhodes, 2010). Or, individuals were attempting to alter fat reserves, either as a result of increased perceived predation risk in the open environments or before making a dispersal attempt (Gentle and Gosler, 2001; Bowler and Benton, 2005). Alternatively, feeding cues may have been misread within the continuous or fragmented habitats, where the provision of food may have resulted in the fragmented habitat being misevaluated as good, and, an ecological trap was created inadvertently, thus provided misleading cues about the quality of the habitat (Gates and Gysel, 1978). This could have resulted in fewer natural resources available and thus, more frequent visits

to the trap were required to obtain sufficient food when compared to the continuous habitat.

Whatever the cause of the increase in activity in the fragmented habitat, higher activity levels might have resulted in higher susceptibility to predation, and thus, this result could be interpreted as fragmentation having a negative impact on *M. minutus* populations, which is what would be expected. Though this cannot be determined here due to the imperfect comparisons between release I and II. This difference could have occurred by chance or a response to another unrecorded variable which acts as a cue for activity, further investigation in this area is required before conclusions can be made.

5.5.4 Recommendations and limitations

The restricted spatiotemporal scales over which the data were collected for this study have limited the application of the findings somewhat. Accordingly, replication of this experiment is required before the findings can benefit the conservation of *M. minutus* and management of their habitats. Nevertheless, it should be noted that data of this type has yet to be collected on *M. minutus* and was only possible with the use of the RFID traps developed as part of this thesis. Progress in terms of research and the collection of sufficient data for *M. minutus* conservation is likely to remain reliant on suitable technological developments, such methods will inevitably require time and sufficient funds. Therefore, continued funding which

facilitates further validation, that can be used to widen the spatiotemporal scales of the data collection would be highly recommended.

With specific reference to fragmentation, repetition of this experiment is required, which simulates additional impacts of fragmented habitats that are not related to physical barriers, for example human disturbance, traffic avoidance, traffic mortality (Jaeger *et al.*, 2005). The addition of camera traps into the experimental design to identify movement attempts would also be recommended, this would allow greater understanding of the effect of gaps greater than 2m on *M. minutus.* Additionally, a number of direct comparisons in relation to movement data from release I and II were presented in this Chapter, yet, due to differences in enclosure design and sample size, these results need to be interpreted with caution. While these data provide evidence that fragmentation causes behavioural adaptation/maladaptation when compared to continuous habitats, further investigation, which account for these differences is recommended.

As per Chapter Four, it is possible that *M. minutus* behaviour post-release was impacted by time spent in captivity (Bremner-Harrison *et al.*, 2004; Luttbeg and Sih, 2010). Genetic variation during captive breeding can cause reduced fitness in wild-born offspring of released individuals, and generations in captivity can potentially cause genetic adaptations which have negative effects on the released individuals (Frankham, 2008; Araki *et al*., 2009).

The assessment of wild *M. minutus* movement would prove highly beneficial in quantifying variables related to their movement ecology. Also, this study has not considered the impact of genetics on movement and movement propensity, it would be of great benefit to consider this in any future research. As fragmentation is only predicted to worsen in the future, quantifying the impact of inbreeding on the movement ecology of *M. minutus* would be beneficial in terms of their conservation.

It is possible that by artificially testing the effect of fragmented habitats on *M. minutus* could have created an "ecological trap" scenario, whereby individuals were misled by the provision of food and water into assessing what should be poorer habitat, as good quality (maladaptation), impacting movement. Although this experiment was not intended to be long-term, these findings could indicate that this species is susceptible to misreading environmental cues, resulting in relative fitness being reduced, and it is possible that similar maladaptation could occur in wild populations. Accordingly, it would be recommended that additional research is carried out which accounts for this limitation, where RFID technology is used in long-term studies to assess the utilisation of production landscapes by *M. minutus* in greater detail, this could also consider source-sink dynamics and their role in adaptation.

Hall *et al.* (1997) discussed the importance of including habitat features into measures of habitat quality, and quality should be based on the habitat's ability to allow individuals and populations to persist, rather than basing measures of quality on presence and density. Much of the data relating to

habitat suitability for *M. minutus* is based on distribution and density (Harris, 1979a; Sargent *et al.*,1997; Meek,2011), but perhaps the scale at which their habitat has previously been assessed was too broad and re-evaluating this at a more local scale is needed before habitat requirements and quality can be fully assessed for *M. minutus* (Kirol *et al.*, 2015).

It is apparent that reducing gaps size between fragments is likely to be fundamental when managing habitats for this species. However, corridors that have previously been used to connect habitat have not always proven successful as the internal/external drivers behind species movement has not always been considered (Baguette and Van Dyck, 2007; Doherty and Driscoll, 2018). Therefore, simply joining suitable habitats with corridors may not be sufficient, and more information in functional connectivity in relation to behaviour would be necessary and this is certainly an area which would require further investigation.

5.6 Conclusion

Cumulatively, these finding suggest it is possible that *M. minutus* adapted their behaviour when inhabiting fragmented environments, and individual responses to fragmentation may differ depending on behavioural type. Yet, it is also possible that these results may be a consequence of experimental design, increased competition compared to release I, or perhaps an unrecorded variable, so, further research is certainly required. Nevertheless, these results present preliminary evidence that *M. minutus* have sufficient motion and navigational capacity to cross gaps in habitat

≤2m, yet as gap width increased, the number of recorded crossings decreased. Thus, gaps >2m may act as a barrier to movement. The findings also indicate that individuals who express slower exploratory behaviour may have an advantage in fragmented habitats, which may be linked to their navigational capacity (Guillette *et al.*, 2011). Thus, it is likely that movement propensity is an individual trait and can vary depending on behavioural type and individual responses to environmental pressures. These findings also support the bimodal movement response suggested by Fahrig (2007), whereby some individuals will not move from their current patch, while others are prepared to, this response can result in a certain level of resilience to changes in habitat. These finding suggest that if distance between patches is too large, movement may be too risky for some individuals, which can impact population persistence, thus, suitable habitat restoration and management should be implemented where conservation of *M. minutus* is a priority. Furthermore, managing habitats based on movement ecology of *M. minutus* would have benefit for other species that have similar movement capacities and perceptual range as this species.

While there are many potential confounding factors related to this experiment, this chapter presents the first steps towards understanding the spatial, temporal and behavioural responses expressed by *M. minutus* in a natural environment. These data cannot account for the genetic adaptations, it would therefore be recommended that a similar study were carried out on wild caught mice. The progress in terms of monitoring methodology presented here should mean that as the framework for

studying movement ecology progresses, so should the knowledge of *M. minutus* movement, and could be obtainable within similar timeframes as other sympatric species if RFID technology was employed.

Lastly, many of the studies of *M. minutus* distribution and habitat are based on nest density or presence/absence data collected at county or national level (Harris, 1979a; Sargent *et al.*,1997; Meek, 2011), which presents two caveats. Firstly, as per Kirol *et al.* (2015) the scale at which these surveys were undertaken may not have been appropriate for the species. Secondly, it is possible that source-sink dynamics created in agricultural landscape have provided a misleading picture of *M. minutus* populations, as per Sargent *et al.*'s (1997) findings. Therefore, as suggested by Brawn and Robinson (1996) alternative measures should be used to assess populations when source sink dynamics are likely to be present, for example reproductive success and demographics (Brawn and Robinson, 1996; Schaub *et al.*, 2010). Ironically, these measures have thus far been unobtainable in sufficient quantities for *M. minutus* and therein lies the challenge presented by the species; a paradox which has begun to unravel with the development of RFID. Perhaps a different perspective is required for developing *M. minutus* autecology, where aims are scaled back, and the understanding of habitat quality revisited using RFID trapping technology on a longer-term basis. *M. minutus* clearly remain a challenging species to study, yet by approaching these challenges with innovation and originality in the future, undoubtedly further developments will be made.

6 Conclusions

The findings presented within this thesis are the first of their kind for *M. minutus* and could influence future research on their autecology and population restorations, as well as to inform management and restoration of habitats. Several key themes have become apparent within Chapters Three, Four and Five of this thesis which require further investigation. These include, the impact of age on detection (scent surveys and RFID trapping), the impact of behaviour on movement and survival, consumer/resource behaviour, optimal foraging strategies in different habitat types, and the impact of agricultural landscapes on their movement ecology. The novel findings presented within this thesis show that progress has been made in terms of monitoring *M. minutus* and developing knowledge of their movement ecology.

6.1 Novel method effectiveness

There is strong evidence that a dog can be trained to detect *M. minutus* and discriminate their scent from other sympatric non-target species in a controlled training environment. When applied to uncontrolled field situations, the remote scent survey proved more effective than nest search surveys by volunteers during the autumn months, which provides preliminary evidence that olfactory indicators could be more efficient than visual clues to establish presence of *M. minutus*. Although these results are encouraging, additional validation in uncontrolled settings is required as there was not sufficient data to draw full conclusions here. Definitive

conclusions on the effectiveness of this method across situations would have been ideal, but the availability of a single detection dog has limited the conclusions that can be made within this thesis. Nonetheless, as far as can be determined from the literature, this is the first attempt to train a dog to detect *M. minutus*, which was a challenging task and the success was reliant on the aid of a specialist dog trainer, novel ideas and approaches, financial support from Moulton College and the PTES and a great deal of patience from the researcher. As this was the first experience which the researcher had of training a dog for this purpose, these findings are encouraging in terms of skill required for future application of this method.

Encouraging results were also observed during validation of the use of RFID for monitoring *M. minutus.* Here RFID trapping was shown to have better results in terms of raw trapping rates over live trapping. A fundamental element of RFID trapping is the PIT tag, which required an animal to be caught before it can be fitted, which highlights a key drawback to the method. Yet, once a PIT tag has been fitted, trapping rates were 27% higher compared to live trapping, but, as with the remote scent survey method, RFID trapping also requires further validation.

There was a possible bias in trapping rates in favour of the anxious individuals. Whether this bias is related to non-anxious individuals experiencing a higher mortality rate, utilisation of natural resources, escape from the enclosures or another unrecorded factor is not clear from these data, nonetheless the findings of Chapter Four indicated that live trapping was no more effective at trapping anxious or non-anxious individuals.

Consequently, the relative effectiveness of RFID should not be underestimated as the detailed behavioural and ecological data that were collected using the RFID are entirely novel and have provided direction and vision for future research.

Combining the remote scent survey and RFID trapping methods could prove beneficial. Firstly, the RFID traps could be used for scent collection and thus validate the effectiveness of the detection dog. Secondly, the scent survey could be used to pin point suitable locations to undertake the RFID trapping. With additional validation, the novel methods presented within this thesis are likely to offer advantages over standard monitoring methods, such as live trapping and nest search surveys. Yet, there are some key caveats to consider. Firstly, the cost of the remote scent surveys and RFID trapping is somewhat prohibitive, while investment would prove beneficial, undertaking further validation of these methods will require sufficient resources and expertise to be able to achieve this. Secondly, each of the tested methods required individual *M. minutus* to visit either a feeder or an RFID trap, which does not entirely move away from the current standardised live trapping methods. The results presented within this thesis suggest that this requirement may present additional complexities, namely, the design of the feeders/RFID traps may not be appropriate across all situations and may depend on behavioural type. Additionally, the presence of other species may confound the result; either by depleting the resources or deterring *M. minutus* from entering. Lastly, individuals may not encounter a feeder as they are too widely spaced. Preferably, in the future

it may be possible to monitor their movements using Global Positioning Systems (GPS), which would significantly improve data collection, yet, this would require progress in terms of reducing the weight of a GPS unit, while retaining battery life, not to mention significant financial support.

6.2 *M. minutus* movement between patches

This thesis presents the first detailed data relating to *M. minutus* movement ecology. The findings indicate that they have sufficient navigational and motion capacity to successfully move over gaps ≤2m, consequently, gaps greater than 2m could limit their movement with possible implications for population persistence. This supports the findings of Meek (2011) and Kuroe *et al.* (2011) who found that habitat connectivity is likely to be a key feature of habitat quality for this species. Information on habitat quality is particularly important for species such as *M. minutus* that face the ever-increasing threat of human induced fragmentation and changing land uses (Kirol *et al.*, 2015).

These findings also suggest that individuals that explore more slowly (slow explorers) may have an advantage when inhabiting a fragmented habitat, which corresponds to the findings of Cornelius *et al.* (2017), with slow explorers engaging in higher risk assessment and spending longer in a patch before moving. Cumulatively, these conclusions indicate that movement propensity is an individual behavioural trait which may vary depending on environmental pressures within habitats, and thus, presents a potentially

novel perspective on their conservation, where conservation decisions are based on behaviour rather than density.

6.3 *M. minutus* conservation and habitat management

Hall *et al.* (1997) noted that habitat quality should not necessarily be based on presence, absence or density of a species, but the features that make it possible for individuals and populations to persist. Therefore, it is possible that methods trying to elucidate population numbers for *M. minutus*, either based on presence/absence or live trapping data should in fact be less of a priority than determining what makes quality *M. minutus* habitat.

Furthermore, the findings related to movement data collected during release I and II and presented in Chapter Five indicated that a bimodal model described by Fahrig (2007) is applicable to *M. minutus*, whereby some individuals are prepared to move, and others are not. This tendency to move may depend on habitat type and behavioural type. Therefore, it could be possible to predict population viability based on the behavioural types present within a habitat and implement conservation measures accordingly. This may be particularly useful to mitigate acute human induced changes to habitat, for example, the findings presented here indicate that slow explorers may be more willing to move between habitat patches, and thus, if a fragmented habitat supported a higher proportion of slow explorers, it may be more efficient to focus conservation resources on improving habitat quality. If fewer slow explorers were present and movement was less likely, emphasis on improving habitat connectivity

maybe more appropriate. This suggestion would rely on behavioural type being present in wild populations of *M. minutus* and an equal probability of capture between the behavioural types, both of which certainly needs further investigation as it is not clear if the fast explorers were choosing not to move, using the RFID traps less or experienced higher mortality as a result of less effective risk assessment. Nevertheless, this approach may provide a cost effective and interesting alternative when prescribing conservation measures based on limited data.

6.4 The future for *M. minutus* in a changing world

To account for the different behavioural types and their potential responses to habitat type, quality and potentially season, it is possible that monitoring methods may need to be flexible in terms of trap design and scale depending on connectivity/season. Thus, a combination of methods may be more useful than a standardised blanket approach, and as discussed previously it is possible that methods not only need to be species-specific but habitat and behaviour specific as well. Based on the evidence presented in this thesis monitoring over finer spatial scales and coarse temporal scales should be investigated as much of what is known about the species distribution is based on large-scale nest density data, which may not be appropriate for *M. minutus*.

Whilst long-term monitoring has been recommended, there are also several research areas that would require fewer resources and could be completed over a shorter timeframe. These include optimising trap design to reduce

the impact of other sympatric species and maximise the use by *M. minutus*, identifying a more appropriate finer scale survey design to account for individuals that are less willing to move, or to develop a procedure to assess behavioural type in wild populations.

It is apparent that there is scope to improve the remote scent surveys and RFID trapping methods, and while each method has its limitations, the data collected for this thesis demonstrates that progress has been made in terms of monitoring *M. minutus* and each chapter of this thesis has presented data which has been entirely novel for this species. Nevertheless, they remain a challenging species and more questions have been asked within the thesis than can be answered, however the sum of this work has provided a clear direction for future research on *M. minutus.*

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8 Appendices

Appendix A – Pilot I testing the prototype RFID traps

To establish if the prototype RFID traps were functioning to the correct specifications and to test the trapping protocol, a pilot study was undertaken. This also allowed other issues relating to the release methodology to be addressed before data collection.

An area of the Moulton College estate, known as Briscoe's Spinney was selected as the most suitable location for Pilot I. This area was in close proximity to agricultural land which was included in Entry Level Stewardship (ELS), but was not a commercial element of the stewardship, allowing restricted public access and habitat protection. The location of the release pen can be seen in Figure 8.1.

During pilot I, 50 *M. minutus* were released into an area of newly planted woodland on the Moulton College estate. Each *M. minutus* had been microchipped in accordance with the protocols described in section 2.5.3. The release took place on the 25th August 2013 into a purpose-built enclosure (Figure 8.2) (10 x 14 m) (52^{o}18'13.29 – 0^{o}52'37.63). This enclosure surrounded suitable *M. minutus* habitat. Principal floral species included bushgrass (*Calamagrostis epigejos*), cocksfoot (*Dactylis glomerata*) and false oat grass (*Arrhenatherum elatius*).

Figure 8.1- Aerial view of the 2013 release site (Briscoe's Spinney), pink line depicts release enclosure, not to scale (Google EarthPro,2016)

Figure 8.2 - Black agricultural plastic surrounding *M. minutus* habitat where pilot I was undertaken.

To minimise the escape opportunities of the released *M.* minutus, black plastic agricultural sheeting was used as the perimeter fence, approximately 1m high, supported by hazel (*Corylus avellana*) stakes on the exterior (Figure 8.2). Plastic was fixed using staples and duct tape. In order to further minimise the individuals escaping, the plastic sheeting was buried underground approximately 5cm, and vegetation closest to the fence was cleared, over hanging branches were removed.

Six RFID traps were available for pilot I. Each RFID trap was fitted to a plastic bottle (380 ml), with the reader fitted over the entrance (Figure 8.3). Their normal Johnson and Jeff Parakeet (Johnson and Jeff, Gilberdyke) feed mix was provided inside the bottle, and as with live trapping, this acted as bait (Flowerdew *et al.*, 2004). The individuals' microchip would be read when they passed through the entrance of the bottle and under the reader, and the data wirelessly sent to the data logger. The RFID trap batteries were changed every 12 hours. Aerocell AA batteries (Lidl, Neckarsulm) were used as the battery life was superior to other brands.

M. minutus were released into the enclosure pictured in Figure 8.2 from a single soft release enclosure, which remained throughout the experiment, offering protection from predation and the elements. To monitor the use of this resource over the experiment, a RFID trap was fitted above a single entrance/exit point.

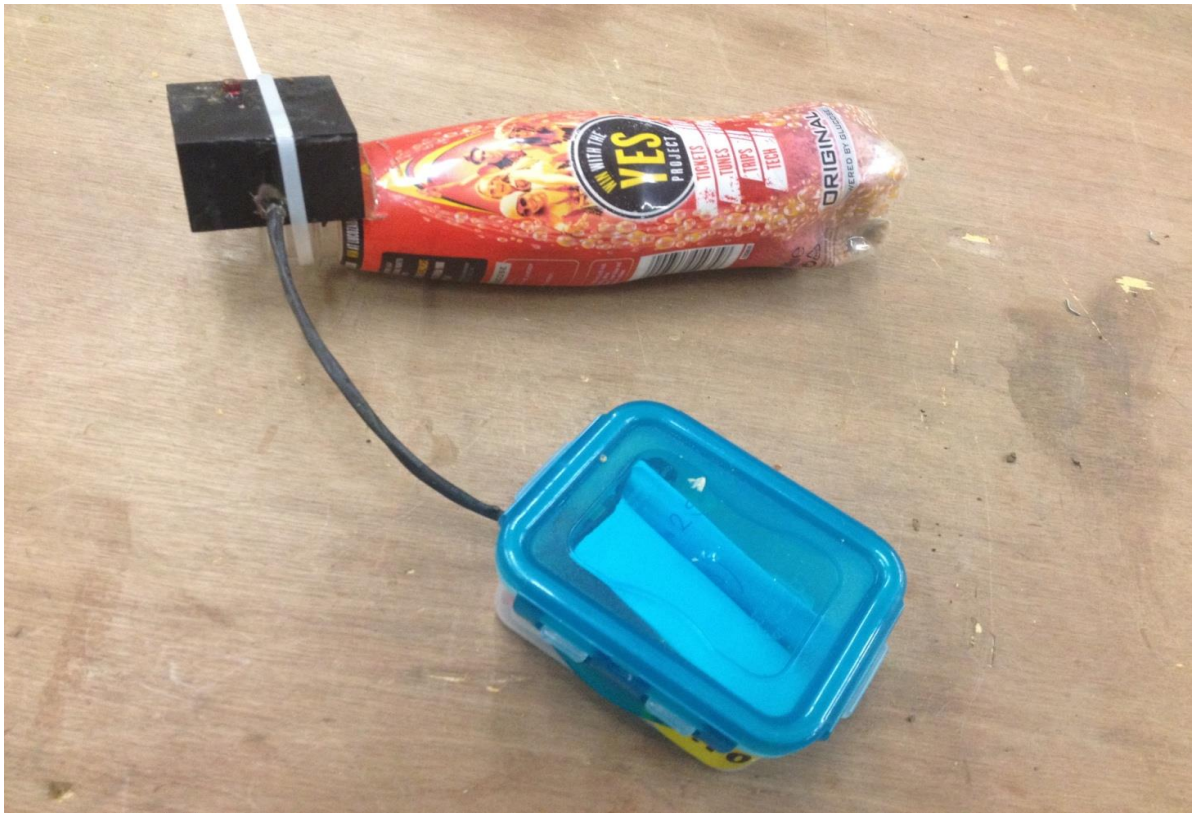

Figure 8.3 - prototype RFID trap. The electronic reader can be seen fitted to the entrance of the bottle and to protect the remaining components these were housed within a waterproof Tupperware box.

A Bushnell Trophy trail camera was set up within the enclosure to observe the *M. minutus* behaviour, the camera trap recorded footage of nest building and intra-specific interactions were observed. A Dell Laptop computer running a Linux operating system was required to extract the data from the SD card. A terminal interface was required, and the specific commands were run to extract the data.

Nest searches were undertaken in all suitable habitat within a 500m radius of the release site between September 2013 and January 2014. These searches were not formal surveys as no time limit was adhered to as per

(The Mammal Society,2013). Ideally, nest searches would have been undertaken the year before pilot I to if establish *M. minutus* were present in the area beforehand, however this was not possible in this scenario. The findings relating to the functionality of the prototype RFID traps and additional results which were useful for streamlining the methodology have been within section 2.5.11. None of the pilot data were used in any of the analyses undertaken in Chapters Three, Four and Five.

Appendix B – Pilot II testing updated RFID traps and *M. minutus* movement over a hostile surface.

Improvements implemented to the RFID traps as per the findings of pilot I were tested during pilot II (Figure 8.4). Initially this study had been devised to contribute to the validation of the RFID method within Chapter Four, and to the movement data in Chapter Five, however technical issues with the data logger meant these data were inconsistent and would not have provided reliable results. Therefore, this study facilitated the testing of PIR motion sensors which had been fitted to the RFID traps and allowed feasibility testing of the aims relating to *M. minutus* movement ecology.

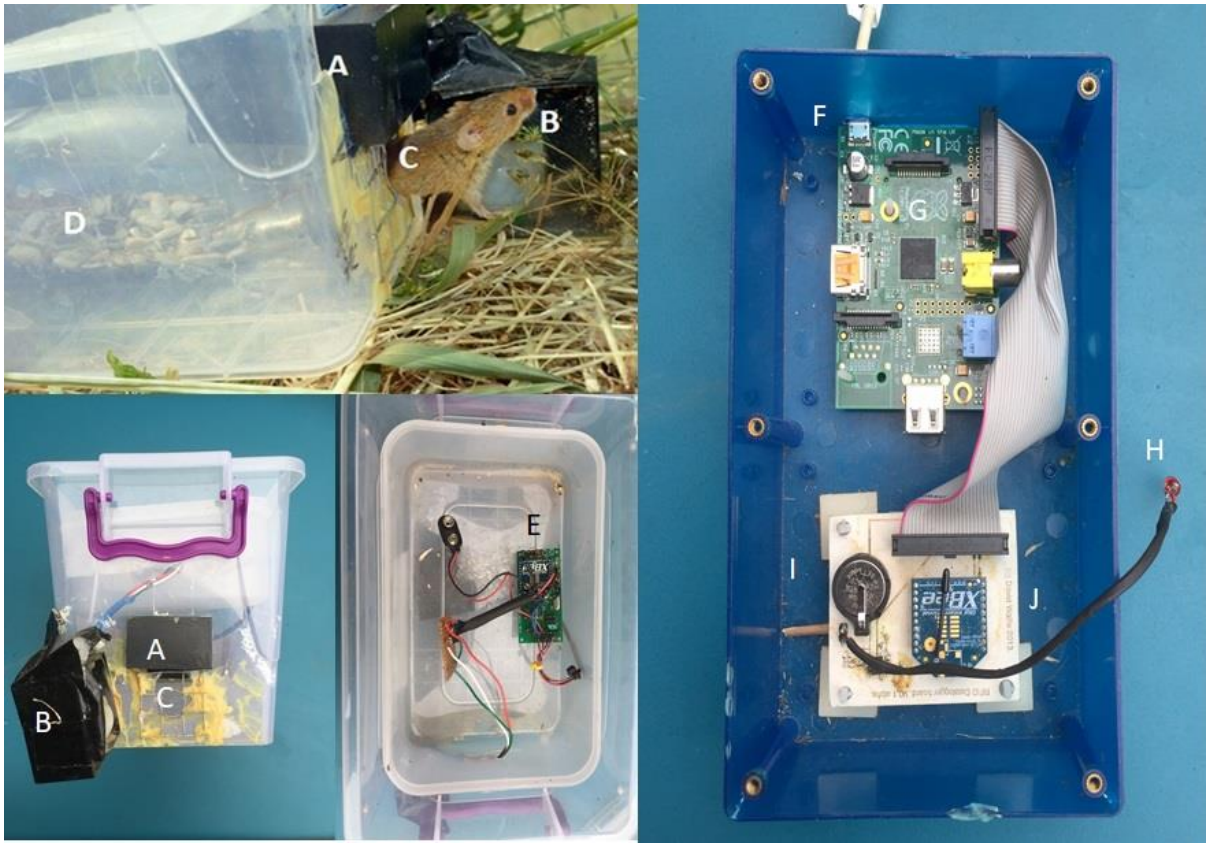

Figure 8.4 - RFID electronic trap with *M. minutus* exiting, electronic trap and data logger. Components include: electronic reader (A), PIR motion sensor (B), trap entrance (C) and baited plastic drinks bottle (D), electronic trap printed circuit board components, this view shows the top of the trap, not accessible to the occupants (E), data logger (F), Raspberry Pi (model A) ® (G), LED data transmission indicator (H), real time clock (I) and Master Xbee radio (J) - (*M. minutus* picture courtesy of Upton (2015)).

To incorporate the movement aims, a second release pen was constructed between May and August 2014 at Bennie's Quarry, Pitsford (Figure 8.5) ($52^{0}17'40.68$ – $0^{0}53'29.50$). Dimensions were 2.4m x 58m, based on the dimensions of the Trakmat used as artificial road surface. Ecofender newt barrier (Hy-Tex, Ashford) fencing was used to enclose the area. Wooden stakes (37x37x1200mm) were placed every 1.5m to secure the newt barrier, fencing was fixed with staples and duct tape. Two cross sections of

artificial road were installed, 4.8m and 7.2m; similar to standard road widths (Murphy,2011). TrakMat® (Marwood Group, Northampton) was used as an artificial road surface, each mat was 2.4 x 1.2m, 10 were needed in total (Figure 8.6). The 4.8m section required four mats, while the 7.2 m section required six. To prevent *M. minutus* crossing the gap under the Trakmat®, roofing felt was placed under the ground at the end of each road.

Figure 8.5 Aerial view of second release site (Bennie's Quarry), pink outline depicts location of enclosure, not to scale (Google Earth, 2015).

To maximise movement data 17 trapping points were identified every 2.5m through the centre of the enclosure, starting at the soft release pen at point A (Figure 8.7), finishing at each road section, commencing on the other side of the road at the same intervals. As habitat was damaged by the inclusion

of a trapping point, 2.5m was thought to be an optimum distance to collect data but to minimise habitat loss. A key drawback here was the range of the Xbee radios was limited to around 10m, which significantly limited the placement of the RFID traps, and resulted in the RFID traps being rotated throughout the enclosure rather than simultaneously monitoring in all of the patches.

Figure 8.6 - Trakmat® utilised to simulate the hostile surface of a road.

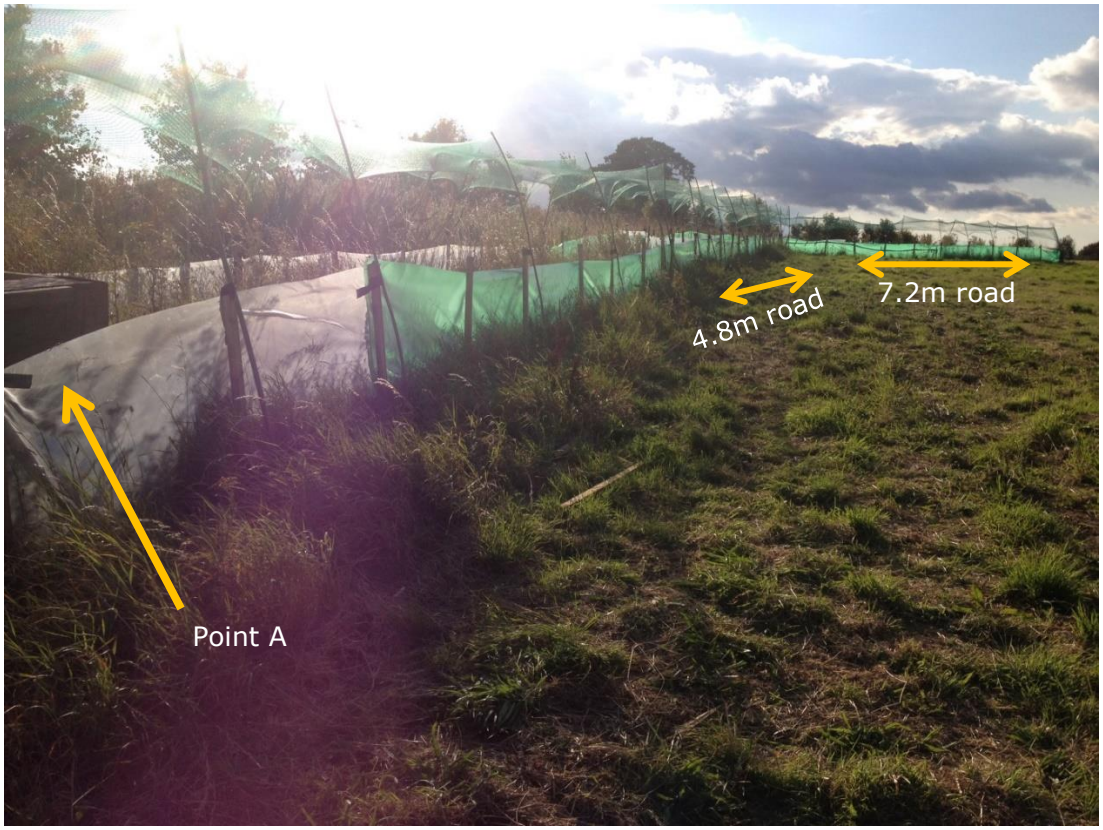

Figure 8.7 – Identifies the position of the soft release pen and the release point (point A), 4.8 m road and 7.2 m road.

To maximise data live traps (Longworth and Plastic Trip Traps) were used for the first 3 days 24/08/2014-26/08/2014. These were placed on plastic boxes 20 cm above the ground, accessible by surrounding vegetation. The RFID traps were placed on the ground as this was how the traps were presented to *M. minutus* when in captivity. RFID traps were utilised for trapping the released *M. minutus* at various times between 24th August 2014 and 2nd October 2014.

At the beginning of the experiment the 4.8m road was blocked off for two days to prevent crossing as a response to a novel environment. Data were

downloaded once a day, batteries were also changed once a day. Technical issues prevented a consistent approach to trapping.

Appendix C - Controlled field testing of the remote scent survey method

The detection dog correctly responded to five of the six samples tested during the controlled field testing. The sample that was missed had a recorded visit by a *M. minutus* however only three reads were recorded by the RFID trap, therefore it is possible that the scent was not strong enough for the detection dog to correctly identify the scent. Furthermore, the detection dog took three runs to identify one of the other confirmed samples. This would have been the first occasion where the target scent had potentially been contaminated with non-target odour. These outcomes suggested it was important to carry out discrimination training, which was implemented, and a formal test completed at the end of the training.

Table 8.1– Results of controlled field tests of the scent survey method using RFID to confirm *M. minutus* presence.

No. of samples tested	No. correct responses	False	Missed
3	3	0	0
2	1	0	1
1	1	0	0

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