Badger-Cattle Interactions in the Rural Environment: Implications for Bovine Tuberculosis Transmission

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Declan T. O'Mahony

Sustainable Agri-Food Sciences Division

Agri-Food and Biosciences Institute, Belfast

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Contents

Executive Summary

1. An integrated research project was conducted on badgers (*Meles meles*) and cattle in Northern Ireland to provide data on the ecology of badgers; quantify the level of direct interactions between badgers and cattle at pasture; and determine the frequency of badger visitation to farmyards and buildings. The project was funded by the Department of Agriculture and Rural Development.

2. The project took place during 2012 and 2013 in a 1,350ha study area in Co. Down that had a regionally high incidence of bovine tuberculosis (bTB) in livestock. The study area was dominated by grassland (68.24%) and arable habitat (20.58%), with relatively little woodland cover (2.25%) and 3.19% of the area consisting of buildings (either farm or domestic). The size of cattle herds varied from <20 animals to >200 animals.

3. A range of ecological studies were carried out including sett surveys, bait-marking and live-trapping to determine the ecology of badgers within the study area. Additionally, Global Position System (GPS) collars that recorded animal locations were deployed on badgers and proximity collars that recorded interactions between cattle and badgers were used.

4. Badgers had a territorial system of social organisation with a mean territory size of 117.80 ± 11.28ha. Estimated minimum social group size varied from 2 to 12 with an overall mean of 3.73 ± 0.95 badgers per social group. Total population abundance estimates were 50.66 badgers (95%CI 29.35-85.26) in the study area or a mean density of 3.62 badgers per km^2. Using GPS derived data from badgers (n=9), mean nightly movement distance was 1,321 ± 34.5m and the maximum was 6,541m.

5. Proximity collars were used to provide the first quantifiable data on the level of inter- and intra-group interactions between free roaming badgers and cattle on the island of Ireland. Data were available from a maximum of 92 cattle and 15 badgers, across several different herds and social groups, and 439,776 interactions were available for analysis. No direct close-range interactions (≤2m) occurred between badgers and cattle during the study. Intra-group contact rates within cattle herds and badger social groups showed considerable variation according to month, herd/group, climate variables and at the individual level.

6. Badger activity and visitation rates to farm buildings in 11 randomly selected farmyards were quantified between July 2012 and August 2013. Motion activated infra-red cameras were deployed at a maximum of 83 locations of potential badger entry, at a range of building types. Badger visits occurred on at least one night in 9 out of the 11 farmyards and during a maximum of 16.13% of surveillance nights in any single farmyard. There was significant variation in badger visits according to farmyard, with a peak during spring and visits were inversely related to temperature. In farmyards that had badger visits on 10 nights or more (n=4, 36.36%), 75.28% of surveillance images were captured from a single camera location only. Badgers positively selected feed stores and significantly avoided cattle sheds during visits to farmyards. Other important species that visited farm buildings included domestic cat, rodents and red fox.

7. This project aimed to quantify potential bTB transmission routes between badgers and cattle in an area of Northern Ireland with a regionally high incidence of bovine tuberculosis (bTB) in livestock. The results of the study indicated that direct contact between badgers and cattle at pasture did not occur and was likely to be a rare event in terms of bTB transmission. In terms of reducing potential bTB transmission between badgers and cattle it is important to concentrate further research into the management of indirect contact venues including setts, latrines, troughs and limiting access to farm buildings.

Acknowledgements

Introduction

1.1   General Introduction and Background

Globally, wildlife-livestock disease dynamics are a major issue with considerable resources placed into ensuring livestock, and livestock products, are available for export (Jones *et al.* 2008; Paton *et al.* 2010; Ferguson *et al.* 2013). Management strategies to minimize interspecific disease transmission between wildlife and domesticated animals can include manipulation of disease agents, cordoning off wildlife areas, removal of wildlife from non-protected areas, vaccination, movement controls, establishing infection zones, and removal of livestock from areas where wildlife graze (Wobeser 2002; Kilpatrick *et al.* 2009; Ferguson *et al.* 2013). Important recent achievements include the eradication of diseases such as rinderpest (Morens *et al.* 2011) but significant issues at the wildlife-livestock interface remain including peste des petits ruminants (Anderson *et al.* 2011), brucellosis from free-ranging cervids and bison to cattle (Cheville *et al.* 1998) and tuberculosis from wildlife to cattle (Barlow 1991; Corner *et al.* 2008a; Hone and Donnelly 2008). Disease surveillance represents a mutually embracing objective; that is to develop effective surveillance and management practices in wildlife whilst ensuring healthy, sustainable wildlife populations (Rhyan and Spraker 2010). Wildlife disease monitoring is largely in its infancy and there is generally a lack of targeted surveillance (Artois *et al.* 2009). Disease management can be particularly complicated as multiple hosts and a range of dynamics can occur (Gortázar *et al.* 2007).

In the UK and Ireland, the Eurasian badger (*Meles meles*) is a group living, social member of the Family Mustelidae. The species is considered an important component of the maintenance and transmission of bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, to cattle although the direction of transmission can also be the reverse (Woodroffe *et al.* 2006a; Hone and Donnelly 2008). Widespread badger culling has occurred to address this issue with interventions such as the Randomised Badger Culling Trial (RBCT) in England (Krebs *et al.* 1997) and culling in the Republic of Ireland (see section 1.2). Outcomes have varied according to jurisdiction, although culling trials have generally shown reductions in bTB in cattle in response to proactive control. Increases in bTB found in periphery areas were found in British studies but have not been recorded in Irish studies (Woodroffe *et al.* 2005; Woodroffe *et al.* 2006b; Corner *et al.* 2008a). Increasingly badger vaccination campaigns are being implemented to address this significant disease issue (e.g. Cater *et al.* 2012) with cattle vaccination also a developing field (Buddle *et al.* 2013).

1.2   Background to Bovine Tuberculosis Management on the island of Ireland

Since the 1970s, badgers have been implicated in the transmission of bTB to cattle on the island of Ireland (Noonan *et al.* 1975). Bovine tuberculosis is an important animal health disease that has significant cost implications for public expenditure. Testing of livestock for bTB was initiated in the 1950s with annual testing of all eligible individuals in every herd taking place in line with EU requirements. This is achieved primarily through use of a single intradermal cervical comparison test (SICCT; tuberculin test) in conjunction with gamma interferon testing (IFNG) (DAFM 2008). Test positive animals are culled from herds and restrictions are placed on herd movements until at least two clear tests are identified within a period of approximately 4 months (Duignan *et al.* 2012). The annual costs for implementing bTB controls and management across the island of Ireland are in the range of £60-80 million / €70-95 million, with the majority of costs associated with compensation for culled animals and testing (DAFM 2008; NIAO 2009; Duignan *et al.* 2012).

In terms of context it is important to consider that the Eurasian badger (*Meles meles*) is a mammal native to Ireland, with its likely origin through natural or human-mediated colonization from refugia in Spain or Scandinavia (O'Meara *et al.* 2012). Increasingly it is being recognized that the species has developed a near 'niche' ecology in Ireland with important differences between conspecifics in other parts of its range in terms of ecology, behaviour, density, diet and reproductive biology (Byrne *et al.* 2012a). It is a strictly protected species with direct interference to individuals or its places of refuge being illegal. Badgers are also listed in Appendix III of the Bern Convention on the Conservation of European Wildlife and Natural Habitats as a species in need of protection but may be exploited in exceptional circumstances. It is estimated that there are approximately 110,000-148,000 badgers across the island of Ireland (Sleeman *et al.* 2009; Byrne *et al.* 2012a; Reid *et al.* 2012)

There are different management strategies enacted in both political jurisdictions (Republic of Ireland (ROI) and Northern Ireland (NI)) of the island of Ireland (Ireland) with respect to the role of badgers in the spread of bTB. The ultimate overall aim is to eradicate bTB from Ireland. In ROI, since the late 1980s there have been major wildlife intervention studies that have involved proactive and/or reactive culling of badgers over large areas in response to farm bTB outbreaks. These have indicated that such interventions can reduce the incidence of bTB in cattle (O'Mairtin *et al.* 1998; Eves 1999; Griffin *et al.* 2005a; Griffin *et al.* 2005b). Further studies have largely mirrored these findings, indicating that reductions in badger density can lead to reduced incidence of bTB in livestock herds (Corner *et al.* 2008a; Kelly *et al.* 2008; Olea-Popelka *et al.* 2009). Current bTB management strategy in ROI is based on

reactive badger culling in areas with chronic bTB levels in livestock, reducing the national badger population by 25-30% and extensive trials of vaccines (O'Keeffe 2006; Corner *et al.* 2008b; Byrne *et al.* 2012b). It is estimated that between 4,000-6,000 badgers (mean 2005-2010 = 5,976) are reactively culled every year in ROI in response to local bTB outbreaks (Anon 2011). As of late 2013, the herd incidence of bTB in cattle in ROI was 3.55%, with a decreasing trend. It is important to emphasize that the control of bTB is part of a multi-faceted strategy involving testing, diagnostics, movement restrictions, farm biosecurity and badger culling. Apportioning causation for the decline in bTB levels to any single factor (e.g. badger culling) is currently not possible.

In NI, the bTB control programme involves stringent testing, culling of test positive livestock, movement restrictions, compensation and increased biosecurity measures. Widespread badger intervention studies have not occurred in NI, with research primarily focused on ecology, population enumeration and biosecurity (Feore and Montgomery 1999; Reid *et al.* 2012; O'Hagan *et al.* 2013). Specifically related to the role of badgers in the transmission of bTB to livestock, the presence and activity of badgers on a farm has been shown to significantly influence herd-level infection (Denny and Wilesmith, 1999; Abernethy *et al.* 2006; Menzies *et al.* 2011). Over 170 different *M. bovis* genotypes have been identified in NI, which display significant geographic clustering (Skuce *et al.* 2010) with some genotypes being potentially more virulent than others but not influencing the overall size of herd breakdowns (Wright *et al.* 2013). It has also been shown using whole genome sequencing that there is a close relationship between strains of *M. bovis* found in badgers and cattle at a very local scale (Biek *et al.* 2012). The bTB status of badgers in NI is primarily assessed through ongoing bacteriological culture of samples obtained from post-mortem of badgers that have been killed by traffic, with an average disclosed infection rate of 15.2% from 1999-2010 (Courcier *et al.* 2012). As of late 2013, the herd-level incidence of bTB in cattle in NI was 6.23%, with a decreasing trend.

To address the role of badgers in the epidemiology of bTB in NI, a five year Test Vaccinate Release (TVR) research project that involves the capture, testing and subsequent release with vaccination of Bacillus Calmette Guerin (BCG) or euthanasia of test positive animals is proposed. The underlying premise is that removing bTB infected individuals and vaccinating the remaining population will build up a sufficient group immunity to reduce levels of bTB in badgers. This may represent a more acceptable strategy for disease management (Smith *et al.* 2011). Recent research has suggested that the BCG vaccine has significant direct protective effects on immunized badgers and indirectly on unvaccinated cubs (Chambers *et al.* 2011; Carter *et al.* 2012).

1.3   Aims of the current study

The main evidence for the involvement of badgers in transmission of bTB to cattle in Ireland is derived from culling trials and efforts to reduce badger population density in areas of high cattle disease incidence. There have been relatively few studies that have attempted to quantify key potential transmission routes for this disease, which is a major impediment in understanding the epidemiology of bTB in the rural environment. The current study addresses the lack of knowledge on interactions between cattle and badgers by undertaking a research project that investigated close-range contact between badgers and cattle at pasture; determined badger intrusion rates into farmyards; and provided data on badger ecology in an area with a regionally high bTB incidence in the cattle population. The formal aims and objective were to:

1. Determine the ecology, abundance and social organisation of badgers in an agricultural landscape that has a regionally high incidence of bTB in cattle

2. Quantify close-range (≤2m) contact rates between badgers and cattle when animals are at pasture and investigate inter- and intra-group contact patterns

3. Determine how often badgers visit farmyards, to include farm building selection, seasonal variation and factors that influence intrusion rates.

This report is laid out into three separate chapters that address each of the key objectives stated with additional general introduction, discussion and recommendation sections.

Badger Ecology and GPS Study

2.1 Abstract

Understanding multi-scale variation in a species' ecology across different landscapes types is a fundamental requirement in managing populations. The Eurasian badger (*Meles meles*) displays considerable heterogeneity in its social organisation, behaviour and ecology across its global distribution. In Britain and Ireland the species lives in related social groups that can range in size from 2 to ≥20 individuals according to habitat and resource availability. The species is implicated in the transmission of bovine tuberculosis (bTB) to cattle and considerable research has been conducted on badgers related to this significant economic and animal health issue.

In the current study, baseline data on the ecology, social organisation, abundance, movement distances and habitat selection of a previously unstudied population of badgers in Northern Ireland is presented. The project was funded by the Department of Agriculture and Rural Development Northern Ireland. Data was collected during 2012 and 2013 in a 1,350ha study area in a predominantly agricultural landscape, with grassland habitat dominant and arable land relatively common. Using a combination of standard techniques including sett surveys, bait-marking and live-trapping, coupled with the deployment of accurate GPS collars on badgers (n=9; 5,253 locations) data were collected on the ecology of the species within the study area.

Badger main sett density ranged from 0.88 to 1.11 main setts per km^2. Territory size estimates were constructed for 10 different groups and ranged from 43.19 to 161.29ha, with a mean territory size of 117.80 ± 11.28ha. Core-ranges of activity determined from fixed kernel analysis, ranged from 2.32 to 12.47ha. Overall mean nightly movement distance was 1,321 ± 34.5m and the maximum nightly movement distance was 6,541m.

Using minimum number alive estimates, the abundance of badgers within the study area during 2012, including adults and cubs, was 2.93 badgers per km^2. Estimated minimum social group size varied from 2 to 12 with the overall mean 3.73 ± 0.95 badgers per social group. Population abundance analysis using closed capture-recapture models provided a total abundance estimate of 50.66 badgers (95%CI 29.35-85.26) in the study area or a mean density of 3.62 badgers per km^2.

Badger habitat selection within the study area was non-random. Woodland and grassland habitat was positively selected and habitats associated with human settlement including

farmyards and domestic buildings were avoided by badgers. For GPS derived location information between August 2012 and May 2013, 0.65% of positions (n=34) were located within farmyard boundaries.

The ecology of badgers within the study area displayed a pattern of social organisation, territory size and habitat selection that is typical of this social mustelid. Density estimates were higher than average national estimates but were within known density limits and considerably lower in comparison to badger populations in the SW of England. This data formed the basis for investigation of badger-cattle interactions and intrusion into farmyards in the study area.

2.2 Introduction

When conducting research on any wildlife species it is a fundamental requirement to have basic knowledge on the ecology, behaviour and biology of the species' of interest to ensure research outcomes are put into context at an appropriate scale. For the Eurasian badger, long-term studies on its ecology have taken place in Britain and are available largely from two locations, Wytham Woods (51.462N, 1.192W) and Woodchester Park (51.711N, 2.298W). A considerable wealth of knowledge on the ecology, behaviour, reproductive biology, genetics, social organisation and epidemiology of bTB has been derived from related studies (e.g. Kruuk 1978; Cheeseman *et al.* 1981; Doncaster and Woodroffe 1993; Rogers *et al.* 1997; Tuyttens *et al.* 1999; Delahay *et al.* 2000; Tuyttens *et al.* 2000; Garnett *et al.* 2002; Macdonald and Newman 2002; Yamaguchi *et al.* 2006; Vicente *et al.* 2007; Macdonald *et al.* 2008; Carter *et al.* 2012; Jones *et al.* 2013). In Britain and Ireland badgers live in social groups consisting of related adults and their young (Dugdale *et al.* 2008), have a facultative social structure depending on food availability (Kruuk and Parish 1982), occupy territories based around setts, have a polygynandrous mating system (Carpenter *et al.* 2005; Dugdale *et al.* 2011) and are omnivorous. There is, however, considerable local and regional plasticity in these parameters across the species range.

Due to an increased output from research projects conducted largely in the Republic of Ireland, a greater knowledge of the ecology and biology of the badger population on the island of Ireland has been achieved. This information is fundamental to the management of the population and understanding the role of badgers in the epidemiology of diseases such as bovine tuberculosis (bTB). Prior to this increased knowledge, data from British studies were generally applied to badgers in Ireland although the relevance of this may now be questionable. The badger population in Ireland originated from different refugia areas (Spain and Scandinavia) compared to the population in Britain (central Europe) (O'Meara *et al.* 2012) and tends to have smaller group size, population density, sett size, less aggression, different foraging strategies and movement parameters (see review by Byrne *et al.* 2012a). The potential consequences of these differences in badger ecology could have 'real world' implications in terms of the management of bTB, exemplified by apparent lack of increased levels of bTB infection in badgers from Ireland following proactive culling (Donnelly *et al.* 2006; Corner *et al.* 2008a). Direct comparisons are complicated by different scales of management interventions applied to badger populations across the different jurisdictions.

Due to the substantial variation that can exist in badger ecology at various spatial scales and in different landscapes, it is important to conduct localised research projects that can contribute to greater understanding of their ecology. In the current study data on the ecology,

social organisation, abundance and habitat selection of badgers in a predominantly agricultural landscape where bTB incidence was regionally high was obtained. The badger population had not previously been studied so this research will contribute data to further the overall understanding of the species ecology across the island of Ireland.

2.3   Materials and Methods

2.3.1   Study Area

The study area was located in Co. Down (54°18'N, -5°38'W), Northern Ireland (Fig. 2.1). The total area consisted of approximately 1,770ha. Field work occurred in 1,350ha where land access was granted by local farmers and landowners. The area was pastoral, with farming enterprise dominated by cattle, predominantly beef rearing with several dairy farms. Sheep farming and horse breeding were also present within the area. In terms of the habitat within the study area, it was dominated by grassland (68.24%) and arable habitat (20.58%), with relatively little woodland cover (2.25%) and 3.19% of the area consisting of buildings (either farm or domestic). Grazing pasture was most common in the area and there was a wide variety of arable crops grown including barley, wheat, potato, kale, oats, maize and turnips. Unimproved land (approximately 5.73% of the area) mainly consisted of rush pastures or marsh habitat. The area was generally flat (altitude range 10-50m), had a relatively mild climate with monthly average temperatures ranging from 2 to 19°C (annual average 8.9°C), and monthly average rainfall from 58 to 92mm (annual average rainfall 843.3mm).

The size of cattle herds varied from <20 animals to >200 animals within the area. In terms of bovine tuberculosis (bTB) status within the cattle population, the study area had a regionally high level of individual (0.76%) and herd level (9.61%) disease prevalence compared to the overall averages for Northern Ireland 0.58% and 6.59%, respectively (DARD Statistics 2013). Blood samples obtained during 2012 from 20 different badgers during the study and tested with the BrockTB Stat-Pak (Chembio Diagnostic Systems, Inc.) indicated that the incidence of test positive badgers was 10%. Using badger post-mortem autopsy data from Northern Ireland for an enlarged area encompassing the study area, the average incidence of bTB infection in badgers was 18% (Maria O'Hagan, DARD)

2.3.2   Sett Surveys

To investigate badger ecology it was necessary to locate and quantify all setts that were present. During February to April 2012, at least 1,350ha of accessible farmland was searched for badger setts and signs of badger activity (e.g. paths, latrines). Trained surveyors walked every linear boundary and recorded the location of setts and badger sign using a specifically designed Geographic Information System (GIS) in ArcPad 10 (Esri Systems, USA) installed on a Trimble Juno (Trimble Systems, USA) field unit. All data was downloaded onto a database in ArcMap 10 (ESRI Systems, USA) for collation, quality assurance and analysis. Each sett was assigned an initial type classification (e.g. main, outlier) using the methodology proposed by Thornton (1998). Additional sett surveys were

undertaken in 2013 and were extended to include land that had previously not been surveyed.

2.3.3   Bait Marking – Territorial Delineation

Bait marking is a traditional technique used to demarcate badger social group territory size. It is based on the deployment of small brightly coloured plastic pellets in a pre-mixed bait, with unique colours introduced to each main sett during a pre-baiting period, followed by surveys of badger latrines to identify where pellets are deposited (Delahay *et al.* 2000). It was used in the current study during 2012 and 2013 to assist in territorial delineation of badger social groups along with data obtained from the deployment of GPS collars (see section 2.3.5). During April-May 2012 and March 2013, a premixed bait of coloured pellets, peanuts and syrup was introduced to a maximum of 8 different main setts for a period of up to 13 days. Subsequent surveys at known latrine locations (see section 2.3.2) were carried out to assess what coloured baits were present and these were recorded in a database. In total, 39 coloured baits were returned.
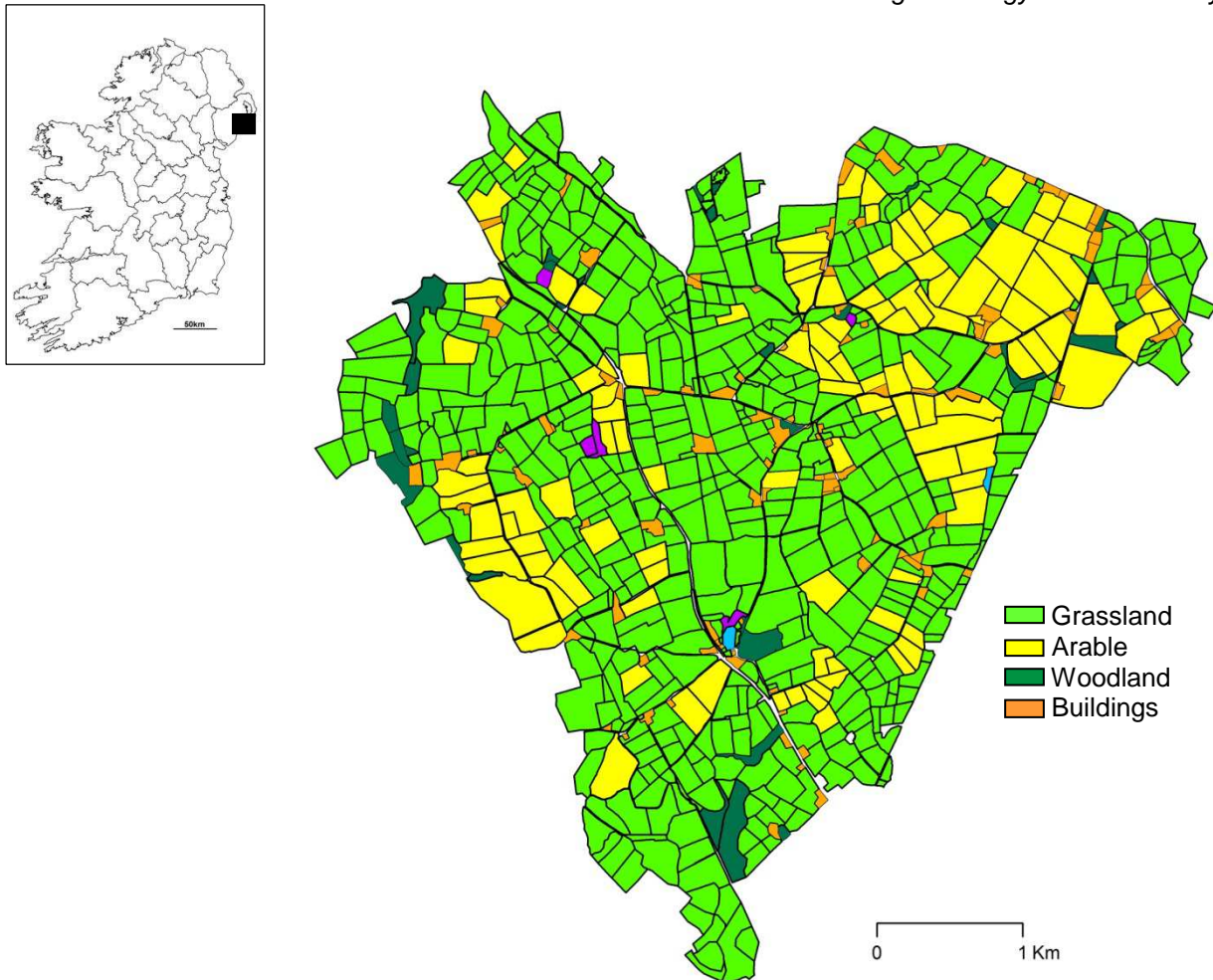
Figure 2.1. Location (inset) and distribution of main habitat types located in the study area.

2.3.4   Badger Live-trapping

All live-trapping, anaesthesia, collar deployment and sample collection that occurred during the present study took place under the conditions of a licence issued by the Department of Health, Social Services and Public Safety (DHSSPS), on behalf of the Home Office UK, according to the Animals (Scientific Procedures) Act 1986. All procedures were approved by AFBI Veterinary Science Division (VSD) ethical review board. Additionally a range of licences were issued by the Northern Ireland Environment Agency (NIEA) to conduct research on badgers in accordance with their protected species status.

Badgers were live-trapped in 2012 to collect data and deploy proximity collars (see Chapter 3) or GPS collars (see section 2.3.5), and during 2013 to remove collars from animals only. Live-trapping followed standard protocols with individual traps (approximately 86cm x 36cm x 36cm) deployed at each sett in proportion to the number of individual badgers potentially present based on an assessment of the number of active sett entrances. Traps were placed *in-situ* for a period of at least 14 days before being activated during which pre-baiting occurred to encourage use by badgers. During 2012 live-trapping occurred during August-

September and December at 13 different sites with the number of traps varying from 2 to 13 depending on the size of respective setts.

Traps were activated during the late evening and were checked at dawn the next day. Any badgers that were present in traps were assessed to ensure they had no injuries and were of suitable size for anaesthetisation. Animals that were not suitable for anaesthetisation were released immediately with a hair sample only being collected. Badgers were anaesthetised with an intra-muscular injection of ketamine hydrochloride (Vetalar, Pharmacia & Upjohn, Crawley, UK) and medetomidine hydrochloride (Domitor, Pfizer, Sandwich, UK). Once a suitable depth of anaesthesia had been reached, each animal was removed from the trap and micro-chipped; various morphometric measurements were made including weight, length, neck and head width; its approximate age determined by physical characteristics and tooth wear (Delahay *et al.* 2011); sex determined; condition assessed; a hair sample taken for genetic analysis (see Appendix One); a blood sample taken for disease status assessment; and a collar placed depending on animal size (GPS collars only on animals weight ≥9kg, proximity collar for animals ≥5kg). When these procedures were finished each animal was left to recover in a protective box at the site of capture to ensure an unobstructed airway and provide shelter from the elements. After a period of at least 1 hour each box was checked to ensure the badger had left and recovered from the procedures.

2.3.5 Deployment of Global Positioning System (GPS) collars

Tellus light GPS collars (Followit, Sweden) that weighed approximately 240g, were deployed on badgers that weighed ≥9kg. These collars are capable of user defined data collection schedules and data were sent using GSM daily, or whenever suitable networks were available (MacWhite *et al.* 2013). In the current study, from April to October (inclusive) 8 locations were scheduled per night (hourly intervals from 21.00-04.00hrs), and for the rest of the year 6 locations were scheduled per night. Accuracy testing of GPS collars (n=9) in the current study indicated that the mean location error was 7.00 ± 0.73m (O'Mahony 2013, unpubl.).

2.3.6   Data Analysis

Badger social organisation and spatial ecology

Data from bait-marking and GPS collars were used to determine home ranges / territory sizes of badger social groups within the study area. Due to variances in terms of the deployment and activation of GPS collars, all data were combined to produce an annual analysis of spatial organisation. All available location data from bait-marking and GPS collars were combined to construct 100% minimum convex polygons (MCP) (Mohr 1947) to investigate local badger social organisation. Further analysis using data from GPS collar only were used to create 95% / 50% fixed kernels (FK) (Worton 1989). MCP analysis is widely used in conventional analysis of location data but has acknowledged limitations in that it can overestimate home-range size. To account for this, fixed kernel analysis at the specified levels was also applied to the data with 50% FK representing 'core' areas of activity for the species (Kauhala and Auttila 2010). Data were analysed in ArcMap 10 (Esri Systems, USA).

In addition to investigating the home range ecology of badgers within the study area, nightly movement distances were also calculated from location data by creating movement paths for each badger that had more than 2 successive location points per night during which collars were deployed. This represented the minimum straight line movement distance between successive points on any single night. The actual distance covered by badgers was likely to have been greater than this. A Linear Mixed Model (LMM) (Genstat 14.0, VSN International, UK) was used to determine the effect of fixed factors (minimum daily temperature, average daily rainfall and month) on the response variate, which was the distance moved per badger per night (continuous). Climate data were obtained from a Met Office recording station located approximately 11.5 km from the study area. For the fixed factor month data from May, June, July and August were removed as data were limited or incomplete. Collar identity was included as a random factor in the model, the dispersion parameter was estimated and a linear mixed model was specified. Significance testing used Wald tests for fixed variables and inference on the direction of effects using *t*-tests, accepted at less than the 5% level. The proportion of points located within 10m of farm buildings was also calculated to provide further inference on farm building use by badgers (see Chapter 4).

Badger density and abundance

Badger density and abundance in the study area was calculated using two different methods based on trapping that occurred only in 2012 during July-August and December. During that time adult badgers, sub-adult and juvenile badgers from the same year were available for capture and it represented the post-breeding (adults and juvenile) abundance for the

population. The first method was based on direct enumeration of animals trapped during the stated period divided by each social group territory size, with a total density estimate produced by dividing the number of animals captured by the study area size (Feore and Montgomery 1999). This is a relatively simple measure of animal density corresponding to minimum number of individuals known to be alive (MNA). Estimates were, however, comparable to data collected on badgers from previous studies conducted in Northern Ireland (Feore and Montgomery 1999; George 2011). In addition to the MNA method a closed population estimator was applied to the data using Chapman's (1951) modification of the Peterson model with variances and 95% confidence intervals also calculated.

Badger habitat selection

Using data from GPS locations, an analysis of badger habitat use within the study area was undertaken. Each location point was assigned a habitat type in ArcMap 10 to calculate habitat use by badgers. Availability was determined as the habitat available within 100% MCP home ranges for collared badgers during respective season, which ensured only relevant data was considered in the analysis. Habitats were divided into 6 broad habitat categories (see Table 2.4) and habitat use was determined for 3 seasons (winter, spring, autumn) using selection analysis incorporating Bonferroni confidence intervals (95%) that employed chi-square goodness of fit tests for observed proportion of use following Neu *et al.* (1974). This method was used as data were not consistently available in terms of sufficient sample size of collared animals within defined periods to allow techniques such as compositional analysis to be undertaken.

2.4   Results

2.4.1   Badger sett density, live-trapping and collar deployment

In total, 191 setts were located in the study area during all surveys with 15 main setts and 152 outliers. Estimated mean sett density varied from 0.88 to 1.11 main setts per km^2. During two live-trapping sessions in 2012 a total of 41 individual badgers were captured within the study area, 19 adults and 22 juveniles (Table 2.1). GPS collar data were available from 9 individual adult badgers with a total of 5,253 location fixes (Table 2.2).

Table 2.1. Summary of the number and age classification of badgers live-trapped in 11 different social groups during two trapping sessions in 2012 within the study area.

Sett	Maximum number of individual badgers captured	Age ratio (Adult / Juvenile)
A	2	2 / 0
B	7	2 / 5
C	2	2 / 0
D	2	2 / 0
E	2	2 / 0
F	3	2 / 1
G	3	0 / 3
H	3	2 / 1
I	1	0 / 1
J	4	1 / 3
K	12	4 / 8

Table 2.2. Summary of GPS collar deployment on nine individual adult badgers to assess spatial ecology within the study area.

Badger ID	Sex	Deployment Dates	Number of locations
1	Male	18 Dec 12 to 23 May 2013	703
2	Male	07 Dec 12 to 24 May 2013	805
3	Male	04 Sep 2012 to 16 Oct 2012	285
4	Female	09 Aug 2012 to 16 Oct 2012	446
5	Female	07 Sep 2012 to 27 Mar 2013	773
6	Female	22 Aug 2012 to 26 Sep 2012	236
7	Male	05 Dec 2012 to 05 Mar 2013	420
8	Female	19 Dec 2012 to 23 May 2013	827
9	Male	07 Dec 2012 to 23 May 2013	758

2.4.2 Badger social organisation and spatial ecology

Using a combination of bait-marking and GPS collar derived location data for 9 badgers it was found that a territorial system of social organisation was present within the study area (Fig. 2.2). Annual 100% MCP badger social group territories were constructed for 10 different groups and ranged from 43.19 to 161.24ha (Fig. 2.2), with a mean territory size of 117.80 ± 11.28ha. Fixed-kernel analysis for individual badgers that had GPS collar location data are shown in Table 2.3. Core-range of activity ranged from 2.32 to 12.47ha (Table 2.3). In terms of nightly movement distances by badgers, the overall mean was 1,321 ± 34.5m and the maximum nightly movement distance by a badger was 6,541m in October 2012 by an adult male badger. There was a significant effect of month (GLMM, $F=31.64$, d.f.=7,807.6, $P<0.001$) on mean nightly distance moved by badgers and temperature approached significance (GLMM, $F=3.55$, d.f.=1,814.2, $P=0.06$). Badgers moved greater nightly distances during April, September and October, and the least during December and January (Fig. 2.3). In total during the complete period for which badger location data was available from GPS collars, 34 positions (0.65%) were located within farmyard boundaries.
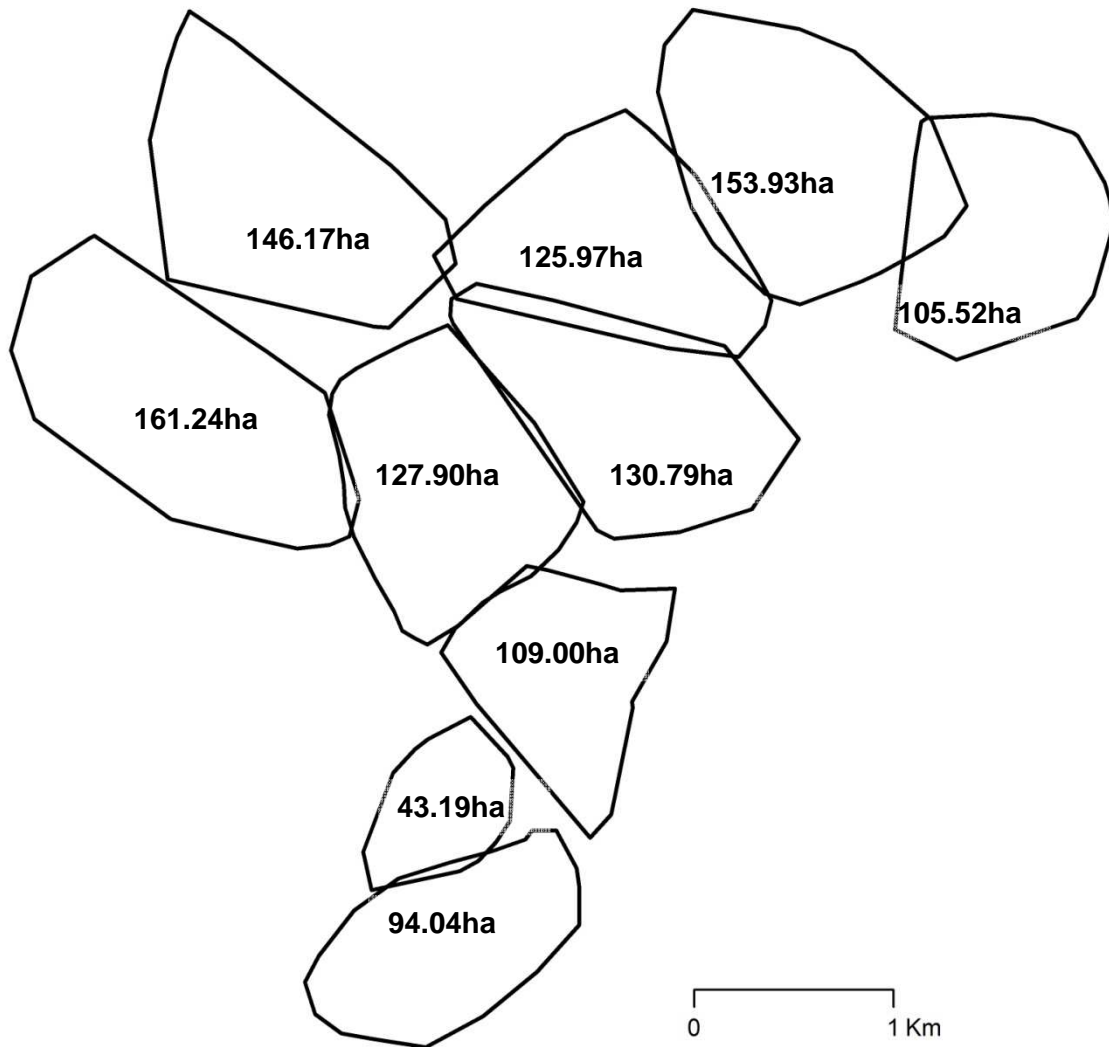
Figure 2.2. Location, distribution and size (ha) of badger social group territories (annual 100% minimum convex polygon) within the study area derived from GPS collar location data and bait-marking data collected during 2012-13.
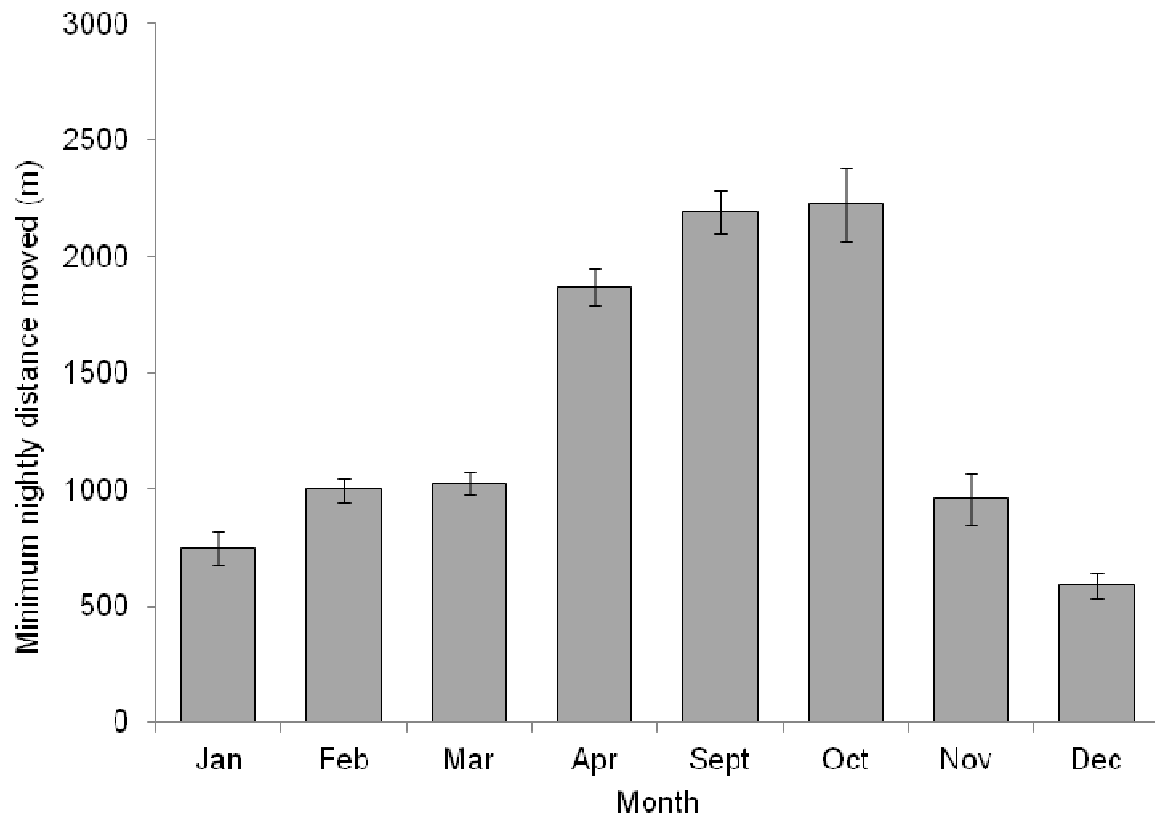
Figure 2.3. Mean nightly distance moved by month for GPS collared badgers (n=9) during 2012 and 2013 (±SE).

Table 2.3. Summary of annual home-range size data (100%MCP, 95%FK and 50%FK) for nine badgers that had GPS collars deployed during 2012-13 in the study area.

Badger ID	100%MCP (ha)	95%FK (ha)	50%FK (ha)
1	115.25	58.47	4.01
2	113.83	55.24	7.23
3	161.24	167.74	12.47
4	153.93	79.64	9.21
5	105.52	55.57	6.64
6	43.19	19.87	2.32
7	71.86	45.61	6.32
8	130.79	83.11	6.41
9	146.17	75.27	3.69

2.4.3   Badger density and abundance

Using minimum number alive estimates, the abundance of badgers within the study area during 2012, including adults and cubs, was 41 or approximately 2.93 badgers per km^2. Estimated minimum social group size varied from 2 to 12 with the overall mean 3.73 ± 0.95 badgers per social group. Population abundance analysis using closed capture-recapture models provided a total abundance estimate of 50.66 badgers (95%CI 29.35-85.26) in the study area or a mean density of 3.62 badgers per km^2 (95%CI 2.09-6.09).

2.4.4   Habitat Selection

Overall habitat use by badgers in proportion to habitat availability in the study area was not random. Habitat selection analyses indicated seasonal differences in habitat use by badgers (Fig. 2.4; Table 2.4). Woodland habitat was positively selected across all seasons and domestic buildings were avoided. Grassland habitat was selected in winter and spring and used less than expected during autumn, which was related to a significant positive selection for arable habitat during autumn (Table 2.4). Farmyards were either avoided (winter and spring) or used in proportion to availability by badgers.
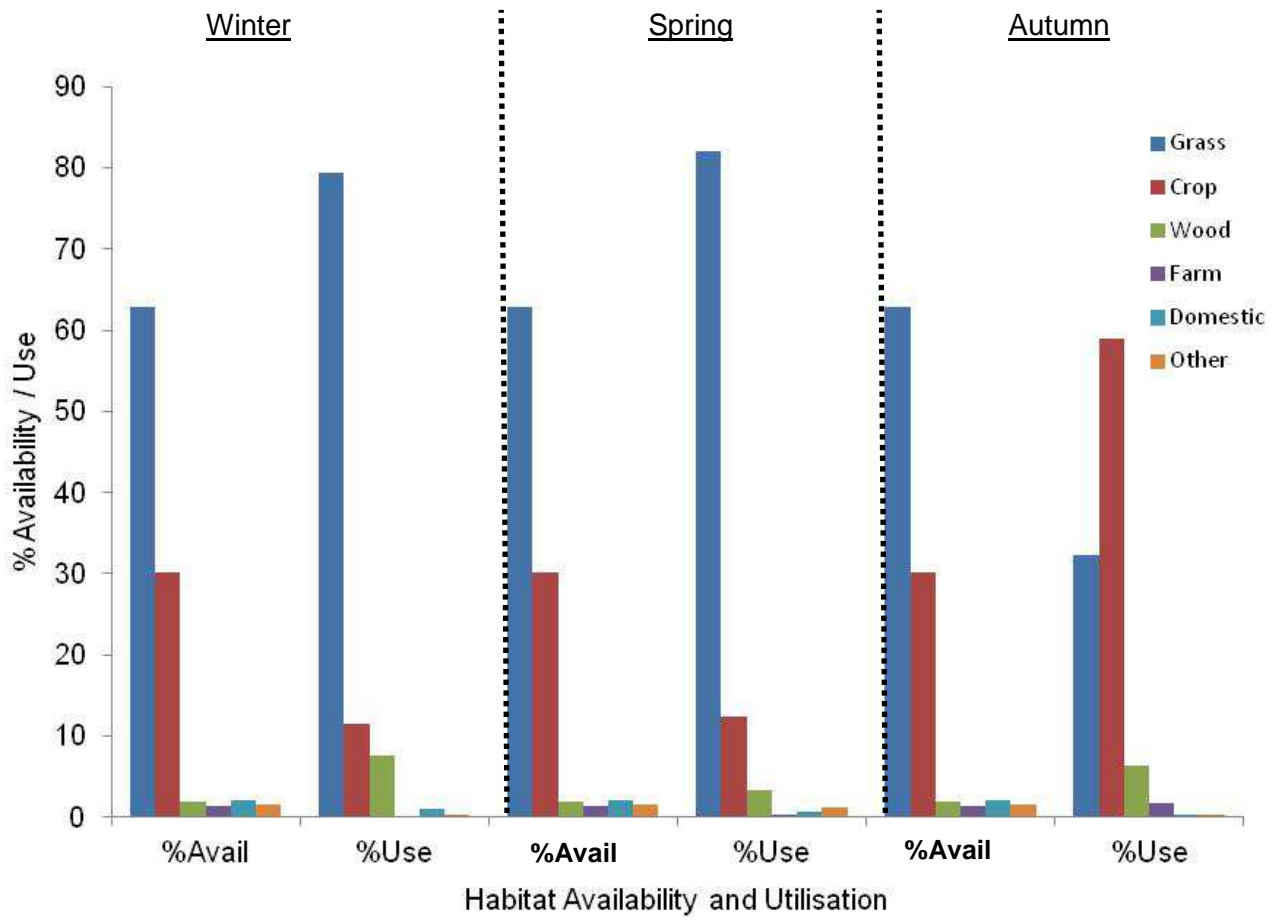
Figure 2.4. Percentage habitat use versus habitat availability, by season, for GPS collared badgers (n=9) within the study area.

Table 2.4. Selection of habitat types, by season, for Eurasian badgers (n=9) in a lowland agricultural landscape during 2012-13. Confidence intervals are 95% Bonferroni, significant selection (P<0.05) indicated by + (positive selection); - (negative selection); 0 (no selection).

Season / Habitat	% Habitat Availability	Observed	Expected	LCL	UCL	Selection
Winter						
Grassland	64.73	1362	1110	0.767	0.820	+
Arable	28.29	196	485	0.0.9	0.135	-
Woodland	2.18	129	37	0.058	0.092	+
Farmyards	0.918	3	16	-0.000	0.004	-
Domestic	2.30	18	39	0.004	0.017	-
Other	1.58	7	27	0.000	0.008	-
Spring						
Grassland	64.73	1695	1338	0.797	0.842	+
Arable	28.29	256	585	0.104	0.143	-
Woodland	2.18	70	45	0.023	0.044	+
Farmyards	0.918	8	19	0.000	0.007	-
Domestic	2.30	14	48	0.002	0.011	-
Other	1.58	24	33	0.005	0.017	0
Autumn						
Grassland	62.93	408	795	0.287	0.358	-
Arable	30.16	746	381	0.552	0.627	+
Woodland	1.95	81	25	0.045	0.083	+
Farmyards	1.36	21	17	0.006	0.026	0
Domestic	2.01	4	25	-0.001	0.007	-
Other	1.58	4	20	-0.001	0.007	-

2.5   Discussion

The current study has provided valuable baseline ecological data on a badger population in a predominately agricultural area, where levels of bTB in cattle were regionally high. It has shown that badgers had a territorial system of social organisation, variable social group and territory size, were capable of relatively long distance movements, had a population density of approximately 3.62 badgers per km^2 and non-random use of habitats available within the study area. This conforms to what we know about badgers in that the species is highly social, have discrete territories and seasonal variation in many aspects of their ecology (e.g. Roper 2010).

Previous studies in Ireland have shown that badger territory sizes can vary from 50 to 345ha, with those in pasture dominated habitat ranging between 69.8 and 127.4ha (see Byrne *et al.* 2012a). A complex interaction between landscape, habitat availability, density, climate, food resources and hunting pressure can influence badger social organisation across their range (Feore and Montgomery 1999; Johnson *et al.* 2002; Kuahala and Holmala 2011; Mysłajek *et al.* 2012). The mean territory size in the current study (117.80ha) was larger than that found in a previous badger population study (50.4ha) of a site located approximately 6.20km north of the current study area (Feore and Montgomery 1999), which may be explained by a greater badger density and more favourable parkland habitat. The smallest territory size determined in the current study (43.19ha) may be an underestimate as it was based on data collected from a GPS collar for 36 days during August and September 2012 and whilst 236 locations were available, seasonal variation in badger movement can be considerable (MacWhite *et al.* 2013). Badger density and social organisation are inherently linked and there is considerable flexibility in the spacing systems of badgers across their range (Frantz *et al.* 2010). 'Loose' systems of badger territoriality (e.g. where there is change in size and little apparent marking behaviour) have been found in relatively low badger density populations (Do Linh San *et al.* 2007; Frantz *et al.* 2010) and in Ireland badgers may have more fluid social groupings (Byrne *et al.* 2012a). Indeed recent intensive studies on badger spatial and movement ecology in the Republic of Ireland, based on deploying large numbers of GPS collars over consecutive years, have suggested that individual badgers have exclusive areas of use, cross territorial boundaries, move relatively large distances and have apparently little aggression towards conspecifics (MacWhite *et al.* 2013), indicating considerable heterogeneity at an individual level in terms of badger social organisation.

In terms of minimum nightly movement distances by badgers, the mean (1,321m) and maximum movement distances (6,541m) recorded within the current study are within the

ranges reported in previous studies (e.g. Elmeros *et al.* 2005; Goszczyński *et al.* 2005; Kowalczyk *et al.* 2006; MacWhite *et al.* 2013; Byrne *et al.* 2014). It is important to emphasise that the vast majority of these movements do not represent direct straight-line distances or dispersal, but rather reflect a pattern of localised space use within a bounded area such as a territory. For instance, the 6,541m movement in the current study occurred in a 100% minimum convex polygon area of 87.54ha. Although there is little known about long distance dispersal attempts in badgers, recent evidence suggests that distances of up to 21.1km can be achieved in Ireland and that there are sex differences in movement frequency and propensity (Byrne *et al.* 2014).

Badger density in the current study was estimated at 3.62 badgers per km^2, including adults and juveniles, which in terms of the Irish badger population appeared relatively high in comparison with average densities at the island scale (Sleeman *et al.* 2009; Reid *et al.* 2012). Local badger densities on the island of Ireland can vary from 0.7 to 11.5 adult badgers per km^2 (Byrne *et al.* 2012a) with recent large scale studies in the Republic of Ireland indicating that densities of approximately ≤1 badger per km^2 may be representative of relatively large areas of the midlands, although culling had occurred in that study area prior to the population being estimated (Byrne *et al.* 2012b). In comparison with density data from local studies in Northern Ireland using minimum number alive estimates, the current study had lower density than that estimated for parkland habitat (17.5 per km^2) within 6.20km of the current study area, and was higher than estimates for marginal upland and upland habitat (Feore and Montgomery 1999). Recent population studies in the same parkland habitat have indicated that group size has declined potentially as a result of an increased number of main setts present in that area compared to previous studies (George 2011). In all recent studies carried out on badgers in Northern Ireland illegal persecution had occurred in respective study areas (e.g. Sadlier 1999; McCann 2002; George 2011; current study) which can influence trapping success and, therefore, abundance estimation.

Badgers throughout their global distribution occupy a wide variety of habitats and landscapes that can have fundamental implications on their ecology and behaviour (Feore and Montgomery 1999; Kowalczyk *et al.* 2003; Do Linh San *et al.* 2007). Across their European range, badgers are considered habitat generalists preferring woodland, pastures, wetlands and riparian areas (Santos and Beier 2009) but in more semi-arid climates the species can select orchards, scrubland and rock outcrops (Lara-Romero *et al.* 2012). In terms of habitat, badgers clearly select habitat types that are associated with shelter and food resources. In the current study, badgers showed significant selection of available habitats, positively selecting woodland and grassland during most seasons and generally

avoiding human dominated habitats such as farmyards and domestic housing. The selection of woodland by badgers is related to their requirement for shelter in terms of sett locations, when this habitat type is available. In Ireland, due to the general low level of woodland cover badgers also utilise linear features and hedgerows as sett locations (O'Corry-Crowe *et al*. 1993; Feore and Montgomery 1999). Arable habitat was only selected during autumn and was not used in proportion to its availability during other seasons and human dominated habitats consisting of farmyards were significantly avoided by badgers. The method of habitat analysis used in the current study operated at a population level, with all data combined from individuals, and involved a relatively small number of badgers. The method has acknowledged limitations such as potential pseudo-replication of data (e.g. Alldredge and Ratti 1992) and by pooling data across study animals individual variation is lost. Despite this, Neu's method is by far one of the commonest used in habitat selection analysis and data from the current study is consistent with that from other research.

The current study has provided important baseline data on badger population ecology within the study area that will form the basis of further research on interactions between badgers and cattle at pasture (Chapter 3) and quantifying farmyard intrusion rates by badgers (Chapter 4) to deliver the overall aims and objectives of the study.

Badger-Livestock Proximity Study

3.1   Abstract

Interactions, both within and between groups and individuals, are becoming a key focus of ecological, behavioural and epidemiological studies of wildlife. Contact networks within populations can have profound individual consequences for animals in terms of breeding opportunities, social status and disease transmission. Bovine tuberculosis (bTB) is a complicated disease issue of cattle, and badgers have been associated with its transmission to cattle. Across the island of Ireland, there is no data available on interaction rates between these gregarious species, which is an impediment to disease eradication.

In the current study, the first quantifiable data on the level of inter- and intra-group interactions between free roaming badgers and cattle on the island of Ireland were collected. Proximity collars were deployed on badgers and cattle, contemporaneously, in Northern Ireland in an area with a regionally high bTB in cattle incidence to assess the importance of close range contact (≤2m) as a possible transmission route for the spread of bTB. The study was funded by the Department of Agriculture and Rural Development. Data were available from a maximum of 92 cattle and 15 badgers, across several different herds and social groups, in a 1,350ha study area where a maximum of 439,776 interactions were available for analysis.

All cattle within herds contacted each other at least once during the study, but there was considerable variation across different herds. Mean intra-group contact frequency (C_{freq}) was 6.35 ± 0.11 contacts/day (min 0.07 − max 194.2), and mean intra-group contact duration (C_{dur}) 139.70 ± 2.47 seconds/day (min 0.20 - max 4,151). Contact rates were significantly influenced by month, herd and climate variables. There were also significant differences between individual collared animals in C_{freq} within 10 of the 11 herds studied.

All badgers within social groups contacted each other at least once, with mean intra-group C_{freq} 2.58 ± 0.05 contacts/day (min 0.25 − max 16.33), and mean intra-group C_{dur} 148.8 ± 6.26 seconds/day (min 0.5 - max 2,080). Contacts between badgers occurred during every hour of the day with a bimodal pattern of intra-group contacts, with peaks from 06.00 to 07.00hrs and 18.00 to 19.00hrs. Month, sett, and sex had a significant effect on badger contact frequency.

Each species interacted to a great extent and with considerable heterogeneity in contact rates within species, even though there was no close-range interaction between badgers and cattle found in the current study. In terms of the transfer of bTB through direct aerosol transmission, this study, and the available evidence suggests that this is likely to be an unimportant route in quantitative terms.

3.2 Introduction

In the environment different animal species interact with each other directly through physical contact, or indirectly through various means such as olfaction, vocalisation or behavioural response. Many interactions between species are of applied interest to science but of principal interest are those of human health or commercial concern, particularly those associated with zoonotic disease (e.g. Bharti *et al.* 2003) or that have an impact on domesticated species (e.g. Pattnaik *et al.* 2012). One of the principal ongoing disease issues of livestock in Western Europe relates to bovine tuberculosis (bTB) in cattle and its link with wildlife reservoirs such as badgers and deer (Donnelly *et al.* 2003; Hardstaff *et al.* 2013). This is a complicated issue that has dominated much of the literature on wildlife-livestock disease system dynamics over recent decades due to its cost, animal welfare and zoonosis implications.

Domestic cattle are complex gregarious animals descended from wild aurochs in the near East some 10,500 years ago (Bollongino *et al.* 2012). Cattle behavioural traits are derived from a complex interaction between genetic and environmental factors (Searle *et al.* 2010), characterised by considerably high individual variation and synchrony, which increases as animals are allowed to freely demonstrate behaviours (Benham 1982; Adamczyk *et al.* 2013). Cattle engage in complex interactions to communicate social status and have a linear hierarchical structure, with social status related to size, age and physical characteristics (Bouissou 1972; Beilharz and Zeeb 1982; Philips 2002). Predominant behaviours are grazing, ruminating and resting (Kilgour 2012). Even though cattle have been farmed through different production methods and have been bred for specific purposes, they retain ancestral behaviours and have individualistic traits that may be important in terms of disease epidemiology. There is an increasing awareness that the amount and structure of individual behaviours within a group or population affects key ecological and evolutionary processes, and their interaction (Wolf and Weissing 2012).

Interactions between badgers and cattle at pasture have been studied over recent decades using a variety of techniques. Initial studies used direct observation of both species and enclosure experiments (Benham 1985; Benham and Broom 1989) to determine interaction rates. More recently, with advances in technology, proximity collars and global positioning system (GPS) collars have been used to quantify interactions (Böhm *et al.* 2009; Mullen *et al.* 2013). Contact between badgers and cattle have also been investigated in farmyards and farm buildings in the current study (see Chapter 4). Although relatively few studies have occurred, and those that have are mainly from England, the available evidence suggests that close range contact (≤2m) between badgers and cattle at pasture are rare events (Benham

and Broom 1989; Böhm *et al.* 2009; Drewe *et al.* 2013) even though in some studies the density of animals available for interaction was high (e.g. Drewe *et al.* 2013). In Ireland, prior to the current study, proximity collars have not been used to investigate interactions between wildlife species. Using GPS collars on badgers and detailed cattle utilisation data of a series of paddocks, it has been shown that foraging badgers avoid paddocks containing cattle (Mullen *et al.* 2013). The available evidence suggests that direct, close range aerosol transmission of bTB may not be a significant disease transmission route between badgers and cattle, instead indirect transmission may be more important in disease dynamics. However, there remains a requirement for larger scale studies to be conducted on this issue to corroborate existing data and examine potential variation according to environmental variables, different farming systems and badger ecology.

The application of technology such as proximity collars to wildlife populations has not only allowed a hitherto unprecedented level of detail in terms of the quantification of between species interactions, it also has allowed fundamental data to be collected on within group interactions and how behavioural responses to disease dynamics may occur within populations. To-date proximity collars have been deployed on brushtail possums, *Trichosurus cunninghami* (Banks *et al.* 2011), Tasmanian devils, *Sarcophilus harrisii* (Hamede *et al.* 2009), racoons, *Procyon lotor* (Prange *et al.* 2011), ungulates (Vander Wal *et al.* 2013), rabbits, *Oryctolagus cuniculus* (Marsh *et al.* 2011), badgers and cattle (Böhm *et al.* 2009). Common to the majority of the studies undertaken has been a high level of individual heterogeneity in contact rates and networks within populations (e.g. Hamede *et al.* 2009; Marsh *et al.* 2011; Drewe *et al.* 2013). For badgers, simultaneous close range radio-tracking has recently shown that badgers within social groups have different contact patterns depending on the type of sett used most frequently (Böhm *et al.* 2008), which may be related to their disease status (Weber *et al.* 2012). Proximity collars on badgers have also revealed intricate behaviours in terms of the isolation of bTB positive badgers from their own social group, with potential consequences for disease dynamics at a population level through increased contacts with other badger groups (Weber *et al.* 2013). Individual contact pattern heterogeneity has also been found within cattle herds (Böhm *et al.* 2009), and in terms of interactions with potentially important infection sources such as badger latrines (Drewe *et al.* 2013). Measuring the complexities of individual animal contact behaviour in a multi-host disease system should be seen as a priority in terms of informing disease management and eradication strategies.

In the current study, the first quantifiable data on the level of inter- and intra-group interactions between badgers and cattle on the island of Ireland are presented. The aim was

to deploy proximity collars on badgers and cattle, contemporaneously, in an area with regionally high cattle bTB incidence to assess the importance of close range contact between these species as a possible transmission route for the spread and maintenance of bTB in both populations. The study also collected data on contact rates within different cattle herds and different badger social groups to determine baseline levels of interactions that occurred within populations.

3.3   Materials and Methods

Proximity collars (Sirtrack, Havelock, NZ) were used to investigate contact rates between cattle and badger in the study area (see section 2.3.1) during 2012-13. Proximity collars are a relatively new technology, which allow interactions to be investigated by emitting and receiving unique collar identity information through a UHF transceiver, within a user defined contact distance interval. Once a contact has been made between two different collars, the date, time, contact duration and collar identity is recorded, which can be downloaded when collars are retrieved. In the current study, 115 collars were deployed on cattle between July and August 2012, when individuals were in farmyards for disease testing, veterinary care or brought in especially for collar placement (Table 3.1). In total, 92 cattle collars were returned, the majority by December 2012 although some (n=19) were deployed until March 2013. For badgers, 25 proximity collars were fitted through licensed live-trapping (see section 2.3.4) during August/September 2012 and December 2012. Badger proximity collars were retrieved and replaced during live-trapping in December 2012 and retrieved only during May and June 2013 (n=17). Of the 17 badgers that had collars returned, 15 (88.23%) tested negative to bTB using the Brock Stat-pak test. Collars weighed ≤2% of animal body weight. Collars were deployed on different farms, different herds within farms and on badgers (Table 3.3) whose territory encompassed respective farms (data obtained from GPS collars and bait-marking; see section 2.3.6). Collars were only placed on cattle that remained at the home-farm during the study to maximise the opportunity for contacts to be obtained. The aim was to have at least 10% of each cattle herd collared during the study. Field trials occurred prior to deployment of both collar types to determine the settings required to record close range (≤2m) interactions, the most important for direct aerosol transmission of bTB between species.

3.3.1   Data Analysis

Interaction data was downloaded from returned collars and placed into a database. To account for acknowledged limitations with collar data, data were amalgamated into 1 minute segments in R version 3.0.1 (R Development Core Team 2013) and any remaining 1 second records were deleted from the dataset (Böhm *et al.* 2009; Drewe *et al.* 2013). Additionally, contact data from the 24hr period after collar deployment was removed for both species, data during badger trapping periods, and from badger collars with less than 20 records (n=2; Table 3.2) were excluded from formal analysis. These procedures reduced the quantity of interaction data available but increased data reliability. Cattle interaction data were calculated as those that occurred during the 'grazing season' (July to November 2012), when animals were out in pasture (free-roaming) for the majority of time and were in sheds rarely. For badgers, the only response variable considered was all contact data recorded

from September 2012 to June 2013. Contact data were analysed for cattle and badgers at the intra-group level only, as inter-group contacts were zero or too few for analysis.

Table 3.1. Summary of the number of cattle proximity collars recovered and interaction data recorded across 11 different herds between July and November 2012.

Farm Code	Collars	Herd Type[a]	Total No. of Interactions	Mean daily C_{freq} (± SE)	Mean daily C_{dur} (± SE)
FA	4	LIM	19,076	18.85 ± 1.84	392.90 ± 36.60
FB	9	CH/BB	25,864	4.11 ± 0.20	81.50 ± 5.25
FC	7	HOL	36,312	9.78 ± 0.44	177.00 ± 8.45
FD	9	HOL	47,966	9.73 ± 0.32	202.70 ± 7.83
FE	10	HOL/BB	31,536	3.22 ± 0.11	57.18 ± 2.46
FF	12	HOL	49,937	3.84 ± 0.06	87.33 ± 1.86
FG	6	HOL	61,130	19.90 ± 0.49	324.30 ± 8.34
FH	4	LIM	15,083	10.80 ± 0.66	200.30 ± 14.33
FI	9	CH/AA	50,705	5.43 ± 0.25	172.00 ± 9.18
FJ	9	LIM	17,320	2.44 ± 0.11	62.95 ± 3.78
FK	13	LIM/AA	71,878	3.79 ± 0.08	122.4 ± 5.57
Total / Means ±SE	92		426,807	6.35 ± 0.11	139.70 ± 2.47

[a] herd type according to breeding codes: AA Aberdeen Angus; BB Belgian Blue; CH Charolais; HOL Holstein; LIM Limousin.

Contact measures were constructed using interaction data to determine the mean daily contact frequency (C_{freq}) and mean daily contact duration (C_{dur}) in seconds, for each collared animal, based on the total number of contacts and total contact duration, respectively, divided by the total number of days each collar was attached to an animal (Böhm *et al.* 2009; Marsh 2011). Both of these measures were then divided by the number of individual animals collared in each cattle herd or badger social group each day, to account for different numbers of collars deployed across the study. Therefore, C_{freq} and C_{dur} corresponded to the mean daily contact frequency and duration for specific individual cattle and badgers with any other collared animal within the same herd or group. These procedures standardised intra-group contact measures for cattle and badgers. All contact measures were log transformed prior to analysis to normalise data. Cattle and badger contact data were analysed separately.

General linear mixed models (GLMMs) (Genstat 14.0, VSN International, UK) were used to test for differences in contact rate measures. Count response variables (C_{freq}) were analysed with a Poisson distribution with log link function and continuous variables (C_{dur}) with a linear mixed model. Fixed factors included month, herd or social group, sex (badgers only), minimum daily temperature (°C), and total daily rainfall (mm). Climate data were obtained from a Met Office recording station located approximately 11.5 km from the study area. Dispersion parameters were estimated for each model to account for potential over-dispersion. Significance testing used Wald tests for fixed factors and inference on the direction of effects using *t*-tests, accepted at less than the 5% level. Individual collar ID was included as a random effect in all models. Additionally for cattle data, General Linear Models (GLMs) (Genstat 14.0, VSN International, UK) were applied to investigate individual animal variation in C_{freq}, within herds with a Poisson distribution and log link function.

Direct contact between cattle and badgers were assessed through contemporaneous deployment of proximity collars on badgers and cattle from September 2012 to February 2013 as some cattle were left out grazing during the winter. Collars were deployed on 58 different cattle, in 6 different herds, located within 4 different badger territories where 11 badgers had proximity collars (7 male, 4 female; 5 juvenile and 6 adult). None of the badgers tested positive to bTB using the Brock Stat-pak test.

Table 3.2. Summary of the number of badger proximity collars and interaction data recorded on badgers across 5 different social groups that had a minimum of two badgers collared, and more than 20 contacts, between September 2012 and May 2013.

Social Group	No. of Badger Collars Retrieved	Total No. of Interactions Recorded	Mean daily C_{freq} (± SE)	Mean daily C_{dur} (± SE)
B1	5	5,248	2.09 ± 0.07	17.76 ± 5.16
B2	2	1,291	3.89 ± 0.20	366.2 ± 37.94
B3	2	724	2.82 ± 0.17	64.19 ± 8.77
B4	2	2,491	3.10 ± 0.16	219.9 ± 16.41
B5	4	3,215	2.44 ± 0.09	150.60 ± 11.15
Total / Means ±SE	15	12,969	2.58 ± 0.06	144.80 ± 6.27

Table 3.3. Summary of contemporaneous data available to assess badger to cattle contact during the study period.

Badger Social Group	Herd No.	Cattle Collars Retrieved	No. of Badger Collars Retrieved	Annual Badger Territory Size (ha)
B1	1	4	4	125.97
B3	1	9	2	146.17
B4	2	15	2	94.04
B5	2	30	3	109.00
Total	6	58	11	

3.4 Results

3.4.1 Interaction data

For cattle, 426,807 contacts were recorded during the grazing season (July-November 2012). The total number of contacts recorded on badger proximity collars between September 2012 and June 2013 was 12,969. In terms of assessing direct contact between cattle and badgers, a total of 376,152 contacts were available, of which 96.87% were from cattle, and 3.13% from badger proximity collars. No close range interactions (≤2m) between cattle and badgers were confirmed during the study.

3.4.2 Inter-group contacts

Inter-group interactions did not occur between cattle herds as they were not contiguous during the study, and were too few for badgers (n=45 / 0.35% of contacts) for formal analysis. Inter-group badger contacts occurred between five neighbouring badger setts, although not with all possible combinations of contiguous setts (Fig. 3.1).
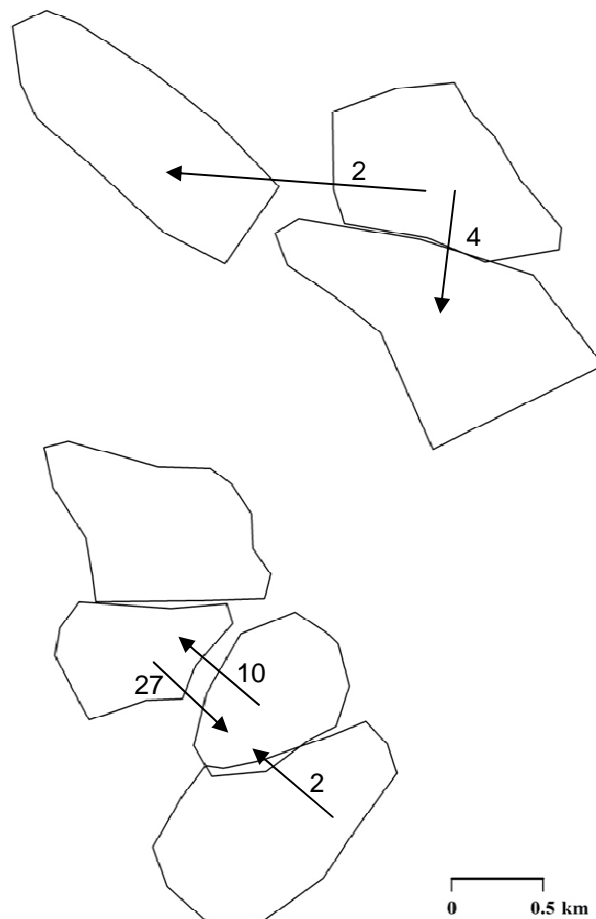

Figure 3.1. Illustration indicating direction (arrow) and frequency (number) of inter-group badger contacts between badger social groups that occurred during the study.

3.4.3 Intra-group contacts between cattle

Mean intra-group C_{freq} was 6.35 ± 0.11 contacts/day (min 0.07 – max 194.2), and mean intra-group C_{dur} 139.70 ± 2.47 seconds/day (min 0.20 - max 4,151). All cattle within herds contacted each other at least once during the study, but there was considerable variation across different herds (Table 3.1). Contacts between cattle occurred in all hours of the 24hr period but were concentrated between 08.00-21.00hr with comparatively few contacts between 00.00-07.00 (Fig. 3.2). All fixed factors considered (month, farm, rain and minimum. temperature) had significant effects on C_{freq} (GLMM, F=43.13, d.f.=4,8075.7 P<0.001; GLMM, F =12.58, d.f.=10,78.8 P<0.001; GLMM, F=95.34, d.f.=1,8061.5 P<0.001; and GLMM, F=75.64, d.f.=1,8062 P<0.001, respectively) and C_{dur} (GLMM, F=30.65, d.f.=4,8071.1, P<0.001; GLMM, F=4.48, d.f.=10,81.4 P<0.001; GLMM, F=16.57, d.f.=1,8062.7, P<0.001; and GLMM, F=61.80, d.f.=1,8063.3, P<0.001, respectively). C_{freq} was lowest in August and September, and two farms had an average of more than 15 intra-group contacts per day (FA and FG; Table 3.3), which were significantly higher than all other farms in the study. C_{freq} was negatively related to average daily rainfall and minimum daily temperature. Similar patterns of differences were observed for C_{dur}.

There were significant differences between individual animals in C_{freq} within 10 of the 11 herds studied (GLM P<0.05; Fig. 3.3; Tables 3.4 and 3.5). Within herds, the maximum difference between the percentages of contacts by individual animals was 2.82% to 32.1% and the maximum % contribution to C_{freq} by any single animal was 48.57% of contacts.

3.4.4 Intra-group contacts between badgers

All badgers within groups contacted each other at least once, with mean intra-group C_{freq} 2.58 ± 0.05 contacts/day (min 0.25–max 16.33), and mean intra-group C_{dur} 148.8 ± 6.26 seconds/day (min 0.5-max 2,080). There was considerable variation according to sett with the mean daily C_{freq} ranging from 2.44 to 3.89 contacts per day, and mean C_{dur} ranging from 17.76 to 366.20 second per day (Table 3.2). Contacts between badgers occurred during every hour of the day and combined data across setts and individuals indicated a bimodal pattern of intra-group contacts, with peaks from 06.00 to 07.00hrs and 18.00 to 19.00hrs (Fig. 3.4). Month, sett and sex had a significant effect on C_{freq} (GLMM, F=28.05, d.f.=8,1642.4, P<0.001; GLMM, F=31.88, d.f.=4,1656.3, P<0.001; GLMM, F=53.17,1656.3 d.f.=1, P<0.001, respectively). Mean daily C_{freq} were lowest in February-May and were greatest in sett B2 and for female badgers. Climate variables had no effect on C_{freq}. Month, sett, and minimum daily temperature had a significant effect on C_{dur} (GLMM, F=7.67, d.f.=8,1657, P<0.001; GLMM, F=57.07, d.f.=4,1657 P<0.001; GLMM, F=9.68, d.f.=1,1657 P<0.005, respectively); (see Table 3.5).
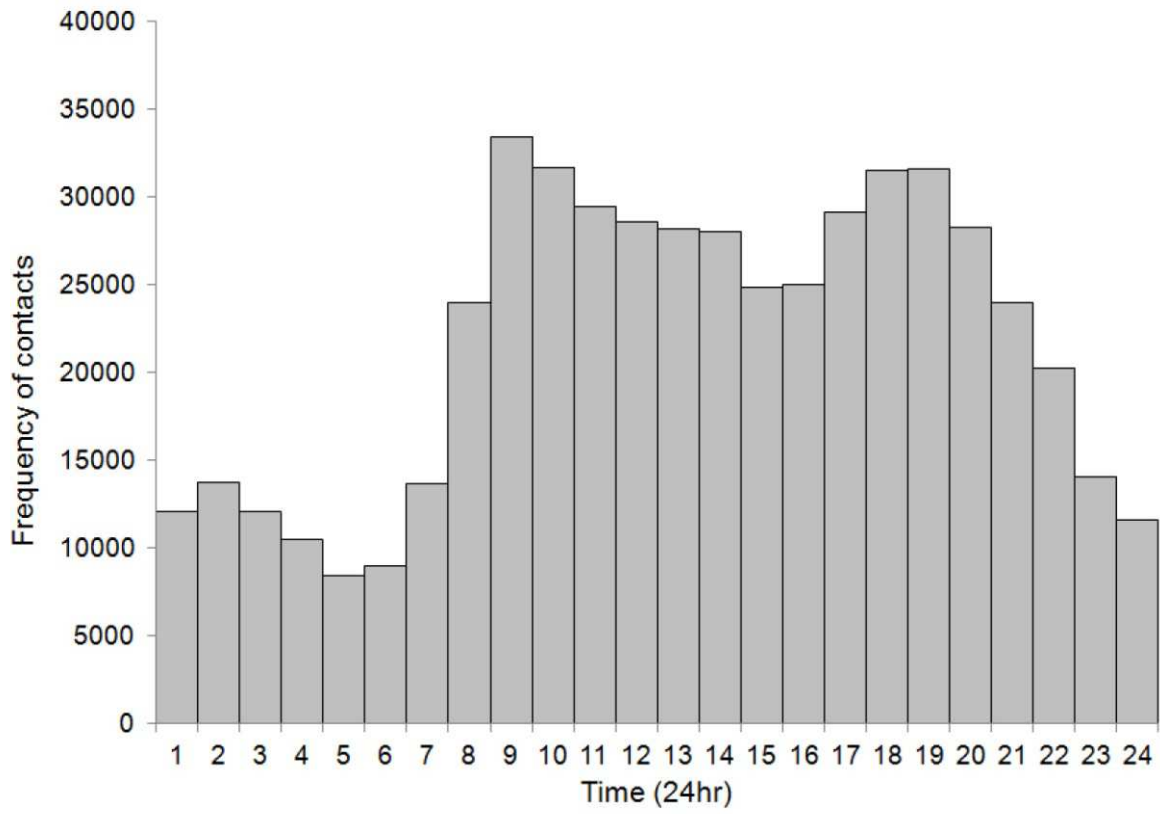
Figure 3.2. Cumulative frequency of C_{freq} for all cattle with proximity collars during the study.
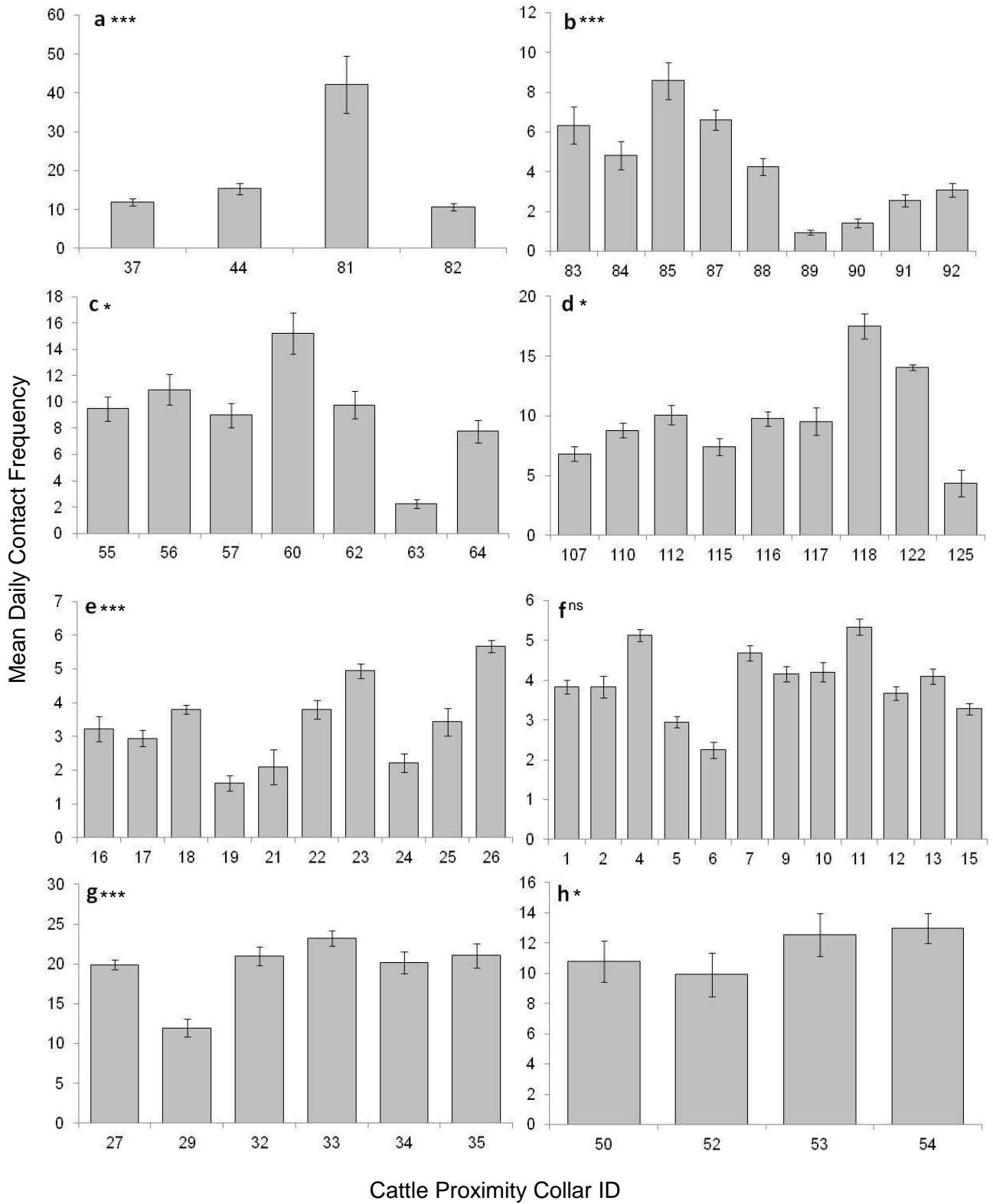
Figure 3.3. Mean C_{freq} between individual cattle in 11 different herds / farms (a – k) between July and November 2012. Error bars are ± SE. *** $P<0.001$; ** $P<0.01$; * $P<0.05$; ns non-significant. *Continued overleaf*
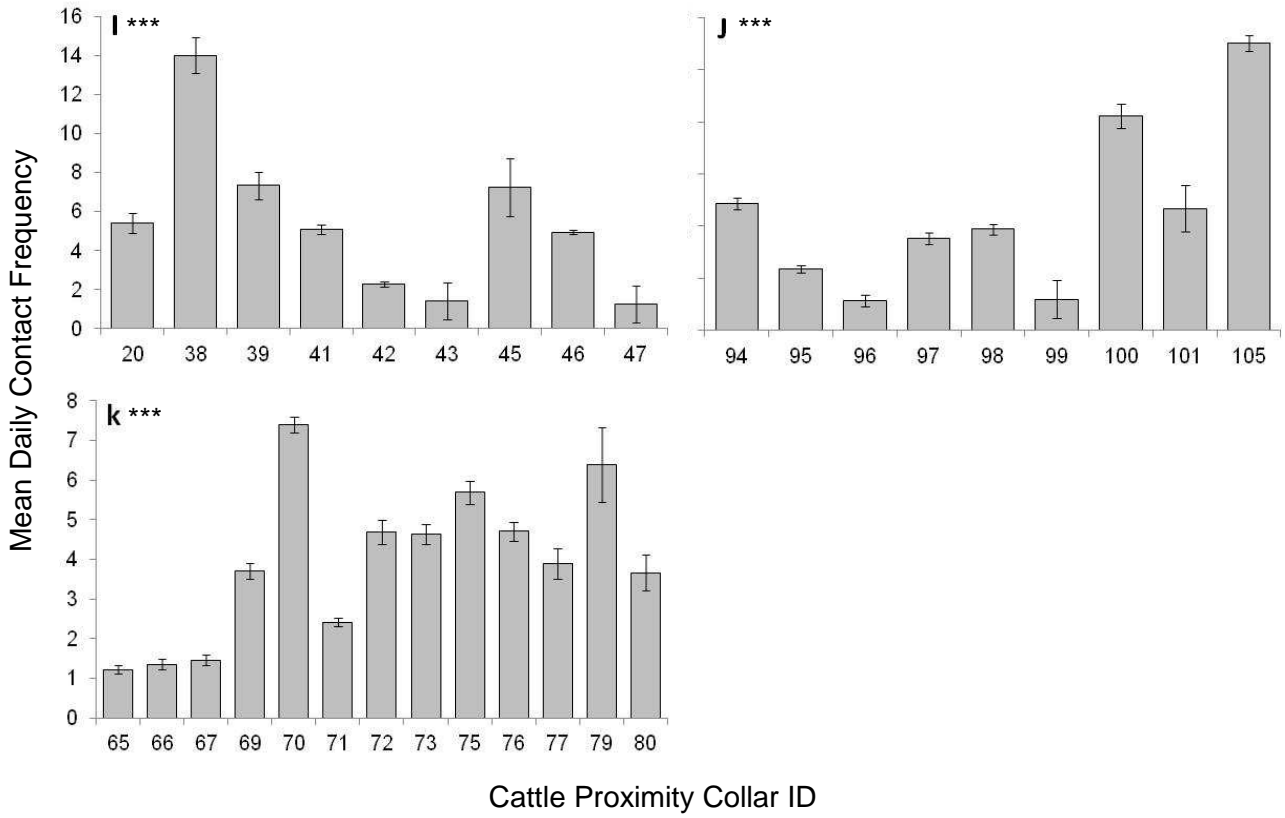
Figure 3.3. Mean C_{freq} between individual cattle in 11 different herds / farms (a – k) between July and November 2012. Error bars are ± SE. *** P<0.001; ** P<0.01; * P<0.05; [ns] non-significant. *Continued from previous page*

Table 3.4. Generalized linear mixed-effects models (GLMMs) for differences in the intra-group daily contact frequency (C_{freq}) and contact duration (C_{dur}) for cattle.

Model 1	C_{freq}			Model 2	C_{dur}		
Variable	Coeff.	t	P	Coeff.	t	P
Intercept	6.31	84.86	<.001	132.31	5.93	<.001
Month						
Aug	-0.2229	-5.56	<.001	-0.3141	-6.07	<.001
Sep	-0.1353	-3.47	<.001	-0.1263	-2.54	0.011
Oct	-0.0743	-1.71	0.087	-0.0165	-0.31	0.76
Nov	0.0286	0.57	0.567	0.1601	2.64	0.008
Farm						
FB	-1.4331	-26.91	<.001	-1.4594	-21.47	<.001
FC	-0.5152	-11.19	<.001	-0.6009	-10.04	<.001
FD	-0.7649	-16.3	<.001	-0.8272	-14.09	<.001
FE	-1.6807	-32.47	<.001	-1.8062	-26.46	<.001
FF	-1.4668	-30.22	<.001	-1.328	-22.24	<.001
FG	0.1712	4.24	<.001	-0.0248	-0.47	0.638
FH	-0.4311	-8.68	<.001	-0.4974	-7.71	<.001
FI	-1.1942	-27.03	<.001	-0.7624	-14.88	<.001
FJ	-2.0386	-33.77	<.001	-1.8429	-26.11	<.001
FK	-1.5693	-35.38	<.001	-1.1326	-22.15	<.001
Climate						
Rainfall	-0.01337	-8.07	<.001	0.00956	-4.89	<.001
Min Temp	-0.03099	-8.4	<.001	0.03562	-8.01	<.001

Factors are explained in the text, reference values are Farm FA and Month July.

Table 3.5. Generalized linear mixed-effects models (GLMMs) for differences in the intra-group daily contact frequency (C_{freq}) and contact duration (C_{dur}) for badgers.

Model 1	C_{freq}			Model 2	C_{dur}	
Variable	Coeff	t	P	Coeff	t	P
Intercept	0.94	13.39	<.001	132.31	5.93	<.001
Month						
Feb	-0.47	-4.42	<.001	-88.57	-3.08	0.002
Mar	-0.45	-4.54	<.001	-65.82	-2.43	0.015
Apr	-0.44	-4.06	<.001	-45.59	-1.55	0.121
May	-0.45	-3.82	<.001	-45.38	-1.40	0.162
Sep	0.49	6.17	<.001	68.53	2.54	0.011
Oct	0.32	4.61	<.001	20.64	0.90	0.366
Nov	0.29	4.07	<.001	-19.76	-0.86	0.389
Dec	0.22	2.71	0.007	39.27	1.44	0.15
Sett						
B2	0.13	1.92	0.055	257.34	10.63	<.001
B3	0.27	3.46	<.001	-12.55	-0.50	0.617
B4	0.64	10.41	<.001	162.12	8.31	<.001
B5	-0.16	-2.79	0.005	54.40	3.03	0.002
Sex						
Male	0.49	-7.7	<.001	-31.89	-1.67	0.095
Climate						
Rainfall	0.00	-0.33	0.741	-0.31	-0.42	0.688
Min Temp	0.00	-0.44	0.657	-6.06	-2.87	0.004

Factors are explained in the text, reference values are Month Jan, Sett B1, Sex Female.

Figure 3.4. Cumulative frequency of C_{freq} for all badgers with proximity collars during the study.

3.5   Discussion

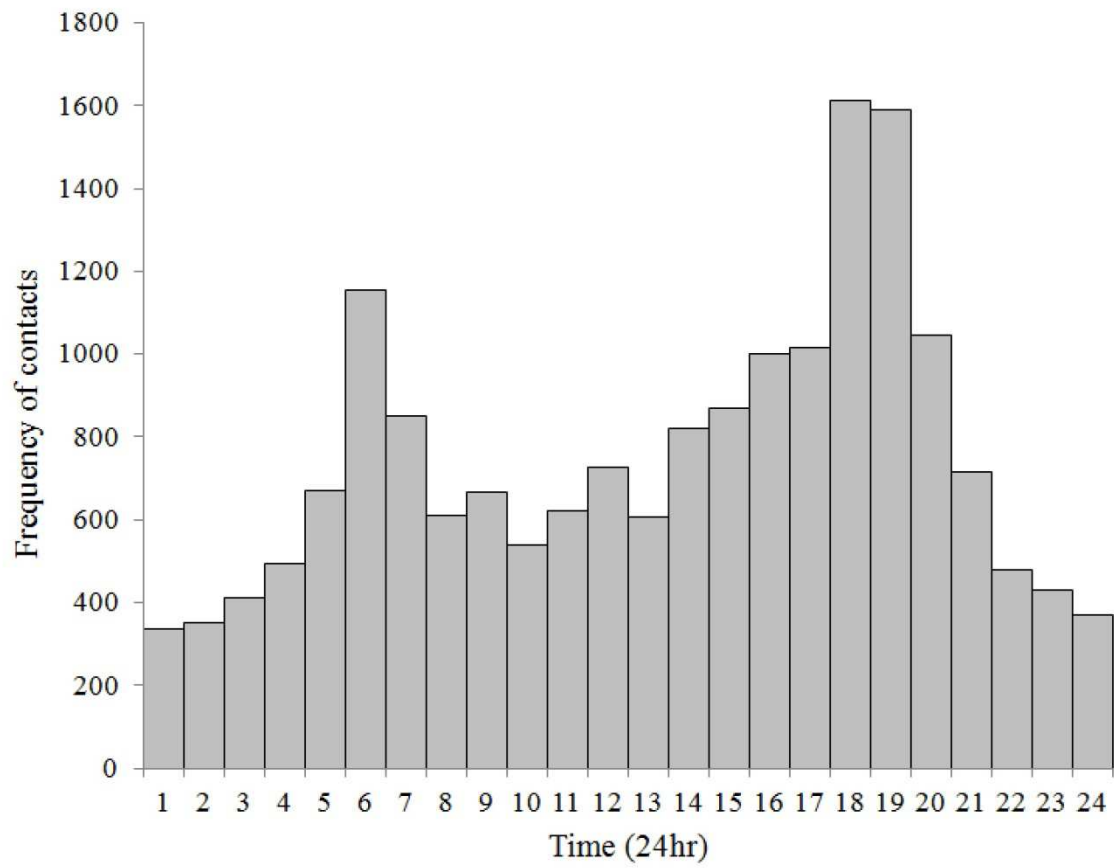Increasingly, variation in individual animal behaviour or unique 'animal personalities' (Gosling 2001) is becoming of key interest in terms of the transmission of disease, parasites and understanding social behaviour within and between populations (Altizer *et al.* 2003; Hamede *et al.* 2009; Marsh *et al.* 2011). Fundamental knowledge on the extent of contact networks, and individual contributions to such networks, is critical in understanding disease epidemiology and assisting in the development and application of management interventions to reduce or eradicate important diseases. Measuring individual contact rate patterns in wild animals is difficult (Hamede *et al.* 2009) and traditionally required long-term observational studies, which has been achieved for some species (e.g. Clutton-Brock and Sheldon 2010; Kappeller *et al.* 2012). Proximity collars potentially offer advantages in determining individual contact patterns as they can be deployed on many individuals within the same group or population and operate continuously, collecting data that otherwise would be difficult, if not impossible to obtain.

The current study has provided for the first time unique data on interactions within and between free roaming badgers and cattle in Northern Ireland. It extends previous research conducted in England by the contemporaneous deployment of proximity collars on several different cattle herds, across at least four known badger territories over the cattle grazing season. It has shown that badger to cattle close range contact did not occur in the study area, even though the density of animals available for interactions to occur was relatively high. In terms of the direct aerosol badger to cattle, or vice-versa, transmission of bTB within the study area the available evidence suggests that this may not represent an important route. Other studies that examined interactions between these species have also found low rates of interspecies contact (e.g. 4 out of 500,000 recorded interactions over a 12 month period; Drewe *et al.* 2013). The current study had a relatively short time-period (3 months) and different animal densities according to cattle herd and badger social group available for contacts to occur, providing a representative sample for the study area during the majority of the period when cattle were grazing. However, whilst the frequency of such contact occurring may be low, the consequences for disease transmission may be high (Drewe *et al.* 2013) if bTB did transfer. Cattle are inquisitive and can exhibit investigative behaviour, therefore, the potential for contact or direct behaviour towards animals such as badgers is possible, particularly when badgers may enter paddocks or fields where cattle are grazing. As cattle can be 20-40 times larger than badgers it seems intuitive that badgers may react adversely to any approach by cattle and the available evidence supports this. Benham and Broom (1989) through direct observation and forced enclosure experiments have shown that badgers avoid or flee from cattle approach, similar to findings with possums in New Zealand

(Paterson 1995). Although it is possible that aberrant behaviour by diseased badgers may increase close range contact with cattle (Paterson and Morris 1995; Ward *et al*. 2010), the extent to which this may occur in the natural environment is poorly understood.

Cattle did not directly interact with badgers in the current study, however, there were over 426,000 contacts recorded within different herds reflecting the gregarious behaviour of these animals. All cattle contacted at least one other individual during the study and displayed significant variation at both a herd and individual level in terms of the number and duration of contacts made. These results illustrate the complexities of within herd interactions between individual cattle and have direct relevance for disease epidemiology at that level. Differences in contact patterns between collared individuals may have been related to their social status within the herd hierarchy, although data on individual characteristics were not available in the current study. Such heterogeneity in contact rates have been found in other studies and it has been suggested that relatively few individuals within a herd may have a high degree of inter-herd connectedness and may be implicated more in relation to between species interactions and possible disease transmission (Böhm *et al*. 2009). The fact the different herds had significantly different levels of contact may suggest that some herds could be more susceptible to within herd spread of bTB after herd exposure, the level of which can be significant (Donnelly *et al*. 2013). Cattle contacted each other throughout the day, but interactions were generally concentrated during daylight hours, during which their predominant behaviour is grazing, whilst at night contacts were generally less, predominantly a time during which cattle are ruminating and resting (Kilgour 2012). Contact measures were negatively related to climate variables but the overall effect was small.

For badgers, the majority of contacts recorded (~99%) were within groups and there were relatively few interactions recorded between groups, even though collared badgers were available for contact in contiguous social groups. This generally reflects the known social organisation pattern of badgers (Roper 2010). Overall contact rates followed a general bimodal pattern throughout the study period with peaks during the early morning and late evening, which probably corresponded to grooming and play behaviour outside setts during animal emergence and return (Fell *et al*. 2006). Contact frequency varied according to social group, month and sex, even at the relatively small scale of investigation indicating how important local studies are when investigating badger behaviour. Badger contact rates were greatest in autumn and least during winter and were higher for females as compared to males, but were lower for both sexes during February to April. In terms of badger ecology these periods are important to badgers from an ecological and reproductive perspective. During the winter/spring, female badgers have given birth and the reduced contact rates

could suggest they largely excluded themselves from other badgers to nurse and protect their young (Weber 2011). Higher contact rates in autumn could reflect increased periods of activity or social interaction outside setts. Badgers were found to be in contact during every hour of the day, although frequencies of contact were generally less between 22.00hrs and 04.00hrs than during the intervening periods. Badgers are typically active at night and can spend considerable amounts of time interacting at dawn and dusk close to their setts. The number of interactions recorded during daylight hours (09.00-18.00hrs) suggest that collars recorded contacts when badgers were resting in their setts. The ability and distance tolerance to which proximity collars can record contacts whilst underground was not explicitly tested in the current study and no details are available in the literature. It is likely that distance tolerance settings from field trials above ground or in laboratory situations would not be relevant to the internal structure of badger setts that often consist of long tunnels, combined with chambers where the UHF signal could be deflected and reflected. Until the performance of proximity collars have been tested in badger setts, it is not possible to comment on whether or not observed contact rates accurately reflect badger behaviour within setts or interference with UHF signals.

The current study has investigated interactions between wild badgers and free-roaming cattle in a region where the incidence of bTB in the cattle was relatively high. It has shown that whilst both species interact to a great extent and with considerable heterogeneity in contact rates within species, there was no close-range interaction between species. In terms of the transfer of bTB through direct aerosol transmission this study, and the available evidence, suggests that this is likely to be an unimportant route, at least in quantitative terms. Although the study took place in a relatively small study area over a short period it does suggest that mitigation or intervention measures that aim to reduce direct contact at pasture may be unnecessary in the study area. It is suggested that, on the basis of the available evidence, indirect transmission routes between these species in terms of direct or indirect contact at focal points such as badger setts, latrines or in farmyards (see Chapter 4) may be more important in terms of reducing bTB transmission between these species.

Quantifying badger visitation rates to farmyards

4.1   Abstract

Bovine tuberculosis is a serious infectious disease of cattle causing animal health issues and considerable economic and logistical hardship to the farming community. A wildlife reservoir for the disease exists in the Eurasian badger (*Meles meles*). Currently there is no quantifiable data on the importance of direct and indirect interspecific transmission routes for the disease, which represents a considerable knowledge gap in terms of disease eradication. In the current study, funded by the Department of Agriculture and Rural Development, badger activity and visitation rates to farm buildings in 11 randomly selected farms in a study area were quantified between July 2012 and August 2013 in Northern Ireland. Visitation rates by other wildlife species were also investigated. Motion activated infra-red cameras were deployed at a maximum of 83 locations of potential badger entry, at a range of building types that occurred in the 11 farms. This was the first study to have conducted continuous surveillance of farms to establish badger visitation rates to Irish farm buildings.

Badger visits occurred in 9 out of the 11 farmyards and during a maximum of 16.13% of nights over the surveillance period. There was significant variation in badger visits according to farm, with a peak during spring and visits were inversely related to temperature. On farms that had badger visits on 10 nights or more (n=4, 36.36%), 75.28% of surveillance images were captured from a single camera location only. Badgers had short duration visits repeatedly to the same farm building types. Badgers positively selected feed stores and significantly avoided cattle sheds during visits. In terms of other species visiting farm buildings domestic cats, rodents and red foxes were of descending importance in terms of visit frequency. Improved farmyard biosecurity coupled with exclusion measures and farmer training should be instigated to reduce the potential for disease transmission on farms.

4.2   Introduction

Despite the various research that has been carried out across the island of Ireland on the role of badgers in the transmission of bovine tuberculosis (bTB) to livestock, there remain significant knowledge gaps in terms of the direction of infection (Biek *et al*. 2012) and importance of direct (close contact) and indirect (e.g. faecal or urinary) transmission routes. It is imperative to address these gaps, as quantifying the relative importance and variability of these routes will lead to requirements for different mitigation strategies to minimize disease spread. Only a few studies have occurred that have considered direct and indirect routes of infection for the disease. Sleeman and Mulcahy (1993) recorded two instances of badgers being near a cattle water trough and milking parlour. Further studies involving 200 lowland cattle herds, using a farmer questionnaire and field survey to determine evidence of badger visits found very low rates (1.4%) of farm visits during winter (Sleeman *et al*. 2008). Using GPS collars placed on badgers and detailed daily locations of livestock that used a series of paddocks on a farm within the collared badger's territory, Mullen *et al*. (2013) showed that foraging badgers significantly avoided paddocks that contained cattle. The authors suggested that indirect transmission was likely to be more important in the disease dynamic. These studies have provided valuable indications of the potential direction of interspecific interactions between badgers and livestock. However, further detailed information is needed to assess the likelihood of contact over a greater number of farms and longer time period to assess potential variation and causative factors.

In terms of disease transmission, farmyards and buildings represent an important area of investigation as they may have features and resources (e.g. animal feed) that could be of interest to badgers. Studies from England have indicated that badger visits can be relatively frequent, involve a variety of buildings, provide a contamination source for animal feed, and be related to climate variables (Garnett *et al.* 2002; Ward *et al*. 2008; Tolhurst *et al*. 2009). The exclusion of badgers from farm buildings may represent the simplest and most effective method of reducing contact and opportunities for disease transmission between badgers and cattle (Ward *et al*. 2010). Exclusions can achieve a high level of effectiveness in reducing badger visits to farm buildings and yards, if properly installed and maintained (Judge *et al*. 2011). Northern Ireland generally has smaller farming practice, scale and herd-size, a milder climate and also different badger ecology compared to Britain. Therefore, it is uncertain if reducing potential contact between badgers and cattle at farmyards is an important area in terms of bTB management and eradication.

In the current study, the first large-scale remote surveillance study of badger activity in farmyards and buildings was conducted in Northern Ireland. The objectives of the study were

to quantify the number of badger visits to farm buildings over a continuous 12 month period; examine what factors may influence visitation rates; and determine what farm building types were selected by badgers. Visitation rates by other species were also quantified at the farm level. These data were required to assess whether badger intrusion into farmyards and buildings represented a potentially important route for the transmission of bTB, requiring specific mitigation measures.

4.3   Materials and Methods

4.3.1 Study site

The study area compromised 11 farms located in Northern Ireland (54°18'N, -5°38'W), in an area with a regionally high incidence of bTB in cattle at an individual and herd level (Fig. 4.1). The study area was approximately 1,350ha in size (see section 2.3.1) with the min-max distance separating farms 0.51-6.37km, respectively. The area was dominated by improved grazing pasture or silage fields (68.24%)) and arable habitat (20.58%), with woodland cover of 2.25%. All study farms had livestock, mainly beef cattle, although there were two dairy farms. Livestock practices in the area generally involved having cattle over-wintered indoors between November and April every year and outdoors in pasture for the rest of the year, although there were a few exceptions. On at least one study farm, beef cattle were kept indoors throughout the year. The number of cattle per farm varied from 20 to 320 individuals.

4.3.2 Camera Surveillance

A survey was undertaken during June 2012 at each farm to quantify the number and type of buildings present and identify potential badger entry points into buildings. The main building types were broadly divided into 5 different categories (Table 4.1). At potential entrance points to buildings, on respective farms, an infra-red motion sensitive camera (Bushnell Trailcamera) was positioned. Cameras were generally placed at least 3m above ground, bolted into place and angled downward to provide a full view of the entrance. The number of cameras deployed on farms varied from one to seventeen, dependent on the number of buildings present. Camera surveillance was initiated in July 2012 and ceased in August 2013, and the mean number of surveillance nights per farm was 381.2 ± 2.38 nights. Cameras were triggered by motion, detected by a highly sensitive passive infra-red sensor (PIR). Prior to being activated each camera whilst in position was fitted with at least a 4GB memory card, checked for correct camera angle, image view, proper operation, date/time and still camera (5MP) mode was selected. Every 3 weeks, each camera was visited, operation and angle were checked, batteries replaced (as necessary) and memory card exchanged. All data were then downloaded to a database for collation and analysis. Each time a badger was observed, the date, time, camera number, location, building type, farm, number of individuals and behaviour were recorded. Data from other contemporaneous studies being undertaken on badgers in the area indicated that study farms were located in at least 10 different badger territories and minimum local badger density was 3.62 individuals per km^2 (see section 2.4).
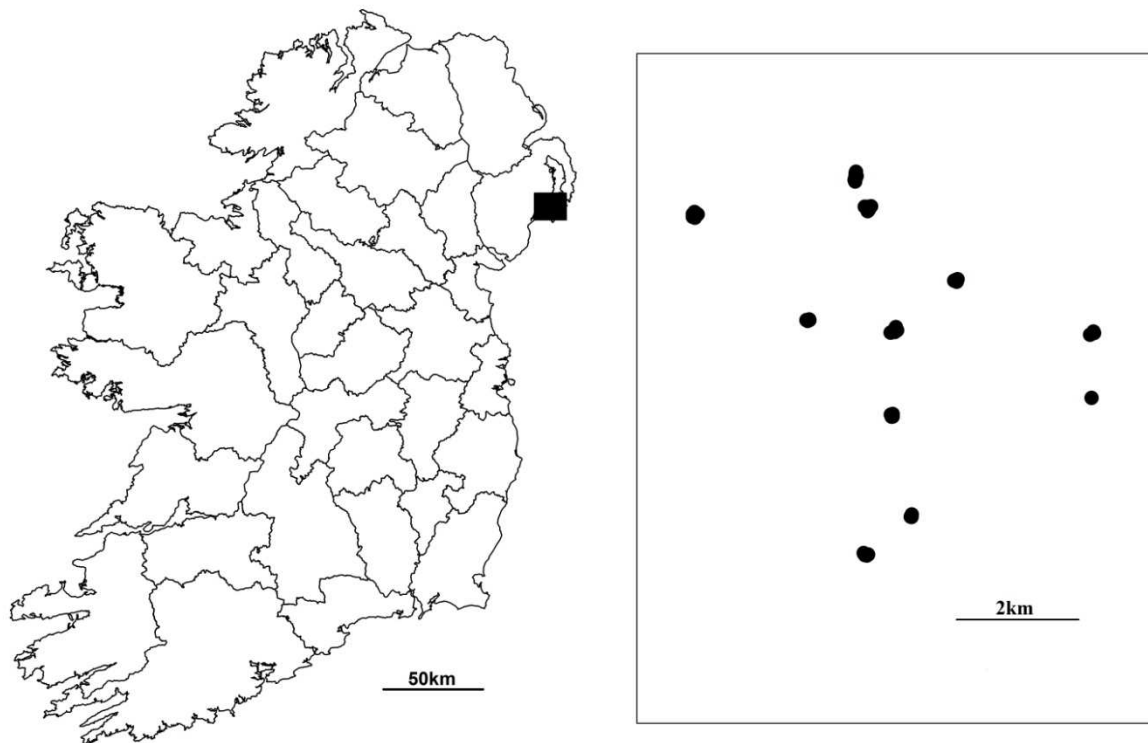
Figure 4.1. Location and distribution (inset) of farms where badger visitation rates into farmyards and buildings were investigated between July 2012 and August 2013.

Table 4.1. Description of the main types of farm buildings encountered during the study.

Building Type	Description
Cattle shed	Building where livestock are placed in confinement for several months of the year
Feedstore	Where animal feeds are stored
Silo	A concrete structure, of variable dimension, that can contain silage, manure, crops etc
Straw/Hay Storage	A building where hay and/or straw is stored
Multi-use	Can contain a variety of contents, including animal feed, straw, vehicles and occasionally livestock

4.3.3 Data Analysis

Data from each camera, on respective farms, were pooled by farm night to produce a farm-level analysis. Badger intrusion into farms was expressed in three different forms: i) a *badger farm-night visit* occurred (1) or did not (0) during each night of surveillance from pooled camera data per farm; ii) *number of badger visits* that occurred on a surveillance night, with

a single badger visit expressed as all those that occurred within a continuous five minute interval, per camera; iii) *duration of badger visits*, where the minimum duration for a single badger image represented one minute and the maximum duration as the total time period during which successive photos were taken, per camera deployed. This level of detail in response variables provided for analyses of basic frequency of badger visits, visitation rates, and visit duration, which are all parameters of interest in terms of possible disease transmission. As not all parts of farms were subject to camera surveillance, data should be seen as minimum level of visits and duration of visits by badgers to farm buildings, during the study.

Variation in the response variables were assessed using General Linear Mixed Models (GLMMs) (Genstat 14.0, VSN International, UK) using a binomial distribution and logit link function for badger farm-night visits (binary); a Poisson distribution with log link function for badger visits (count); and a linear mixed model specified for duration of badger visits (continuous). Fixed explanatory variables included in the model were season (winter = December, January, February; spring = March, April, May; summer = June, July, August; and autumn = September, October, November), total daily rainfall (mm) and minimum daily temperature (°C) obtained from a Met Office recording station located approximately 11.5 km from the study area. Individual farm identity was included as a random effect in each model and number of active cameras deployed per farm night was included as a log-transformed fixed covariate in all analyses. Dispersion parameters were estimated for each model to account for potential over-dispersion. Significance testing used Wald tests for fixed variables and inference on the direction of effects using *t*-tests, accepted at less than the 5% level.

To examine any selection of building types, data were combined across all farms, the total number of active camera nights (equivalent to survey effort) per building type was calculated and total frequency of badger farm-night visits to building types determined. A selection analysis using Bonferroni confidence intervals (95%) that employed chi-square goodness of fit tests for observed proportion of use (badger images) and availability (active camera nights per building) following Neu *et al.* (1974) was undertaken. Where individual animals were identifiable through deployment of proximity collars (see Chapter 3) then their contribution to badger visit rates were investigated.

Although badgers were of primary interest in the study, visitation rates by other important species to farm buildings were also quantified during the 12 month study period. Farm visits by red fox (*Vulpes vulpes*), domestic cat (*Felis catus*), rodents (including rat *Rattus*

norvegicus; and Muridae (*Mus* and *Apodemus* spp.) were recorded as binary data; either a visit occurred (1) or did not occur (0) during each night of surveillance on each farm from pooled camera data. This represented the minimum intrusion level by other wildlife species into farmyards during the study. Variation in the response variables (red fox, cat, and rodent, respectively) were assessed using General Linear Models (GLMs) (Genstat 14.0, VSN International, UK) using a binomial distribution and logit link function. The only fixed factor considered was individual farm ID. Additionally General Linear Mixed Models (GLMMs) were used to assess variation in response variables (red fox, cat, and rodent, respectively) with fixed explanatory variables included in the model specified as season, total daily rainfall (mm) and minimum daily temperature (°C). Individual farm identity was included as a random effect in each model and number of active cameras deployed per farm night was included as a log-transformed fixed covariate. Dispersion parameters were estimated for each model to account for potential over-dispersion. Significance testing used Wald tests for fixed variables and inference on the direction of effects using *t*-tests, accepted at less than the 5% level.

4.4 Results

Throughout the surveillance period, badgers visited 9 out of the 11 farms on at least one night and on between 0 to 16.13% of farm surveillance nights (Fig. 4.2). Four farms (36.36%) had badger farm-night visits on more than ten nights during the monitoring period. Badgers visited farms between 18.00 and 06.00, with the median time of visits occurring at 00.15 (Fig. 4.3).

The frequency of badger visits varied significantly with season (GLMM, F=10.08, d.f.=3, 3522.9, P<0.001, Fig. 4.4), being greatest in spring and was negatively related to minimum daily temperature (GLMM, F=25.29, d.f.=1,3525.6, P<0.001). A single farm accounted for 38.81% of all badger visits during the study. The effect of total rainfall on variation in badger visits to farm buildings approached significance (GLMM, F=3.91, d.f.=1,3522.8, P=0.05) with badgers most likely to visit yards during periods of low rainfall. The duration of badger visits to farm buildings was negatively influenced by temperature (GLMM, F=11.42, d.f.=1,3525.6, P<0.001). Annual frequency of badger farm-night visits and badger visits were not correlated with distance to nearest main badger sett.

The estimated total duration of badger visits recorded during surveillance monitoring was 626 minutes (10 hrs 43 mins.). In total, 56% (349 mins.) of the duration of all badger visits occurred on a single farm. Individual visit duration ranged from 1 to 39 minutes. In three of the four most visited farms (i.e. on 10 nights or more) the frequency of badger visits to surveillance camera locations/buildings was significantly different from random (χ^2 = P<0.001 in all cases), with a single camera on each farm recording on average 75.28% of the badger visits. In the other most visited farm, 100% of badger visits occurred at the location of a single surveillance camera (feed silo), despite nine cameras being deployed during the study.

During badger farm-night visits to farm buildings, meal stores were positively selected and cattle sheds were avoided (Table 4.2), although badgers were observed close to cattle sheds on 31 different farm nights. On the most visited farm where 38.81% of all badger visits occurred, an individual badger was identified and accounted for 91.2% of all visits to that farm.

The main other species that visited farm buildings were cat, rodents and red fox (Table 4.3). Cats visited all farmyards during the study, ranging from 11.22 to 94.13% of surveillance nights, with rodents and foxes recorded on fewer occasions. Visit data combined across all farms for the four species over the duration of the study indicated that cats were the most

frequently observed species (60.33% of visits) and badgers were the least (3.89% of visits) (Figure 4.5). There was significant variation in cat (GLM, F=349.9, d.f. = 10, P<0.001), red fox (GLM, F=117.8, d.f. = 10, P<0.001) and rodent (GLM, F=176.06, d.f. = 10, P<0.001) visit rates across the different study farms. For cats, variation in visitation rates to farmyards was negatively related to temperature (GLMM, F=7.60, d.f.=1,4295.4, P<0.005) and there was significant seasonal variation (GLMM, F=24.39, d.f.=3,4297.1, P<0.001) with visits least during winter and greatest during autumn. For the red fox, variation in visitation rates to farmyards was negatively related to daily rainfall (GLMM, F=6.01, d.f.=1,4294.9, P<0.005) and there was significant seasonal variation (GLMM, F=5.58, d.f.=3,4296.2, P<0.001) with visits least during winter and greatest during summer. For rodents, variation in visitation rates to farmyards was positively related to daily rainfall (GLMM, F=9.51, d.f.=1,4295 P<0.005), negatively related to temperature (GLMM, F=33.14, d.f.=1,4295 P<0.001) and there was also significant seasonal variation (GLMM, F=38.19, d.f.=3,4300.7, P<0.001) with visits greatest during winter and least during summer.
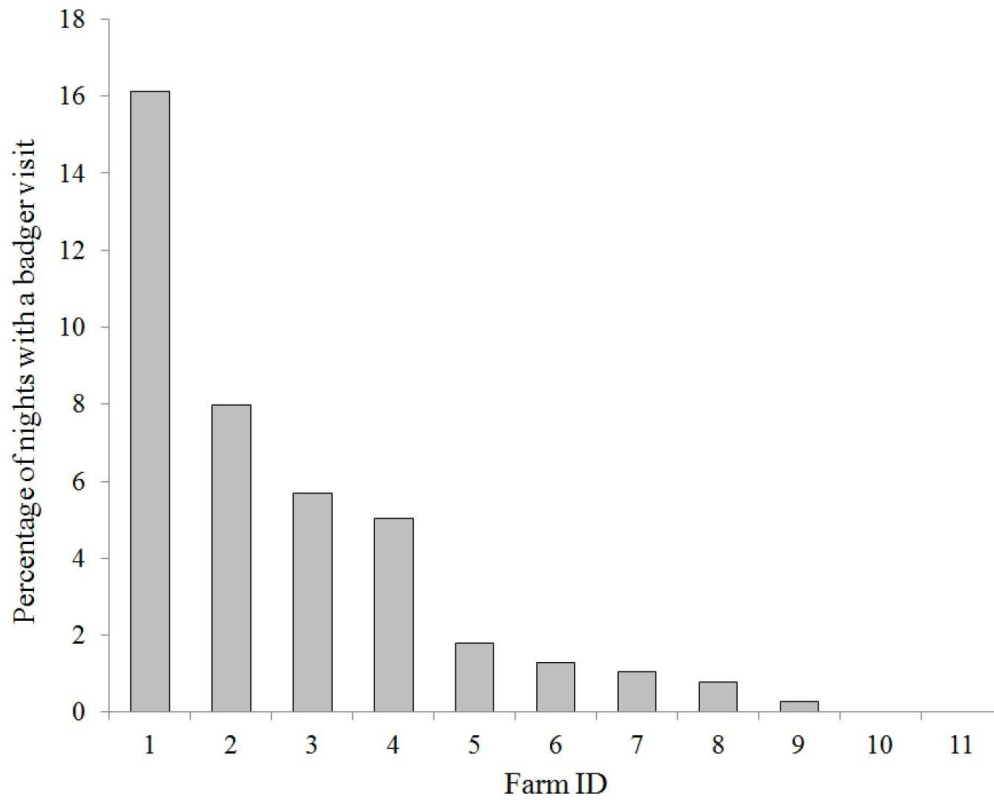
Figure 4.2. Percentage of badger farm-night visits to eleven study farms between July 2012 and August 2013.
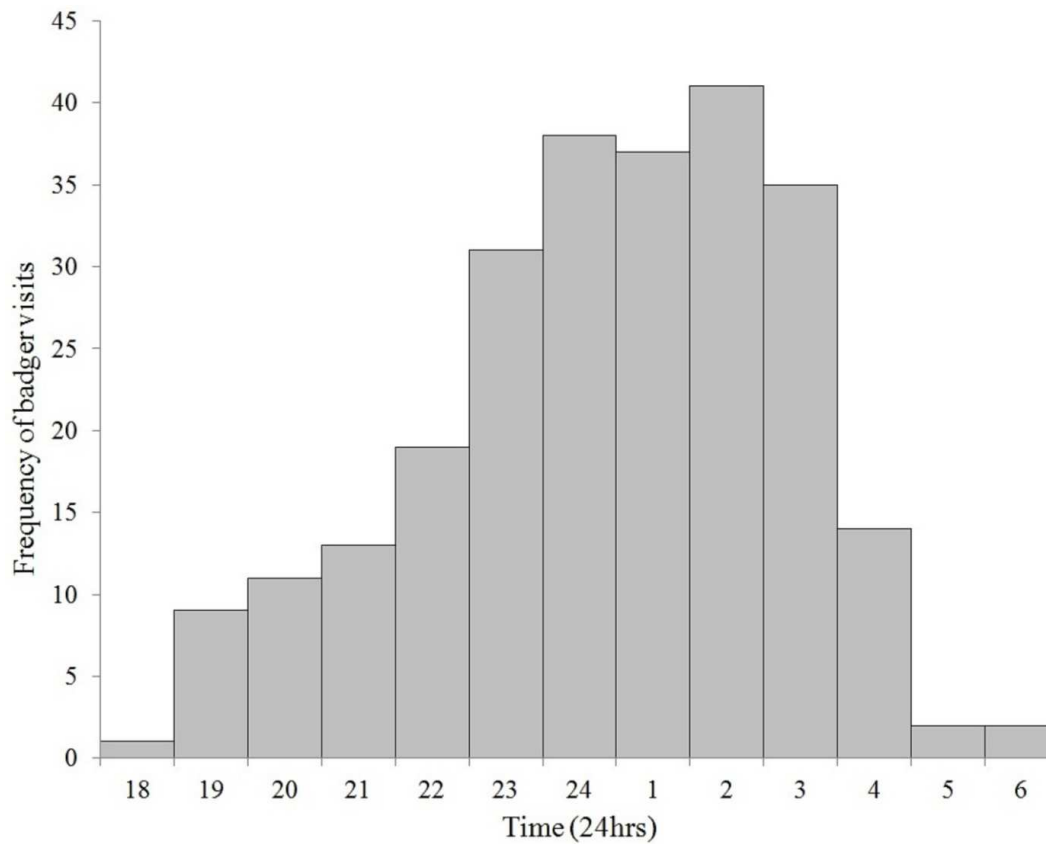
Figure 4.3. Cumulative frequency of the time of badger visits to farm buildings over 12 month period.
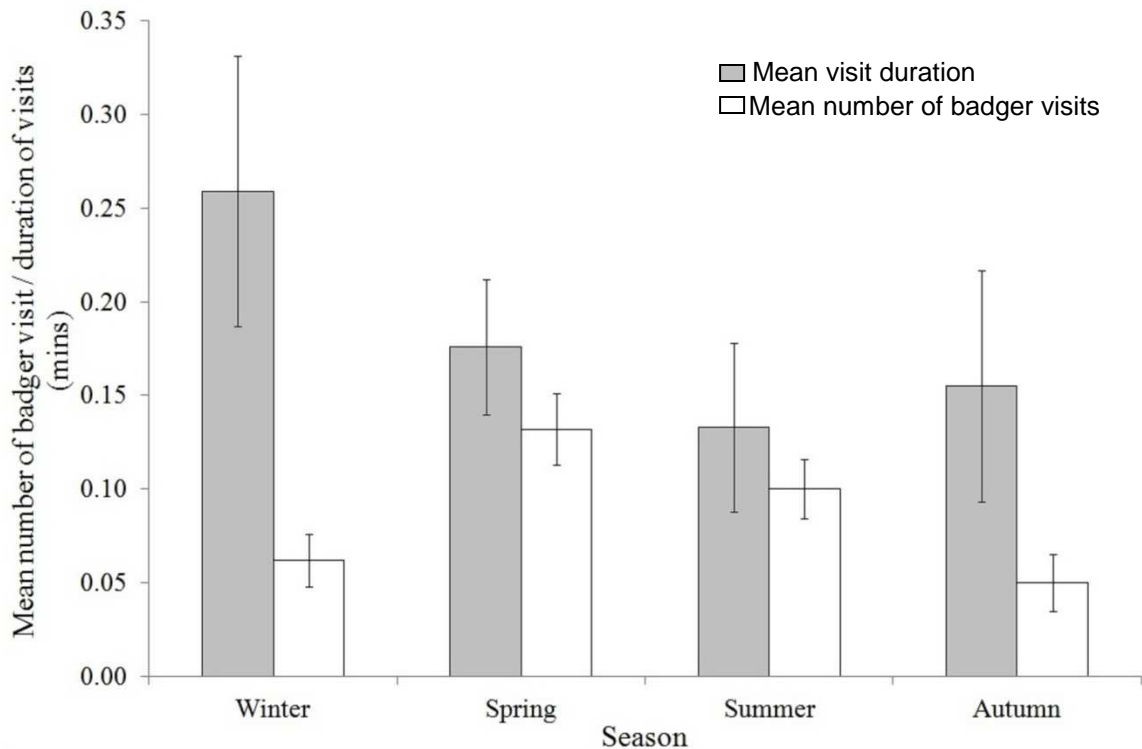
Figure 4.4. Mean daily number of badger visits and duration of visits (in minutes) by season, across 11 study farms during 2012-13. Data is pooled across all farms ±SE. Y-axis scale is equivalent for daily frequency (count data) and visit duration (minutes)

Table 4.2. Selection of buildings types by the Eurasian badger (*Meles meles*) during farm-night visits to 11 farmyards between July 2012 and August 2013. Confidence intervals are 95% Bonferroni, significant selection ($P<0.05$) indicated by + (positive selection); - (negative selection); 0 (no selection).

Type	% Camera Nights	Observed	Expected	LCL	UCL	Selection
Cattle	0.61	31	95	0.11485	0.280054	-
Meal	0.06	55	9	0.213251	0.487386	+
Multi use	0.04	4	7	-0.03237	0.083325	0
Silo	0.21	45	33	0.08453	0.488718	0
Straw	0.08	22	12	-0.04271	0.322968	0

Table 4.3. Percentage of surveillance nights during which cat, red fox and rodents were recorded on at least one camera, during each surveillance night across 11 study farmyards between July 2012 and August 2013.

Farm ID	Cat	Red fox	Rodents
1	92.35	5.87	0.51
2	67.60	0.51	34.95
3	50.00	34.95	15.05
4	40.56	12.24	25.51
5	85.97	18.62	9.69
6	82.40	16.58	0.26
7	94.13	7.40	40.05
8	11.22	7.40	12.50
9	35.46	46.43	52.55
10	26.02	17.09	0.51
11	19.90	0.51	0.00

Figure 4.5. Percentage (in brackets) of all visits by different species to 11 farmyards between July 2012 and August 2013.

4.5   Discussion

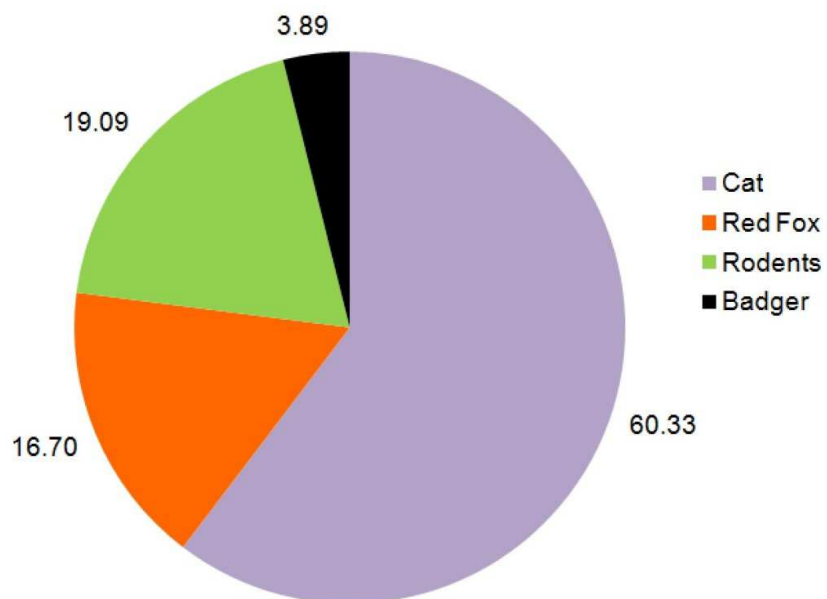This is the first study undertaken to quantify badger intrusion rates into farmyards and buildings across the island of Ireland through continuous surveillance monitoring over a twelve month period. It has been shown that badger visits to farmyards and buildings occurred rarely, or not at all in 64% of the farms investigated, but was relatively frequent in the remainder of sample farms. Overall, badger visits occurred at levels greater than previously thought (Sleeman *et al.* 2008) but not as often as conspecifics in Britain where visits have occurred on up to 71% of surveillance nights (Judge *et al.* 2011), compared to a maximum of 16.1% in the current study, although results may not be directly comparable. The majority of British studies have been undertaken in areas with greater badger density (5.8 to >30 per km^2; Cheeseman *et al.* 1981), compared with an estimated badger density in the current study of 3.62 per km^2. Seasonal variation in badger visits to farms, with a peak in spring, has been found in other studies and may be related to aspects of badger ecology and energetics rather than food availability (Tolhurst *et al.* 2009; Judge *et al.* 2011). Spring coincides with a peak in badger territory delineation (Delahay *et al.* 2000) during which badgers can move large distances that could provide an increased opportunity for the species to visit farms. However, badgers were shown to visit yards all year round and therefore, whilst inherencies in badger ecology may influence visitation rates during some periods, other factors could have had an impact throughout the year.

In the current study, badger visits to farmyards were more likely at low temperature, although the effect was relatively small. Although the temperature extremes in the study area were not particularly pronounced (-3.5 to 21.6°C; 18 nights with minus °C temp.) it may suggest that resource availability could have been an important factor influencing badger visits. In Ireland during seasons when temperatures are generally lower, important prey items include noctuid and tipulid larvae that overwinter in soil (Cleary *et al.* 2009) and may be relatively unavailable to badgers leading to increased farms visits. Badger movements during periods of low temperature are also generally reduced (Goszczyński *et al.* 2005) and visits to farmyards, in what was a relatively compact study area (mean distance from study farms to nearest main sett was 353.4 ± 42.8m), may provide a valuable trade-off in terms of energy expenditure/gain. In England, badger visits to farms are influenced by periods of increased temperature and low rainfall, which may reduce the abundance and availability of a key prey item, the earthworm (*Lumbricus terrestris*) (Tolhurst *et al.* 2009). Although the current study did not show similar climate relationships, rainfall was in the same direction but not significant, it did have similar outcomes i.e. badgers were more likely to visit buildings during conditions that potentially affected prey availability. Climate also had an influence on the duration of badger visits to farm buildings, suggesting longer visits were more likely during

dry periods. Seasonal visit duration by badgers to farm buildings in the current study was lower than those found in England (Tolhurst *et al.* 2009).

In terms of other factors influencing badger visits to farm buildings during the study, the exploitation of potential on-farm food resources was important as indicated by the positive selection of feedstore building types during farm-night visits. Badgers are opportunistic foragers and it is not surprising that they visit farms when foraging (Garnett *et al.* 2002). In the four most frequently visited farms in the current study, badger activity was concentrated at buildings where potential sources of food including spilt grain outside an animal feedstore, crimped wheat silo, hay/straw storage sheds and manure piles were located. In these farms, 86% (n=121) of badger farm-night visits were located at buildings where food was potentially available. In England, badgers also visit feed stores significantly more than cattle sheds (Tolhurst *et al.* 2009; Judge *et al.* 2011). The buildings visited tended to be used repeatedly on different nights, for short duration visits. Badgers did not tend to visit multiple buildings during visits, as attested by the few visits to more than one camera on any single surveillance night across study farms (~10% of visits). The current study did not investigate the behaviour of badgers inside buildings although it did monitor entrance points and found little evidence of badgers entering buildings, irrespective of whether they were associated with cattle. There was no direct 'nose to nose' (≤10cm contact) contact between badgers and cattle observed during the study, the avoidance by badgers of buildings associated with cattle may have limited the opportunity for this.

An individual component to visits was determined in at least one farm, where the same individual repeatedly visited the same building (feed store) on multiple occasions throughout the surveillance period, accounting for over 90% of all visits to that farm. Therefore, learned behaviour either through general foraging in yards or perhaps an inter-generational taught component may have been involved. A small proportion of a wildlife population may be responsible for most visits to farms and transmitting disease pathogens, as was suggested for white tailed deer (*Odocoileus virginianus*) in parts of the USA, where 19% of individual deer were responsible for 88% of yard visits (Bertensen *et al.* 2013). In high density badger populations (25.3 adults per km^2; Rogers *et al.* 1997), up to 26 individual badgers have been observed visiting two farms in England (Garnett *et al.* 2002). Therefore, there is likely to be considerable variation in any individual component to visitation rates to farms but future studies should incorporate individual animal identification to assess this.

Increasingly studies in England have focused on assessing badger access and utilization of farm buildings as a potentially important source of disease spread. These studies have

confirmed that in areas with relatively high badger densities and where bTB has a high prevalence, badgers can regularly visit farm yards and buildings, contaminate animal feeds and have close contact with cattle (e.g. Garnett *et al.* 2002; Roper *et al.* 2003; Ward *et al.* 2008; Tolhurst *et al.* 2009). In some instances bTB badger carcases have been found in farms that have tested positive for *M. bovis* (Ward *et al.* 2008) with limited evidence suggesting that badgers found dead on farms have a higher level of bTB than those killed by cars on roads (Cheeseman and Mallinson 1981).

Other species visited farm buildings during the study, with domestic cats recorded with the greatest visit frequency and badgers with the least, in overall terms. Other important species that visited farmyards were rodents and red fox. Individual species variation in visitation rates by season and climate variables may reflect differences in resource availability and potential inter-specific interactions between the species including predator avoidance. For instance, rodents were more frequently recorded during winter, when red fox visits were least frequent. The importance of other species in the epidemiology of disease transmission to livestock is poorly understood but all the different species groups that visited farm buildings in the current study have been recorded as being infected with *M. bovis* (Delahay *et al.* 2007), albeit at low levels. Other pathogens including *Salmonella* and *Cryptosporidium* are known to occur in red foxes (Tolhurst *et al.* 2011) and rodents can carry a variety of diseases, viruses and parasites. The recent finding of cat to human transmission of *M. bovis* in England highlights the potential importance of other species in the epidemiology of disease transmission even though the risk of cat to human transmission is deemed to be very low (HAIRS 2014). The majority of cats observed in the current study were likely to have been feral, a status which may influence disease status. Active disease surveillance programs do not generally occur for wildlife species other than badger, and the extent of undiagnosed, sub-clinical *M. bovis* infection in non-bovine species is unknown and warrants further epidemiological research (Broughan *et al.* 2013).

The use of infra-red surveillance cameras in the current study proved successful in establishing baseline information on levels of badger activity at farm buildings. There were limitations with camera use, however, including that not all parts of the farm could be monitored, they required regular maintenance, are potentially obtrusive to farmers, have natural blind spots and are relatively expensive. Additionally, a number of cameras had to be replaced (approximately 15%) due to either damage from farm equipment, malfunction or technical failure. Surveillance cameras used in wildlife studies have a number of technological limitations but they remain an exceptional scientific tool (Rovero *et al.* 2013). Given strategic placement to cover key points of interest, regular maintenance and a reserve

stock of at least 15-20% of deployed units, infra-red surveillance cameras offer a hitherto unparalleled opportunity to rapidly quantify wildlife intrusion rates in farmyards and buildings, offering site specific data that can be incorporated into disease management strategies.

4.5.1   Management Implications

The process of disease transmission between wildlife and livestock is a complicated, multifaceted process that involves bidirectional interactions among animals, pathogen communities, and environments (Alexander *et al.* 2012). In the current study badgers were found to visit some farms relatively frequently but there was substantial variation across farms, even at the relatively small scale of investigation. This highlights the requirement for site specific information on local patterns of badger intrusion to inform management strategies. Whilst this may not be possible in all occasions, it should be seen as a priority in establishing the likelihood of badger visits to farms and buildings. If resources were insufficient to allow detailed medium to long-term studies, then concentrating survey effort in spring could serve as a proxy to obtain relevant data.

Although badgers in the study farms appeared to utilise farms in a manner that led to little direct contact with cattle, there remained the potential for contamination of feeds and yards with badger excretia that requires further attention. Comprehensive disease management or eradication strategy requires a multi-faceted approach to limiting opportunities for disease spread both inter and intra-specifically. Therefore, increased farm biosecurity should be considered an essential part of overall disease management, and to the protection of herd health (Mee *et al.* 2012). Apart from the protection of feedstores, biosecurity standards on study farms, in terms of limiting badger entry to yards and buildings, were generally poor. It has been shown that relatively simple exclusion measures once deployed and maintained appropriately, can be 100% effective in excluding badgers from farm buildings and reducing badger activity in yards (Judge *et al.* 2011). Similarly, exclusion of badgers from crop fields using fencing can be very successful (Poole *et al.* 2002). Reducing badger activity and intrusion into farmyards and buildings can cause shifts in animal ranging behaviour and core activity areas, which do not result in extra-territorial movements suggesting that farm resources may not be a critical food source for badgers (Tolhurst *et al.* 2008). A greater emphasis should be placed on increasing farmers awareness of biosecurity issues and the cost-benefit of installing and maintaining exclusion measures be assessed.

Another potential area of investigation could be the potential role of farm dogs in limiting wildlife entry into farms. Livestock protection dogs have been used for thousands of years to protect livestock from predators (Gehring *et al.* 2010a) and increasingly it is being suggested

that they may also be useful in limiting contact between wildlife and livestock in terms of disease transmission (Gehring *et al.* 2010a). In the USA white-tailed deer have been implicated in the transmission of bTB to cattle (Schmitt *et al.* 2002) and deer can be frequent visitors to farm pastures and yards (Berentsen *et al.* 2013). Livestock protection dogs can reduce direct and indirect contact between deer and livestock at pasture and feedlots (VerCauteren *et al.* 2008; Gehring 2010b). Farmyard dogs are common across the island of Ireland, and interactions between, or deterrent effects on badger visits to farmyards are poorly understood. In the current study only two farmyards had continuous dog presence, and any impact on badger visitation rates could not be assessed, but it may be worth considering in future studies.

Although there was considerable variation in badger visit rates between farms, there was some consistency in terms of the pattern of visitation throughout the sampling period. Badgers visited farmyards and buildings more frequently during spring; were more likely to visit during periods of low temperature; had a tendency to visit the same building types and parts of the farm regularly; had relatively short duration visits; positively selected feed store and straw/hay buildings and avoided buildings associated with cattle. There was also limited data that indicated an individual behavior component in badger visits to farms. This data can be used to support the development of mitigation measures to limit potential interspecific disease transmission in farmyards across the island of Ireland.

General Discussion

5.1 General Discussion

This research project has carried out an integrated study of a badger and cattle population that inhabited an agricultural landscape with a regionally high incidence of bovine tuberculosis (bTB) in livestock. It aimed to quantify the importance of potential disease transmission routes for the spread of bTB between badgers and cattle, concentrating on i) direct transmission through close-range contact of badgers and cattle at pasture, and ii) assessing badger visitation rates to farm buildings. The study has also gathered ecological data on this previously unstudied badger population and combined traditional field survey techniques with recent technological advancements including GPS collars and proximity devices.

In terms of the ecology of badgers, the study found that estimated mean sett density varied from 0.88 to 1.11 main setts per km^2. Badgers lived in social groups that ranged in size from 2 to 12 individuals with the overall mean 3.73 ± 0.95 badgers per social group. Population abundance analysis using closed capture-recapture models provided a total abundance estimate of 50.66 badgers (95%CI 29.35-85.26) in the study area or a mean density of 3.62 badgers per km^2. Badgers had a territorial system of social organisation with individual territories ranging from 43.19 to 161.29ha, with a mean territory size of 117.80 ± 11.28ha. Data from GPS derived locations for nine individual badgers indicated that the mean nightly movement distance was 1,321 ± 34.5m, with the maximum recorded in any single night as 6,541m. Badgers moved the least distance during winter months and had non-random use of habitats, generally selecting woodland and grassland and avoiding farmyards and domestic buildings. The general patterns of social organisation and spatial ecology determined for badgers in the study area conformed to what we know about the species in the UK and Ireland.

Through the deployment of proximity collars on badgers and cattle in the study, unique data quantifying interaction rates was collected from Northern Ireland. In total, 426,807 contacts were recorded for cattle and 12,969 for badgers within the study area during 2012 and 2013. Direct, close-range contact (≤2m) did not occur between badgers and cattle during the study indicating that aerosol transmission of bTB within the study populations was unlikely to have occurred. This corroborates the findings of other studies carried out in England (Drewe *et al.* 2013). Inter-group contacts did not occur between cattle during the study as contiguous herds were not collared, and occurred at too low a rate for badgers (0.35% of all contacts) for analysis. Interactions between badgers occurred during every hour of the day and had a

bimodal pattern with peaks from 06.00 to 07.00hrs and 18.00 to 19.00hrs, corresponding to peak activity levels at sett entrances when badgers tend to groom and exhibit play behaviours. Contact rates significantly varied by month, social group and sex with lowest rates in winter/spring, and greatest for females compared to males. Cattle interacted more than badgers in terms of the mean number of daily contacts, with most contacts occurring between 08.00-21.00hrs. Contact rates for cattle where influenced by month, farm and climate variables. There were also significant differences in contact rates at the individual level within herds, indicating considerable individual heterogeneity within contact networks, which may have direct relevance to disease epidemiology within herds. Relatively few individuals within a herd may have a high degree of inter-herd connectedness and could be implicated more in relation to inter-species interactions and possible disease transmission (Böhm *et al.* 2009).

Badger intrusion rates into farm buildings in Northern Ireland were unknown prior to the current study, which represented the largest-scale continuous farm surveillance project undertaken anywhere across the island of Ireland. Individual buildings within 11 farmyards were monitored between July 2012 and August 2013 using motion activated infra-red cameras at a maximum of 83 locations of potential badger entry to buildings. Throughout the surveillance period, badgers visited 9 out of the 11 farms on at least one night and on between 0 to 16.13% of farm surveillance nights. Four farms (36.36%) had badger farm-night visits on more than ten nights during the monitoring period. Other data from GPS collars indicated that 0.65% of locations where located within farmyards. Badgers visited farms between 18.00 and 06.00 and the frequency of visits varied significantly, being greatest in spring and were negatively related to minimum daily temperature. A single farm accounted for 38.81% of all badger visits during the study. Annual frequency of badger farm-night visits and badger visits were not correlated with distance to nearest main badger sett. Badgers had short duration visits repeatedly to the same farm building types. Badgers positively selected feed stores and significantly avoided cattle sheds when visiting farmyards during the study. No close range contact between badgers and cattle in farmyards were found during the study. The frequency of other species visiting farm buildings particularly domestic cat, rodents and foxes merits further research in terms of potential implications for disease transmission. Although badgers in the study farms appeared to utilise farms in a manner that led to little direct contact with cattle, there remained the potential for contamination of feeds and yards with badger excretia. Improved levels of farmyard biosecurity coupled with exclusion measures and farmer training should be instigated to reduce the potential for disease transmission at farmyards and buildings.

5.2   Conclusions

This project aimed to quantify potential transmission routes between badgers and cattle in an area of Northern Ireland with a regionally high incidence of bovine tuberculosis (bTB) in livestock. The results clearly indicated that direct contact between badgers and cattle at pasture did not occur and probably represents a rare event in terms of bTB transmission between badgers and cattle. Whilst the study area was relatively small and number of animals collared moderate, these results are supported by other comparable studies and showed consistent patterns in terms of direct contact being negligible. Whilst direct contact may of course occur between these two species, it is likely to be at a low frequency and would be difficult to manage without direct intervention through initiatives such as culling or large-scale wildlife exclusions.

Other than direct transmission between badgers and cattle at pasture, the study also investigated badger visitation rates to farm buildings as this may have been an important venue for interaction and disease spread between these species. The majority of farmyards were visited infrequently by badgers over 12 months and there was considerable variation in visitation rates across farms. Four farmyards were visited on more than ten nights by badgers and the tendency was for relatively short duration visits, repeatedly to the same building types. Building selection analysis indicated that badgers significantly selected building types associated with feed and food availability may have been the driving factor influencing badger visits to farms. Whilst nose-to-nose (<10cm) contact was not observed during the study, there remained the potential for disease transmission at this interface.

It is suggested that in terms of reducing potential disease spread between badgers and cattle it is important to concentrate further research into the management of indirect contact venues for these species. Important areas to consider are setts, badger latrines and limiting access to farm buildings. Relatively simple measures could be instigated to reduce interactions at these key potential indirect routes of bTB transmission and effectively limit contact to very low levels. The cost-effectiveness and efficacy of such measures also needs to be further assessed.

5.3   Research Recommendations

There are three principal recommendations from the current research:

i)   Quantify the levels of indirect contact that occur between cattle and badgers at setts, latrines, feed and water troughs, mineral licks and other potential interfaces to understand indirect transmission routes of bTB between badgers and cattle.

ii)   Evaluate the cost-effectiveness and efficacy of introducing measures to limit badger access to farmyards and buildings.

iii)   Provide further data on heterogeneity of contact networks at an inter- and intra-group level for badgers and cattle.

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Appendix One

Population Genetics of the Eurasian Badger (*Meles meles*) in the study area

Introduction

In addition to the research detailed in the main body of this report, population genetic parameters were tested among the badgers surveyed. These data had the capacity to inform on the population structure of the area surveyed, specifically to indicate if there was any differentiation observed between setts, which may be indicative of territorial behaviour.

Materials and Methods

The laboratory was supplied with 47 individual hair samples from the study area during 2012. Genomic DNA was extracted from these, and they were subjected to analysis of short tandem repeat (STR) loci from 15 locations in the badger genome. Markers used have previously been characterised by Carpenter *et al.* (2003). Random 5% retyping of isolates was undertaken to ensure repeatability of DNA profiles obtained and to quality assure data.

DNA profile data from individual animals were subsequently analysed for standard population genetic metrics including allele frequency, observed heterozygosity, expected heterozygosity fixation indices by Genepop (Rousset 2008) and Fstat (Goudet 2001) programs. Identification of potential sub populations was carried out using the program Structure v2.3.4 (Pritchard *et al.* 2000) and the method of Evanno *et al.* (2005) as implemented in the web application Structure Harvester (Earl and vonHoldt, 2012) was used to determine the most likely number of sub populations.

Fixation statistics between identified sub population fragments were determined using FSTAT and the nmod package (Winter, 2012) in the R environment (R Development Core Team, 2011). Assessment of likely full sibling and half sibling relationships between individual animals was assessed by the program Colony (Jones and Wang 2009).

Results

From the 47 hair samples tested, 4 were unable to be genotyped because of poor DNA quality. The remaining 43 samples produced full good quality DNA profiles at all 15 genetic loci tested. 10 samples were repeat samples of already tested animals on account of their profile being completely identical at all tested loci. Repeat samplings of individual animals were observed to occur from the same sett at which they had previously been sampled.

The remaining 33 samples were unique to the dataset and represented individual animals associated with the setts they were captured at. A full breakdown of numbers of hair samples per sett are shown below:

Sett A – 8 animals; Sett B – 6 animals; Sett C– 4 animals; Sett D – 2 animals; Sett E – 2 animals; Sett F – 2 animals; Sett G – 2 animals; Sett H – 2 animals; Sett I – 2 animals; Sett J – 2 animals; Sett K – 1 animal.

5% repeat genotyping of random samples produced identical DNA profiles indicating consistency of allele calling and quality of data. Expected heterozygosity across the whole Lecale area, if Hardy Weinberg random mating / panmixia was in evidence, was estimated to be 0.496. Observed heterozygosity across the whole area was 0.461.

After Structure analysis and implementation of the Evanno *et al.* (2005) method, two distinct sub populations were observed to reside in the study area. Population 1 was centred on Setts A and D which were in close proximity to one another (approx. 1.5km). Population 2 was centred on the Sett B with satellite setts C, E, F and K. Sett G, H, I and J appeared to accommodate badgers from both populations in a small area of land between the population 1 and population 2 sett associated locations (Fig. 1).

Fixation indices between population fragments 1 and 2 as defined by Structure were assessed by both FSTAT and nmod programs. F_{ST} as calculated by FSTAT between both subpopulations was 0.1538 ($p<0.05$) indicating significant genetic differentiation. G''_{ST} as calculated by nmod was 0.26. Both metrics indicated substantial genetic differentiation between sub populations. Data analysis with Colony indicated that there were 13 full sibling relationships among the 33 sampled animals.Ten of these relationships (77%) were observed within sett A, with the remaining 3 (23%) found in sett C.

Figure 1 – Structure output indicating population assignment of individual badgers from each sett to either of the two sub populations identified by the Evanno *et al.* (2005) method.

Seventy-four half sibling relationships were observed. Animals from setts A and B (sub population 1) accounted for 24 (30%) of these relationships. Of these half sib relationships observed for these setts, the majority (13 / 55%) were found to be within sett occurrences, whilst of the remaining 11 inter sett half sib pairs, the majority (7) were observed to be between sett A and D. Forty-one (55%) of the total number of half sib relationships were observed to occur in the setts that constituted sub population 2. Of these, 12 were within sett occurrences with the remaining 29 being inter-sett in character.

Discussion

The preliminary data presented here are indicative of badger sub population differentiation over a small distance in this region of County Down. The initial finding that the 10 observed duplicate re-samplings of the same animals occurred at the same sett location of initial capture was suggestive, albeit not very robustly of philopatry / territoriality perhaps being a feature of the population under study. The observed heterozygousity being approximately 4% less than that expected was also initially indicative that some level of inbreeding was

occurring which may be consistent with non random mating / panmixia – again this may be a feature of population divided into smaller sub-populations.

Structure analysis seemed to confirm this hypothesis by indicating the presence of two distinct sub populations which exhibited significant genetic differentiation. Sub population 1 was the smaller of the two and most limited in geographic range, taking in only the 10 animals of setts A and D. By comparison, sub population 2 was more geographically widespread taking in the 15 animals of setts B, C, E, F and K.

Colony analysis seemed to complement the previously discussed data by suggesting that the animals from sub population 1 exhibited a greater level of genetic isolation from sub population 2. Evidence for this included the fact that animals from these setts accounted for most cases of full siblings across the whole dataset. Where incidents of half sibships occurred, they were primarily within sub population 1 setts, or to lesser degree between them. By contrast, sub population 2 exhibited very few cases of full sib ships, and whilst intra sett half sib ships were common, inter-sett half sib ships across the sub population's range were observed to predominate.

These data are consistent with badger sub population differentiation occurring at a very local scale over short distances. This may be a result of territoriality, a feature also observed in other badger populations in Britain and Ireland. Social structure like that observed here has been observed to be prone to disruption / perturbation by culling.

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