

# **Badger social biology and its effects on bovine TB control**

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## ABSTRACT

It is now widely regarded that in the United Kingdom and the Republic of Ireland that the European Badger (*Meles meles*) plays a role in the maintenance and transmission of bovine tuberculosis (bTB), in populations of wild and domestic animals, particularly cattle. The complicated nature of badgers' social behaviour and social structure has been shown to have implications for disease transmission and therefore in attempts to manage the disease. As a result of this, the effect badger social biology has on attempts to control bTB warrants further research. This dissertation investigates some of the gaps in our knowledge relating to how badger social biology influences two different management strategies that aim to target bTB incidence in badger populations. Firstly I investigate how culling, in a badger's social group, changes individual movement (Chapter 2). To do this I employ two novel measures to quantify this movement. Badgers were from two adjacent areas, one that was the subject of culling and one that was not. Badgers from the area which had been culled returned radio tracking fixes 44.5% further from their main sett, on average, than individuals from the area not subject to culling. There was no difference found between populations using the second measure, which aimed to quantify the amount of movement around an individual's range. Secondly, I investigate how the social composition, demography and activity of a badger's social group influences the consumption of baits that are part of research to develop an oral bTB vaccine (Chapter 3). I found that age class, the proportion of cubs in a social group, the proportion of other individuals eating bait, and sett activity levels have a significant effect on the consumption of bait. Finally I discuss the implications of these results on our understanding of how badger social biology affects bTB control and how the results may influence the design of future research and management strategies (Chapter 4).

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## CHAPTER 1: INTRODUCTION TO WILDLIFE DISEASE

Disease is most often described in its simplest form as any impairment of normal functions to an organism due to a disease agent [1]. It is something that is ever present in natural ecosystems and plays an important role in limiting population growth as well as in natural selection [2], it can affect a number of processes including behaviour, fecundity, growth or metabolic requirements, sometimes resulting in mortality. The exact nature of the disease depends largely on the agent causing it. The agents of disease can be split into two general categories, non-infectious (e.g. non-infectious cancers or toxic substances) and infectious (e.g. prions, viruses, bacteria or fungi). Infectious diseases can be transmitted directly between organisms or with indirect contact through the environment or through vectors. Because of this they pose a significant threat to populations of humans and animals and as a result, are the target of most disease management programmes.

Throughout history and to this day, infectious diseases have a major role in shaping society in human populations. Worldwide pandemics have caused large death tolls, resulting in upheaval to the economic, cultural and religious environments at that time. For example the Spanish influenza pandemic that took place between 1918 and 1920 is thought to have killed 50 million people worldwide, although this still may be an underestimation [3]. In the early 20<sup>th</sup> century, this may have been around 2.5%-5% of the world's population. As a result of the sheer number of those killed and because around 50% of those who died were young adults, (20-40 years of age) [4] it is not difficult to imagine the effect this pandemic would have had on many aspects of society, for example the available workforce. Due to outbreaks such as this, attempting to manage diseases that affect humans is not a recent phenomenon. However, of the 1400 diseases that have been identified to affect humans, 61% are zoonotic [5], which means they can be transmitted between species, from animals to humans or in the opposite direction. For example, the Black Death between the years of 1348 and 1350 is estimated to have reduced the population of Western Europe by a third, this disease is thought to have been spread by the black rat (*Rattus rattus*) and highlights the importance of wildlife vectors in causing disease in human populations. More recently, in 2012, 627,000 people are estimated to have died from malaria [6], which is spread by mosquitos

(*Anopheles* spp.). Therefore, management of diseases may be complicated where pathogens exist in wild animals and can be transmitted to the organism upon which management is focused.

The realisation of a need to manage infectious diseases in wild animals is relatively recent. The need for which is no doubt affected by anthropogenic activities disturbing the natural order of ecosystems [7]. Increased human population growth and agricultural expansion and intensification has led to direct encroachment and degradation of natural habitat in all corners of the globe, as well as this, climate change and increased global travel has led to the emergence of novel diseases at a scale not seen before. Human, domestic animal and wild animal populations have been affected because of an increase in pathogen burden and disease transmission between species. The management of disease in wild animals normally occurs because the disease also has a potential impact on the health of a human or domestic animal population, or the wild animal in question is of political, economic, ecological or conservation importance [1].

### 1.1 MANAGEMENT OF WILDLIFE DISEASES TO PROTECT HUMANS

Of the 1400 infectious diseases that have been identified to affect humans, 61% are zoonotic, this figure increases to 75% when including diseases that are thought to be of emerging importance [5]. When designing management plans for wildlife disease outbreaks, particular importance is put on the risk to human health. Much of the research that has been carried out and the approaches adopted, are as a result of attempting to protect humans from diseases transmitted in some way from wildlife. An example of an effective management strategy that significantly reduced the risk to human health, of a disease carried by mammals, is the oral bait vaccination of red foxes in Europe against Rabies.

Rabies is a neuroinvasive type of Lyssavirus that is usually transmitted between mammalian hosts in the saliva, from a bite by an infected individual. The disease is nearly always fatal if not treated before symptoms start to show. Around 55,000 people die of rabies each year, mostly in Africa and Asia. Around 15 million people annually, across the world, receive a post exposure vaccination to prevent the disease; this saves hundreds of thousands of lives [8].

In Europe a strain of sylvatic rabies is thought to have developed in 1939 on the Polish-Russian border; this strain spread rapidly through the red fox (*Vulpes vulpes*) population. At the point where a decision was made to tackle rabies in the 1960s, the disease was almost endemic within the fox population of Europe [9]. Not only did this have implications for the status of the fox but as human cases of rabies began to rise, it also posed a significant threat to human health.

European governments first implemented a culling strategy to reduce the density of the fox population, which aimed to decrease the number of susceptible individuals an infected animal could come into contact with. However, because of the large geographic spread of the disease and the inaccessibility of some endemic areas, this practise was deemed not cost effective and largely unsuccessful in treating the disease [10]. This meant that another solution was needed: an oral bait vaccine. The first field trial of an effectively potent, safe and attractive bait was carried out in Switzerland in 1978 [11]. This first generation of vaccine, however, lacked efficacy and there were also concerns over a potential reversion to virulence [12]. By 1986, a new vaccine had been developed that utilised recombinant vaccinia virus clones expressing protective rabies virus glycoproteins. This improved vaccine was shown to confer protection when delivered via the oral route, be stable in the environment and safe to non-target species [12]. By 1996, 8.5million baits had been deployed, in Europe, by hand and aerial means [13]. This programme was successful in a dramatic reduction in rabies in a targeted wild animal population (red fox), thereby significantly reducing the risk to the human population and is seen as a bench mark for vaccination programmes.

## 1.2 MANAGEMENT OF WILDLIFE DISEASES TO PROTECT SPECIES OF IMPORTANCE

The definition of an important species varies depending on what the importance is being based on. A species may be important ecologically, because it is a keystone or umbrella species (its survival in an ecosystem indirectly results in the survival of other species). An organism may also be considered to be important because it is endangered and the loss of such a species would be a major loss to global biodiversity. A third definition is because it is important

politically or economically, for example because of revenue it attracts, in tourists travelling to view the species. Often an endangered species may satisfy all of these definitions, for obvious reasons.

Disease outbreaks in endangered species can be particularly difficult to manage, as, by definition, the population size will be small, therefore the genetic diversity may be lacking to naturally counteract the disease. Such is the case in the Tasmanian devil (*Sarcophilus harrisi*), the largest surviving marsupial carnivore, which suffers from Tasmanian devil facial tumour disease (DFTD). There is clear evidence that a loss of genetic diversity in the major histocompatibility complex (part of the mammalian genome that discriminates between 'self' and 'non-self') is causing DFTD to spread rapidly through the population [14]. DFTD was first observed in the mid-1990s, the disease has spread from its point of first detection in north-eastern Tasmania to now being present in most of the devil's distribution. The tumour cells appear to be the infective agent [15] and are thought to be transmitted by a susceptible individual with wounds or exposed flesh in or around its mouth, biting an infected individual's facial tumour [16]. The management strategy currently in use is to maintain insurance populations of disease free animals (for reintroduction, in case of extinction in the wild), in situ management (development of vaccines and removing captured, infected individuals) and detecting and spreading devils showing natural resistance to the disease (no firm evidence that animals are totally or partially resistant to the disease [14]) [1]. Only time will tell whether all, or any of these approaches, will have a positive impact in counteracting the disease. However, using a multi-faceted approach and not relying entirely on just one method gives it the highest chance of success. An important lesson to learn is that of early recognition, although this disease was first observed in 1996, its general recognition as an infectious agent and its identification did not occur until 2006. Earlier recognition of the infectious nature of this disease would have assisted in its management, particularly for the effectiveness of a culling strategy [17].

### 1.3 MANAGEMENT OF WILDILFE DISEASES TO PROTECT LIVESTOCK

Disease in domestic animals or livestock can have a significant impact on the productivity of the animals affected, which can result in substantial economic losses for the livestock owner. In some instances the ability for disease control is limited because of the transmission and maintenance of disease in a wild animal source. Being able to quantify the contribution of transmission from wild animals to domestic animals and vice versa, is of particular difficulty. This presents problems in deciding how to tackle the disease effectively.

One such example is the case of bovine tuberculosis (bTB) infection in domestic cattle in the United Kingdom and the Republic of Ireland. Pasteurisation of cow's milk and the regular tuberculin skin testing and subsequent slaughter of infected individuals are the main methods of controlling the disease in cattle herds [18]. It is of great importance to control this disease, due to the economic cost to a farm, of not only the loss of infected individuals but also the trade restrictions imposed on infected farms. The infectious agent causing bTB is *Mycobacterium bovis* (*M. bovis*), this slow, growing, aerobic bacterium has been found in many mammalian hosts in the UK [19]. It is however, the European Badger (*Meles meles*), which is widely regarded as a major bacterial reservoir of the disease and implicated in transmitting the disease to cattle [20]. Badgers often overlap directly with cattle because of their habitat and feeding requirements, providing an opportunity for the transmission of bTB. As well as this the badger's social structure and the fact that they live in damp, dark setts provide prime conditions for the disease to spread from badger to badger [20].

Attempts to reduce disease incidence, by culling badgers around a bTB herd breakdown, have been carried out since the 1970s; despite this, the disease has gradually increased nationally [20]. In order to evaluate the role that culling of badgers could have in the managing of bTB, the Randomised Badger Culling Trail (RBCT) was carried out between 1998 and 2005. This large-scale ecological trial, involved 30 areas of 100km<sup>2</sup> separated into 10 sets of three, termed 'triplets'. Within each triplet, one area was the subject of proactive culling (annual culling of badger populations across all accessible land), one was the subject of reactive culling (on or near farmland where a recent herd

bTB breakdown had occurred) and one was a control or survey only area (received no culling treatment) [21]. Overall, almost 11,000 badgers were culled. The reactive culling treatment was stopped in November 2003 after it was associated with a 20% increase in confirmed bTB breakdowns of resident herds [22]. After 5 years of culling inside areas subjected to the proactive treatment, there was a 23% decrease in the number of herd breakdowns, however, there was a 25% increase in the area  $\leq 2$ km outside. More recent analysis shows that the beneficial effects increased up to two and half years post culling and that the detrimental effects outside the area subsided [23]. The perturbation of, once stable, badger social groups as a result of culling is widely regarded as causing an increase in transmission between badger social groups and cattle herds [24], subsequently causing the failure of the reactive strategy and the short-term increase in bTB incidence in land neighbouring culling zones.

The first piece of research within this document investigates two novel methods to quantify perturbation. As mentioned, this phenomenon is an important factor when considering the culling of badgers as a potential management strategy to reduce the incidence of bTB in cattle. However, perturbation and the behaviour associated with it can be difficult to quantify. I detail the use of two novel indices to measure the movement of badgers in an area that has been the subject of culling and an area that has not. The use of these indices should be to add to other, previously documented methods with the aim of understanding perturbation in as full a capacity as possible, to help shape future management plans.

The modest improvement in cattle bTB incidence, from the large-scale culling of badgers in the RBCT and widespread unpopularity of this method with the British public, suggests other methods should be researched and trialled. The use of vaccination to reduce the number of susceptible individuals is another possibility. Intramuscular vaccination using an *M. bovis* strain Bacillus Calmette-Guérin (BCG) vaccine [25], is one method to achieve this. However, because of the potential cost of a large-scale vaccination programme and the lack of research on how the protection against bTB in badger social groups will relate to bTB incidence in cattle herds [12], there are no plans for a large scale roll-out of this strategy. A vaccine delivered via an oral bait, however, has the potential

to be easier to distribute over a large geographic area and could, in theory, be done so at a lower cost than culling or intramuscular vaccination [26].

In recent years, both laboratory and field trials have been carried out to identify potentially attractive bait types [27], that could hold a vaccine and how best to deploy baits to maximise uptake. Despite this, a viable oral vaccine, within a palatable bait, deployed using a robust strategy to immunise badgers in setts of all shapes and sizes, is a long way off.

The second piece of research within this document investigates the effect of sett activity on the consumption of bait and how group composition and demography influences bait uptake. The difference in badger social groups in terms of their size and their composition, of age and sex classes, may have a significant impact on the uptake of bait. The findings should help to inform a strategy of bait deployment that will be successful in maximising the uptake of bait across all badger setts.

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## CHAPTER 2: CULLING-INDUCED CHANGES TO BADGER MOVEMENT

### ABSTRACT

In the 1970s, in England and Wales, the European Badger (*Meles meles*) was identified as a significant host of the disease bovine tuberculosis (bTB). Since then efforts to control disease incidence in cattle, by culling badgers have had varying degrees of success. A reason for the lack of consistency in reducing bTB incidence in cattle is perturbation, this is the resulting disruption to badger social groups and change in individual movement that can cause the disease to spread. This study investigates the movement of badgers in response to culling, using data derived from radio tracking individuals in an area that was part of the UK Government's Randomised Badger Culling Trial and an area adjacent to it. Two proxies were created to quantify perturbation. Badgers from social groups which had been the subject of lethal control yielded radio tracking fixes which were on average 44.5% further from their main sett than badgers from the un-culled area. No significant difference between un-culled and culled populations was found for our second proxy, this investigated the level of movement within an individual's range. In using new ways to measure perturbation, this research shows increased movement of badgers because of culling, the subsequent spread of bTB may negate any possible benefits of reducing population numbers.

Keywords- Perturbation; bovine tuberculosis; Randomised Badger Culling Trial; *meles meles*; radio tracking.

### 2.1 INTRODUCTION

The management of disease can follow three main paths; preventing the introduction of disease, limiting the existing disease or complete eradication [1]. Of the 1400 infectious diseases that directly affect humans, 60% can be transmitted between species [2], from animals to humans or in the opposite direction. Attempts to control disease in human or domestic animal populations can be complicated by the persistence of disease in wildlife [3]. Often symptoms of disease or sickness are not obvious in wild animals and when the focus of control is turned to a wildlife host, the opinion of the public can cause conflict amongst decision makers. Conflicting views on the scientific evidence

surrounding the subject only adds to the task of deciding the correct course of action.

One such example is the case of bovine tuberculosis (bTB) in the United Kingdom. This disease carries a threat economically to the cattle industry as well as posing a risk to human health [4]. In 2012 in Great Britain 37,754 cattle were destroyed as a result of testing positive for bTB, an increase from 34,245 in 2011 [5]. *Mycobacterium bovis* (*M. bovis*), the causative agent of bTB has been shown to infect many mammalian hosts in the UK [6]. However, since the first infected individual was discovered in Gloucestershire in 1971 [7], it is the European Badger (*Meles meles*) that has been labelled as the major bacterial reservoir in the wildlife population, in Great Britain and the Republic of Ireland. Badgers are able to sustain infection with bTB for a number of years, therefore increasing the probability it is passed on [8]. Badgers also live in social groups, in damp, dark setts which provide prime conditions for bTB to spread amongst the group [8], as well as this, as a result of their feeding habits and habitat requirements they often interact directly with cattle on pasture land as well as venturing into farm buildings [9]. As a result of these factors, combined with the current population of badgers, it is now generally regarded badgers spread and transmit the disease to cattle. There is, however, some scepticism on the matter and it has been challenged in a recent publication suggesting the distribution of bTB in cattle herds over the last few decades does not match that of bTB positive badgers picked up in road traffic accidents [10]. However because of a wealth of evidence, badgers are at the heart of any debate and policy regarding bTB in the UK.

It is a difficult task to work out the best method to manage this complicated disease with minimum conflict. Proceeding with a strategy underpinned by a sound scientific basis must be the first step [11]. Lessons can be learned from the approaches adopted in other countries; culling of the wildlife reservoirs of water buffalo (*Bubalus bubalis*) and feral cattle (*Bos taurus*), in Northern Australia was of fundamental importance in their bTB eradication programme [12]. In the Republic of Ireland, implementing 2 different badger culling strategies, across 4 paired study areas resulted in fewer confirmed herd breakdowns where a proactive strategy was adopted [13]. Lower badger density than areas of high bTB incidence in England and natural boundaries to cull

areas may have played a part, however, in the success of this trial. In the UK the culling of badgers to control bTB has been a contentious topic for decades and between 1975 and 1997 more than 20,000 were killed, in an attempt to reduce transmission to cattle. Despite this, there has been a national increase in disease incidence [8]. The Randomised Badger Culling Trial (RBCT), carried out from 1998 to 2005 was set up to examine the role culling could have in managing bTB [14]. It was, arguably, the single largest ecological trial ever carried out [15], with almost 11,000 badgers killed [14]. The final conclusions were that culling did indeed reduce bTB incidence in cattle inside culling areas, however it increased it to a similar level in areas  $\leq 2$ km outside [16]. With successive culls the benefits inside the boundary increased and the detrimental effects outside decreased, resulting in a modest overall reduction in bTB incidence in cattle [16]. The detrimental effects on land bordering cull areas was largely because culling can cause disruption to badger social groups leading to changes in individual movement. The result can be badgers occupying new areas, increasing ranging behaviour, mixing of once relatively stable social groups and therefore elevating the risk of transmission between badgers and cattle; this has been termed perturbation [17-20].

The phenomenon of perturbation is not something that applies only to small scale disease dynamics, such as the individual movement of badgers, it also plays a role at a much larger scale. The very emergence of novel diseases globally can be as a result of perturbation [21]. This can occur when there is movement of a disease in response to a disturbed system, often caused by land use change or other human disturbance. The result can be a change in the rate of transmission across species, exposing naïve hosts and in a worst case scenario, resulting in a pandemic.

The UK government has agreed plans for a pilot badger cull across two 150km areas in England as a means of controlling bTB [22]. This not only highlights the relevance of the topic but evidence from the RBCT suggests that even culling 70% of the badger population, as proposed, may result in an increase in bTB incidence in surrounding areas [16]. Also recently announced is that Northern Ireland will attempt a 'test and vaccinate or remove (TVR)' approach in order to reduce levels of bTB in badgers and cattle [23]. A complete understanding of perturbation will be important in this combined approach as models suggest the

detrimental effects of perturbation could reduce the effectiveness of this strategy [24].

A challenge arises in how best to measure and quantify this complicated phenomenon. The rate of change in range size and range overlap between individuals or social groups are common proxies, derived either from radio tracking individuals [18,20] or from bait marking [19]. The median distance from a sett to its associated bait return was also used to look at ranging behaviour by Woodroffe and colleagues [17]. Pope *et al.* [25] used dispersal as a proxy for perturbation, by comparing the genetic signatures of badgers taken from an initial cull, assumed to be a relatively stable population to those taken in the follow up cull. As demonstrated there are a number of ways in which perturbation can be quantified.

In this paper the way perturbation can be quantified is investigated by employing two novel indices of individual movement that, to my knowledge, has not been done so previously. This is done in an area that has been the subject of culling and an adjacent area that has not. It is known from a previous study that the area experienced perturbation [26]. Therefore the focus of this research is not to identify whether and why perturbation has taken place but rather if a more detailed account, of the effect of culling on individual movement, can be gained through the use of the two novel indices proposed. I hypothesise that badgers from the culled area will have increased individual movement as a result of perturbation [17]. I also predict that over three years of culling operations the culled population will become increasingly perturbed.

## 2.2 METHODS

The data from which this project is drawn was collected in a study titled 'The demographic, ecological and epidemiological consequences of culling badgers' completed in 2007. Any relevant methodology is described below, however for a complete description see [26].

### Study area

An area consisting of 27.34km<sup>2</sup> of land in South Gloucestershire was the focus of this project. It was predominantly arable and agricultural grassland and largely flat. 16.47km<sup>2</sup> of this area was contained within the proactive triplet I2 of

the RBCT [14]. This was subjected to four years of successive culling from 2002 to 2005. The remaining 10.87 km<sup>2</sup> was adjacent to the triplet and was not the subject of culling operations involved with the RBCT. From this point on the former will be referred to as the culled area and the latter the un-culled area.

#### Live capture and handling

In order to collect epidemiological and demographic data, badgers in the study area were live trapped and subjected to clinical sampling. Social groups were trapped, on average 4 times throughout the year, apart from a closed season from February to April inclusive, when females may have dependant cubs [27]. All captured, adult badgers were fitted, once under anaesthesia, with radio collars. This totalled 40 badgers, 13 in the culled area and 27 in the un-culled area. Badgers with severe lesions or wounds to their neck were not fitted with collars. The collar was made up of a TW-3 transmitter with a closed loop antenna (Biotrack Ltd, Furzebrook, Wareham, Dorset, UK), this was encased in epoxy resin and set in a leather collar. The weight of the whole unit was well below 5% of the animal's body weight, as recommended for radio-tracking studies [28]. As a proportion of the study area was in the RBCT some individuals that had been radio collared were trapped at a later date by Defra's Wildlife Unit (WLU) and destroyed (for details of this procedure see 14).

#### Radio tracking

Radio tracking took place both by car and on foot using a hand held Yagi-flexible-element antenna (Biotrack Ltd, Furzebrook, Wareham, Dorset, UK) connected via a coaxial cable to a TR-4 receiver (Telonics Inc., 932E Impala Avenue, Mesa, AZ, USA). When possible, landmarks were used to document the position of the individual carrying out the radio tracking and/or the badger being tracked, if absent, bearings were taken using a mirror compass and the observer location was recorded with a handheld Garmin E Trex H GPS unit (Garmin Ltd, Olathe, KS, USA). Two bearings were taken at least 100m apart but in the shortest time possible in order to triangulate the position of the animal. Vantage points from which a clear signal was known to be detectable were recorded and mapped using ArcGIS 9.1 (ESRI, 2005). Because badgers were tracked using this method of triangulation, it was not possible to collect continuous movement data, however fixes were recorded at least 15min apart.

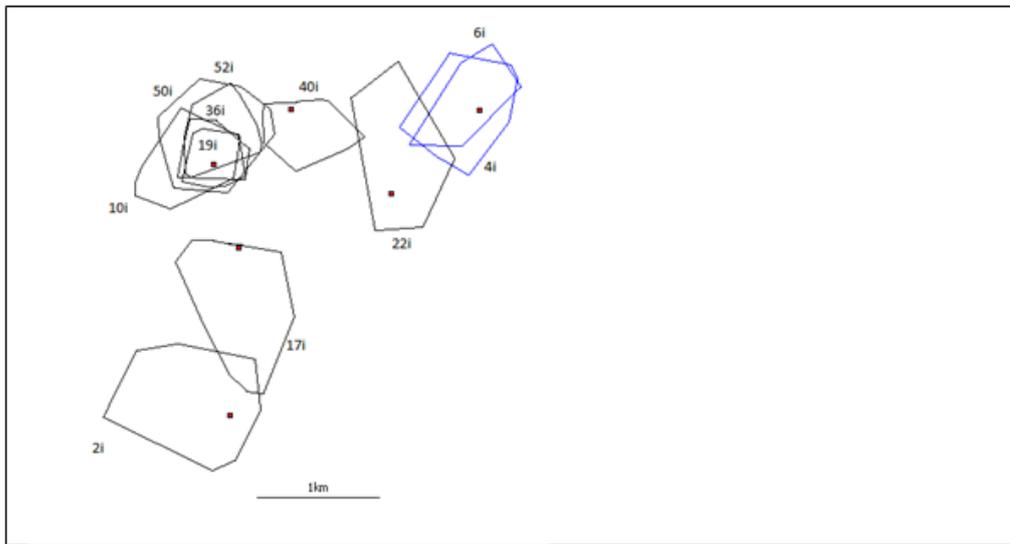
Collecting fixes using such a short sampling interval may result in potential autocorrelation but as movement is a non-independent phenomenon, all locations are included in an effort to gain as much movement information as possible [29]. Radio tracking was carried out at night, mainly between the hours of 21:00 and 05:00 adjusted according to season and subsequent emergent patterns, this timespan was chosen to take into account early evening and early morning peaks in activity [18,30]. Three periods of radio tracking were carried out from June 2004 to Oct 2004 (termed post-cull period two), from Nov 2004 to July 2005 (termed post-cull period three) and finally from July 2005 to December 2006 (termed post-cull period four). The initial cull commenced in September/ October 2002 and subsequent culls occurred between each of these periods. The approximate number of radio fixes collected was 3000 during more than 1,200 hours of nocturnal tracking. The error of the telemetry used varies with habitat [31], therefore it was estimated in the four main habitat types present in the study area; hilly woodland, flat pasture, hilly pasture and flat crop . A collar was placed in a location in each of the habitats unknown to the observer. The exact location of the collar was recorded using a handheld GPS device (accuracy >6m), the observer then took two bearings to estimate the location and the distance between the exact GPS reading and the telemetry bearings calculated. The results for average error in each habitat were; hilly woodland 67m, flat pasture 47m, hilly pasture 57m and flat crop 22m. Therefore the overall mean telemetry error is 48m. It is likely that in practise, error will be increased, because of the difficulty of tracking a moving animal, of which its speed and predictability will be affected by habitat type as well as the challenge of the observers themselves having to travel through different habitats. However these sources of error are difficult to fully quantify and therefore are not accounted for here.

### Individual range analysis

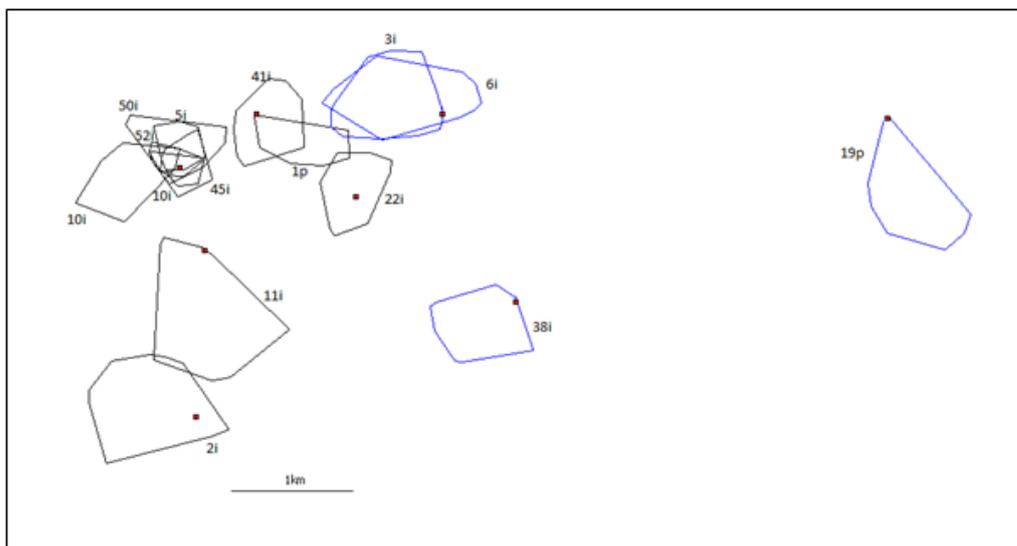
For each individual, in each period, a home range area curve was calculated to test for asymptotic home range. An individual is said to have reached an asymptote if each additional fix does not produce more than a 1% increase in area, therefore ten observations do not cause an increase by more than 10% [32]. As the location data were discontinuous, due to the irregular sampling interval, fixes were added to the analysis randomly [33]. All animals were tested

and an average number of fixes for asymptotic individuals produced (37). Therefore 37 fixes is enough to adequately describe an individual's home range. The only problem encountered was that as the very phenomena being investigated is categorised by an unstable home range and a move towards a more transient individual, for a time at least, one might reject certain individuals that do not satisfy these aforementioned conditions [33]. Many studies of this type use a previously reported adequate number of fixes and do not test individuals in their study [29,34,35]. Because of this practise and as the very existence of asymptotes has been questioned [36,37], the following step was added to the analysis. Any individuals that had more than the 37 fixes but that had not formed an asymptote, within the range required, were kept in the analysis. These individuals were examined case by case and only included if the number of fixes obtained were at least two times that required or at least the last 5 fixes for an individual, in a given time, produced less than a 5% increase in area [38]. Three individuals were rejected from post cull period two leaving eleven, ten individuals rejected from post cull period three leaving fifteen, nine rejected from post cull period four, leaving fifteen (fig. 1).

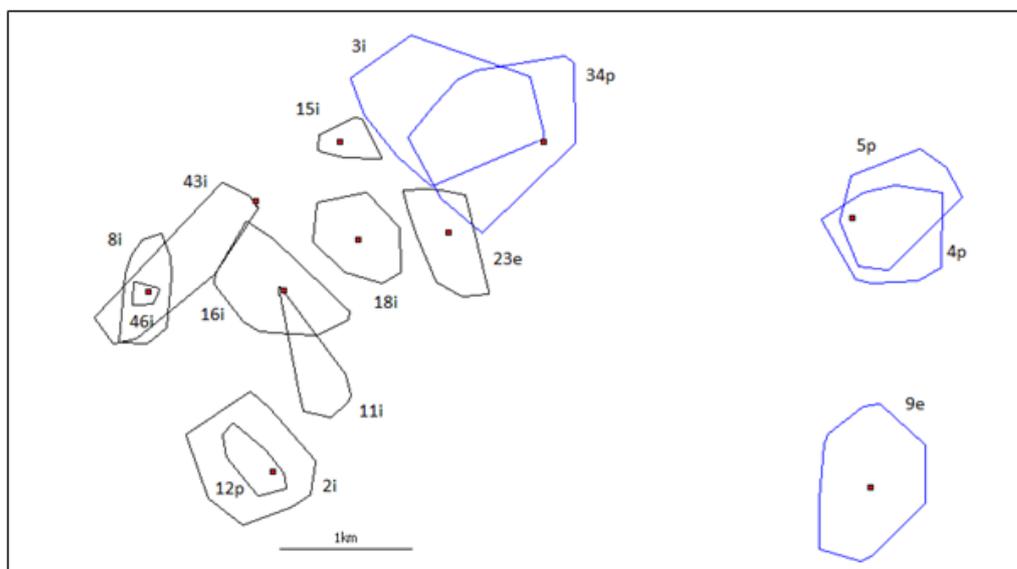
To represent the home range of each animal 95% minimum convex polygons (MCPs) are used [39,40] (fig. 1) Ranges8 v2.9 software (Anatrack Ltd. <http://www.anatrack.com>) was used. MCPs were chosen to represent badger home ranges over location density estimators (LDEs) for a number of reasons: they are comparable with other studies; the former make no statistical assumptions of the distribution of the data set; MCPs are more robust when the number of fixes used is relatively low [33]. The focus of this study is not on intensity of use within an animal's range or habitat use therefore using a type of location density estimator would be of no real benefit. Due to the temporal and transient nature of the phenomenon investigated, MCPs were not deemed appropriate to include in the statistical modelling. The two novel indices however attempt to form a more independent, dynamic measure of perturbation and to represent individual movement within and around an individual's range more accurately than using an MCP could, especially with respect to the perturbation effect.



A-Post-cull period 1



B-Post-cull period 2



C-Post-cull period 3

Figure 1. 95% minimum convex polygons for each individual, per post cull period. A- post cull period two, B- post cull period three, C- post cull period four. The blue polygons represent individuals from setts that were subjected to culling and black polygons are those that were not. Also included are the identification codes assigned to each individual, the red squares show the position of the individual's main sett.

## Statistical Analysis

### Data Analysis

From individuals kept after the asymptote analysis, two measures were employed to look into the movement and ranging behaviour. Firstly, all fixes less than 30metres from the main sett were removed as they were not deemed to represent any sort of dispersal or ranging behaviour. The first measure aimed to look at the distance badgers were ranging from their main sett. The distance from the GPS coordinates of the animal's main sett to the coordinates of every fix, in each post-cull period, were calculated and the mean taken. Ranges8 v2.9 was the software used to do this. The purpose of the second measure was to investigate an individual's movement around its range, rather than just how far away it travelled. Microsoft Excel (2010) was used to randomise all the fixes obtained for each individual, in each period. From this the fixes were put into random pairs. The number of random pairs formed equalled the number of fixes of that individual, in the given period, therefore not exaggerating the sampling effort for that animal. The mean distance between the fixes in each pair was calculated and a mean, from all pairs, produced. The data was then tested for normality and transformed if necessary [41].

### Statistical Modelling

Two linear mixed models fitted using restricted maximum likelihood estimation were carried out using IBM SPSS Statistics version 20. The response variable of the first model was the mean distance (log transformed) from the sett to each fix. Individual and social group were set as random factors. Sex, treatment (culled or un-culled), post-cull period and bTB infection status (results from ELISA and Culture tests) were fitted as fixed factors. Included, as a covariate, was the number of fixes per individual. The interactions tested were treatment and post-cull period, treatment and sex, bTB infection status and treatment. The second model only changed in its response variable, the average random pair distance. The best model was chosen using a forward step approach based on the Akaike information criterion (AIC) to evaluate the explanatory power of different models [42].

## 2.3 RESULTS

Individuals from the setts subjected to culling had an average home range (95% MCP), over the 3 periods, of 72.28ha (n=9, s.e=10.37) compared to the 48.33ha (n=22, s.e=7.02) of individuals from the un-culled setts, this did not represent a significant statistical difference (Student's *t* test:  $t = 1.94$ , d.f. = 29,  $p > 0.05$ ).

A total of 444 fixes, from within 30m of the main sett were removed from the analysis, 139 of these were from individuals from the culled treatment (n=9) and the remaining 305 from the un-culled treatment (n=22). With regards to the data for average sett to fix distance, the model to best fit the data therefore producing the lowest AIC score, contained all terms except the number of radio tracking fixes per individual. The only term having a significant effect on the data was treatment (table 1) therefore badgers from the area subjected to culling had a significantly larger mean distance (488 metres) from sett to fix value than individuals that were not (337 metres) (fig. 2), an increase of 44.5%. The second measure, average distance within fix pairs, produced no such significant result from a model containing all terms as it gave the lowest AIC score. None of the terms from this model had a significant effect on the response variable.

Model Term	F	d.f.	<i>p</i>
<b>Treatment</b>	4.414	1, 31	<b>0.044</b>
Sex	1.856	1, 31	0.183
Post-cull period	0.05	2, 31	0.951
Tb-status	1.443	1, 31	0.239
Treatment X Post-cull period	0.637	2, 31	0.535
Treatment X sex	0.034	1, 31	0.854
TB-status X treatment	0.788	1, 31	0.381

Table 1. Output from a linear mixed model analysing the effect of treatment, sex, post-cull period, TB-status, treatment and post-cull period, treatment and sex and TB- status and treatment on mean sett to fix distance. (F= F-statistic).

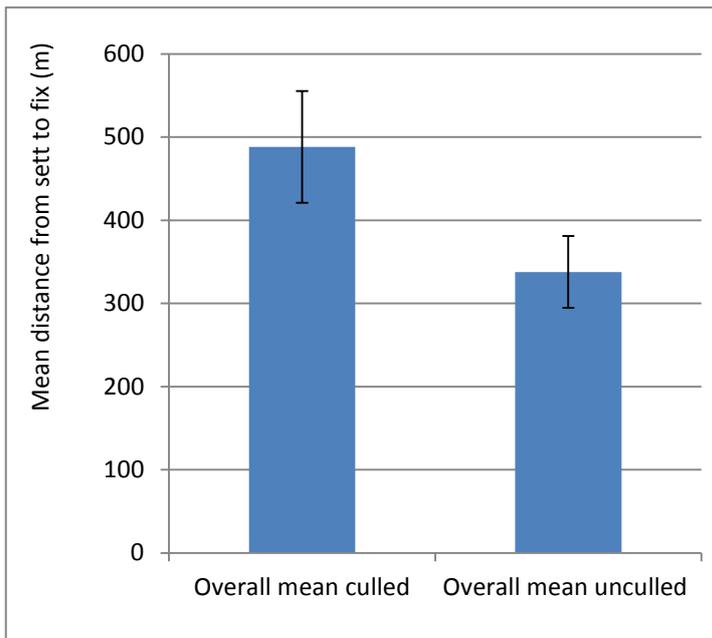


Figure 2. Mean distance from sett to an individuals associated fixes, showing average for culled and control areas. Standard error bars are shown.

## 2.4 DISCUSSION

Our study shows that individuals within groups subjected to culling have similar ranges but have increased movement within that range, compared to adjacent groups, not subject to culling. Of the two proxies used to represent this movement; mean sett to fix distance showed a significant difference between the culled and un-culled groups, random fix pair distance, however did not. Tuytens *et al.* [18] found changes in bait marking returns that they attributed to culling, similar to our findings, but also could not pick up any changes in the movements of individually radio collared badgers. To answer the second main prediction mentioned in the introduction, there was no evidence of individuals from culled social groups becoming more perturbed as the 3 years of culling operations progressed.

Our results are consistent with the findings of previous studies looking into the effects of culling on badger movement [17-20]. The recent study by Riordan *et al.* [20] also used data from the radio tracking of individual badgers and found a similar increase of 43.5% in the home range size of surviving badgers from groups subjected to culling. A study by Woodroffe and colleagues [19] used a similar proxy of distance from sett to bait return and also found significant differences between groups from culled and un-culled populations. No evidence

was found to support the idea that badgers infected with bTB have larger home ranges than their uninfected counterparts, as has been discovered in previous studies [25,43]. An explanation for this could be that our study did not look at the habits of individual movement in the same way and it did not possess the same statistical power in the study design, as Garnett *et al.* [43] in particular. Tuytens *et al.* [18] found males have significantly larger ranging habits, however, when a habitat is left vacant, as is often the case in a culling scenario, females have been found to be the most likely to be the first to recolonize. There was no evidence in this study of a significant difference in movement behaviour between sexes.

The way data was collected imposed some limitations on this study. Namely some individuals were represented more than others as a result of the radio tracking regime, this has proved problematic in some studies [19] but not so much in others [34,40]. Employing the asymptote analysis and including the number of fixes per individual, as a covariate in the analysis should have accounted for this. The radio tracking was carried out in a discontinuous and irregular fashion, this limits how much information can be gathered from the data. Tracking every individual for a constant time period and recording radio tracking fixes in a continuous manner would not only give a more accurate representation of movement but also allow more assumptions to be made about the rate of movement [33,40]. Also to consider is that as the radio tracking began after the second cull there is no baseline data on the movement of individuals before any culling took place. The study area was assumed to be uniform in habitat type, however, individuals from the social groups culled could have always ranged further than individuals from the un-culled area [44]. Furthermore, analysis from the RBCT shows the perturbation effect present not only in populations subject to direct culling, but in adjacent populations [15]. Therefore the behavioural differences noted in this study may have been between two perturbed populations, especially at un-culled setts closest to where culling operations were taking place. The result of this may be that the differences in movement, picked up in this research, are not as pronounced as it may have been between a culled and a truly un-culled, un-disturbed population.

Using the average distance from an individual's main sett to the radio tracking fixes, recorded for that animal, has been successfully used in this study to detect differences in badger movement, between culled and un-culled populations and therefore attempt to quantify perturbation. Many studies that have investigated perturbation have utilised bait marking as their main investigative tool, [18,19,45]. Bait marking exploits a behaviour shared by an entire social group and therefore may underestimate the degree of disturbance at an individual level. Proxies such as those used in this study should add to already well-established methods to effectively and efficiently understand perturbation. Utilising both proxies again in a larger study with a number of different areas, paired to appropriate controls would be the natural progression to this study.

In conclusion, the results of this research suggest that culling can have a significant impact on the movement of badgers. This movement may result in an increase in the transmission of *M. bovis*, not only to other badgers but also to cattle [14]. In areas subjected to culling where badger numbers can be significantly decreased, a reduction in the incidence of bTB in cattle has been observed [16,47]. However adjoining areas may be subject to immigration of surviving badgers, which as shown in this study are thought to travel further from their main sett; in turn this may increase the risk of bTB in these areas and negate any overall benefit of reducing population numbers [16]. This is especially true of localised culling such as that carried out in response to a cattle herd breakdown [14,46]. These findings can be used to inform, when determining a role for the culling of badgers in future management plans that aim to reduce bTB incidence in badgers and cattle.

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## CHAPTER 3: HOW THE COMPOSITION, DEMOGRAPHICS AND ACTIVITY OF BADGER SOCIAL GROUPS INFLUENCE BAIT UPTAKE

### ABSTRACT

In the United Kingdom and the Republic of Ireland attempts to eradicate bovine tuberculosis from domestic animals have been hampered by its presence in wildlife, most notably in the European Badger (*Meles meles*). Research on the control of bovine tuberculosis in badgers includes investigation into the potential of an oral bait, containing a vaccine, to immunise badgers against the disease. Recent research has focussed on vaccine formulation and the design and deployment of a palatable bait. In this study the uptake and disappearance of bait, by badgers, was investigated, in relation to social group composition and demographic variables and also sett activity levels. Data from three previous, large-scale field trials in which biomarkers were contained within bait, to quantify uptake, were analysed, as well as video recordings of badger behaviour from a study carried out in 2013. The results show that age class, the proportion of cubs in a social group, the proportion of other individuals eating bait, and sett activity levels have a significant effect on the uptake or disappearance of bait. The results from this research should help to inform further research into the efficacy and implementation of an oral bait vaccine.

Keywords- bovine tuberculosis, *Meles meles*, biomarker, oral vaccination, oral bait

### 3.1 INTRODUCTION

The disease Tuberculosis (TB) is still a problem globally and in 2012 8.6million people fell ill with the disease and around 1.3million people died [1]. Bovine tuberculosis (bTB), is a form of the disease caused by the bacterium *Mycobacterium bovis* (*M. bovis*) and is a zoonotic infection affecting humans, cattle and other animals [2]. In many countries regular tuberculin skin testing and subsequent culling of infected cattle has helped to control the disease in cattle [3]. However, management is complicated in countries where infection persists in a wildlife reservoir [4]. This is the case in the United Kingdom and the Republic of Ireland where badgers are implicated as a major source of infection [5]. Due to the badger's social structure, physiology and foraging habits not only

are they likely to come into contact with cattle and contract or transmit the disease but also likely to harbour infection and pass it on to other badgers, especially within their social group [5]. The evidence from a long term study of the epidemiology of bTB in a high density badger population in Woodchester Park, south-west England [6], suggests that where badger social groups are not disrupted, infection persists in certain groups with limited transfer of infection between neighbouring groups.

Since first discovering a badger infected with bTB in 1971 [7] various strategies have been employed in an attempt to reduce infection in badgers and therefore incidence in cattle. The most extensive of these was the Randomised Badger Culling Trial, which involved testing two potential culling strategies. Overall, nearly 11,000 badgers were killed [8]. The proactive culling of badgers, reduced the incidence of bTB in cattle, inside the culling areas. On adjoining lands, however, that were not the subject of culling there was a temporary increase in the incidence of bTB in cattle, although this detrimental effect did decrease with successive culls [9,10]. An increase in movement of surviving badgers because of the disruption, to their social group, caused by culling, has widely been cited as having the possibility to spread the disease and therefore could be the cause of this increase, in adjacent areas [11]. Intramuscular vaccination of badgers against bTB, is another management option, using an *M. bovis* strain Bacillus Calmette-Guérin (BCG) vaccine [12]. This vaccine is licensed in the UK and is part of the Welsh Government's most recent attempts to tackle bTB [13]. More research is needed, however, to identify the most efficient and cost-effective method of large-scale vaccine deployment and the effect on the incidence of bTB in cattle [14].

An advantage of vaccination as a response to this disease is that it is likely to be less contentious and enjoy increased public support compared to the culling of a wildlife host [15]. This should not be overlooked as it not only shapes the decisions of the policy makers but also how efficiently a management programme can be carried out, with regards to the levels of public disruption that might be encountered. Vaccination, by intramuscular injection, is however considered to be the more expensive option [14]. An oral badger vaccine has the potential to be easier to distribute, especially over a large geographic area, than the intramuscular injection [15]. It is also not likely to cause the

perturbation effect associated with culling. Badgers seem a prime candidate for the delivery of an oral vaccine due to their varied diet which presents opportunities for novel bait types. As well as this their existence in groups, in obvious setts, means they could be easily targeted. Some research has been carried out into creating a palatable bait design capable of containing an oral vaccine and the most effective way to deploy baits, to badgers [15]. However, more work needs to be done not only into the chemistry behind maintaining a vaccine in bait but also how to be cost-effective in deploying baits and trying to get enough badgers to eat the bait as to confer “herd immunity” in the population [12]. Evidence from previous wildlife disease scenarios treated using an oral vaccine suggests that continued research would be worthwhile, for example, the almost complete eradication of rabies in meso-carnivores in North America and Northern Europe. The large geographic spread of rabies present in wildlife and the inaccessibility of some endemic regions meant that culling operations, carried out up until the mid-1970s, had largely been unsuccessful [16]. The first field trial of an effectively potent, safe and attractive bait was carried out in Switzerland in 1978, after this, further improvements in the palatability of bait and the production of the vaccine lead to millions of baits being distributed by hand and aerial means [17]. This long term vaccination programme was mainly focussed in targeting the important vector of the red fox (*Vulpes vulpes*), in Europe, but also successful in treating raccoons (*Procyon lotor*), coyotes (*Canis latrans*), and gray foxes (*Urocyon cinereoargenteus*) in North America [18].

There are many challenges that face the development of an oral bTB vaccine, and a strategy of deployment, to treat badgers, if it is to be as successful as the oral rabies vaccine. With regards to the delivery of the vaccine, the difficulties lie in producing an effective bTB vaccine and bait that will keep the immunising bacilli viable from the point it is administered into the bait to the point of immune induction, in the badger. Once past this stage the vaccine must be able to maximise the likelihood that the consequent immune response is sufficient to confer protection against bTB. Once these problems are solved, the effectiveness of an oral vaccine would be dependent on the proportion of susceptible individuals that eat the bait and therefore receive the vaccine [19]. The social behaviour and feeding preferences of the badger adds some

complications to this process. Badgers live in social groups of different sizes, commonly from 2-10 [20] and in different habitats [21], as well as this each group may be composed of different ages and sexes of individuals. The effectiveness of an oral vaccination programme might be affected by how these factors influence bait uptake. Knowledge of this is currently limited and this research attempts to investigate some of these elements.

The key questions I plan to address are:

1. Does the uptake of bait differ between setts of different sizes?
2. Are certain age and sex classes of badgers more likely to eat or not eat the bait?
3. Do differences in the composition of the social group (interaction between age and sex classes and group size) have an effect on bait uptake?

These questions are important because the cost-effective but sufficient deployment of baits would be key to an oral vaccination programme. To enable this nature of questions to be investigated, the identification of individuals who have consumed baits is necessary. One method to enable this is to incorporate chemical markers into bait deployed for badgers. Iophenoxic acid (IPA) has been used successfully when combined with various ingestible products of interest, thus allowing analysis of their uptake. For example, it has been shown to be an effective long term marker in wild boar [22] as well as being used previously to study the uptake of baits in the badger [23,24]. After the period of bait feeding a blood sample must be taken from the animal and then high performance liquid chromatography is performed to detect the occurrence of IPA in the serum, from the blood [25].

The key questions, as previously identified, will be investigated using two sets of data, based on different response variables related to bait being eaten by badgers. The first part of this study is using data collected from video recording of badgers, from a bait preference study carried out during August 2013 that targeted 16 setts. I aim to investigate the effect of badger activity around a sett on bait disappearance. Information and analysis relating to this will be referred to as 'the effect of badger activity on bait disappearance' or the 2013 study when referring to the data set. As biomarkers were not used and badgers not captured, the amount of bait eaten was quantified as the number of baits that

had been removed by badgers each night of study. Badger sett activity can be loosely linked to group size [26]. This is because the more active a sett the more individuals are likely to be present in that sett, therefore the overriding question links to whether the amount of baits eaten differ because of the number of individuals within a sett. The second part of this study is utilising data from three relatively large-scale field trials carried out in England in 2010, 2011 and 2012. The aim is to investigate the effect of age and sex on bait uptake and how different compositions of these factors in a social group effect bait uptake, the influence of group size on bait uptake will also be analysed. Information and analysis relating to this will be referred to as 'how badger social group demographics influence bait uptake' or the three previous field trials when referring to the dataset. These field trials, included a biomarker in the bait so uptake could be identified.

## 3.2 METHODS

### Study Sites

#### The effect of badger activity on bait disappearance

Prior to the study 16 main setts were identified based on the appearance of active sett entrances, badger runs and latrines. A main sett can be defined as a sett permanently in use, with multi entrance burrows and that is used for breeding [27]. The badger populations were naïve, to the best of our knowledge, to being feed any sort of bait and were all located in the county of Gloucestershire, southwest England. Of the setts used in the study, most were found in woodland of varying sizes as well as some in pasture and arable land. The study ran for 10 days from the 6-16<sup>th</sup> of August 2013.

#### How badger social group demographics influence bait uptake

The data from which this part of the study is drawn comes from field studies carried out over three years; 2010, 2011 and 2012. Each trial aimed to investigate a different aspect of bait palatability and the most effective way to deploy bait in order to maximise uptake. Table 1 summarises the main facts relevant to each of these studies.

## Materials

### The effect of badger activity on bait disappearance

Prior to the trial starting, 24, labelled paving slabs of dimensions 20cm x 20cm were positioned randomly around active areas of the sett. Bait was placed under these slabs to deter non-target species. Using a slab has been previously described as suitable to accomplish this but still easy enough for a badger to move [28-30]. During each day of the study, a bait was placed in a small depression under each slab, to avoid crushing the bait. As the main purpose of this trial was looking into the palatability of a candidate vaccine bait\*, four different presentations were used, six baits of each type were deployed each day. The baits consisted of three different presentations of an 8g candidate bait and a control bait of peanuts mixed with syrup of equal weight. The position of each bait under each numbered slab was randomly allocated for the first day and each bait rotated daily so there would be no positional bias of some baits always being put in the same location. In order to minimise non-target interference, slabs were checked and baits replaced every afternoon. Un-eaten baits were removed and a record made of the fate of the bait the previous night.

At each of the 16 setts, two Bushnell trail cameras (Bushnell Trophy Camera model 119435) were secured to trees and aimed at active parts of the sett, also in view were varying numbers of the paving slabs complete with the corresponding bait underneath. These motion sensitive cameras were set up to take 60 second videos when tripped and have the minimum amount of time possible (1 second) between the end of a video and the capability for it to be tripped again. In reality the gap between videos was a few seconds longer as the camera needed time to write the video it had just recorded to the memory card. Daily checks were carried out on each camera and batteries and memory cards replaced when necessary.

### How badger social group demographics influence bait uptake

The exact details between studies varied (table 1), however, they all followed the same format, detailed as follows. Once the number of setts necessary had been located, prior to the start of the study the required equipment was deployed at the setts, this may have included slabs under which bait would be

\*Full details of the candidate bait cannot be provided due to commercial sensitivity and because uptake data being collected will form part of the evidence provided to the Veterinary Medicines Directorate for any future licence application.

placed or cameras in instances where badger behaviour was being recorded. New baits were deployed daily and, where baits had been deployed under slabs, daily disappearance was recorded. Biomarker (one of the three IPA analogues) was added to the bait to indicate whether or not the badgers had ingested bait. Once each study had been completed badgers were trapped in steel mesh box traps located on or near badger runs or active parts of the sett. This occurred after a period of one week's pre-baiting and 10-14 days after feeding the marked baits in 2012 and on two occasions two weeks and four weeks after feeding of the marked baits in the other two years. Trapped badgers were then transferred to a holding cage and transported back to a laboratory for anaesthesia and examination. Once recovered from the anaesthesia all badgers were released at their point of capture.

Year and months of study	Main locations	How many social groups targeted	Bait type deployed	How many baits per day	Factors investigated	How many badgers caught	Biomarker used
2010 May, June, July and August	Bath (Avon), Cirencester (Gloucestershire) and Langford (Bedfordshire)	48	Peanuts and syrup	1 <sup>st</sup> day 5, 2 <sup>nd</sup> day 10, 3 <sup>rd</sup> day + 15. At 100ml.	Where baits should be deployed (main setts or all setts), how baits should be deployed (above ground or down holes), when baits should be deployed (spring or summer).	269 badgers (100 adults, 169 cubs)	Propyl- lophenoxic Acid
2011 May, June and July	Tiverton (Devon), Cheltenham and Tetbury (Gloucestershire)	12	Candidate bait	15 a day. 3g baits.	How baits should be deployed (above ground or down holes)	67 badgers (38 adults, 29 cubs)	Propyl, Ethyl and Isobutyl- lophenoxic Acid
2012 July and August	West Sussex	40	Candidate bait	8 or 16. 8g baits	Pre-baiting duration (4 or 8 days), packaging presentation (perforated or unperforated) and number of baits deployed per day (8 or 16)	76 badgers (49 adults, 27 cubs)	Propyl, Ethyl and Isobutyl- lophenoxic Acid

Table 1. A table summarising the main facts from each of the three field trails. Accumulatively they constitute the data set I am using to investigate how social group demographics influence bait uptake.

## Statistical Analyses

### The effect of badger activity on bait disappearance

In order to quantify the difference in activity between badger setts, in relation to the disappearance of bait, two measures were applied to the video footage recorded at each sett and for each night. The first of these was how long badgers were captured on camera having interacted with the bait or with the slab. This interaction time included sniffing at the slab, moving the slab, eating the bait and sniffing at the bait packaging. This measure was controlled for how many slabs were in view by dividing the total amount of time badgers had been recorded having interacted with the bait by the number of slabs that particular camera had in view. The second measure aimed to quantify overall movement around the sett, independent of bait consumption. It was simply the number of times a badger passed the camera and didn't interact with the bait.

Generalised linear mixed models were used to investigate the effect of these two measures and others on the number of baits that had been eaten by badgers per sett per night. Baits were assumed to have been eaten by badgers if the slab had been moved and bait removed. Therefore the response variable was the number of baits eaten per sett per night up to a maximum of 24 (all the baits deployed had been eaten by the badgers at that sett during that night). Variables included in the model were the day of study (1-10), the two measures of activity (time spent interacting with bait and number of passes not interacting with bait) and two two-way interactions between 'time spent interacting with bait x day of study' and 'number of passes not interacting with bait x day of study'. Sett name and the number of cameras each night effectively collecting data (1 or 2) were included as random factors and the distribution was specified as Poisson as the response variable had a set limit and was count data [31]. Candidate models produced from all the different possible combinations of these variables were compared using Akaike's information criterion (AIC) to assess which model/s best explained variation in the response variable. The lower the AIC value the better the relative fit of the data to the model, a difference in model AIC values of more than two is considered a significant reduction in fit to the model [32]. Using a cuff-off, therefore, of more than two AIC points, the models generated were reduced to those that best explained the

variation in the response. Of these the coefficients were averaged to produce the final output. In order to describe the goodness of fit of these models to the data an  $R^2$  value was calculated using the procedure specified by Nakagawa and Schielzeth [33]. All analyses were carried out using R software version 3.0.2 [34] and the packages lme4 [35], MuMIn [36] and arm [37].

#### How badger social group demographics influence bait uptake

The second section of this study which aimed to investigate the influence of badger social group demographic characteristics on bait uptake was analysed in a similar way as the above. The response variable was whether a badger tested positive for the uptake of a bio-marked bait and therefore was binomial (0 or 1). The factors included in the model were age class (cub, adult), sex, year, group size (number of badgers trapped), proportion of cubs in each social group and proportion of males in each social group. Interactions between these factors that were included were; proportion of males and proportion of cubs in each social group, age and group size and sex and group size. One three-way interaction of group size, sex and age was also included in the model. Social group was included as a random factor. Group size was included in the models as a measure of intra-group competition (the number of other individuals which might also consume bait). However in some cases no matter what the group size, none or only a small proportion of badgers consumed bait, therefore this on its own is not a reliable measure of intra-group competition. In order to investigate this idea further, the proportion of badgers in each social group testing positive for a biomarker and group size was included as an interaction term. The proportion of badgers in each social group testing positive for a biomarker will obviously be related to the response variable, as the likelihood of bait consumption will always be higher in social groups where overall uptake is high (e.g. 80%) than one where it is low (e.g. 30%). However, we would not predict that the interaction would be significant. For example, the likelihood of bait uptake for an individual in a group of four animals where two were bait positive (50%) would be equal to that of an individual in a group of eight animals where four were bait positive (50%). What does differ between these two scenarios is the potential amount of bait consumed (i.e. four animals eating bait compared to two) and therefore the remaining bait available for other group members. A significant interaction may, therefore, suggest an individual is more

likely to test negative for consuming bait in a larger group where a certain proportion of the group members are bait positive than in a smaller group, possibly due to bait being limited.

Social groups in which only one individual had been caught were removed from the analyses because of the binomial nature of the response variable and the skew this would have imposed when looking at variables related to group uptake. All analyses followed the same model selection and averaging procedure as detailed previously and were carried out using R software version 3.0.2 [34] and the packages lme4 [35], MuMIn [36] and arm [37].

### 3.3 RESULTS

#### The effect of badger activity on bait disappearance

Of the 16 setts where baits and cameras were deployed during the 2013 study, badgers were recorded, from camera footage or from evidence of the consumption of bait (i.e. slab flipped over or moved significantly) as being present at 12 setts. Of these, a further five were removed. Three of these setts did not show any evidence of badgers consuming baits and at two setts, badgers were thought to have eaten some of the baits but no badgers were confirmed as being present from the camera footage. Consequently these setts were obviously not suitable for inclusion in a study looking at badger activity and bait disappearance, therefore seven setts remained that recorded sufficient bait disappearance and activity levels, during the 10-day study period. From these seven remaining setts, badgers from six were eating all 24 of the baits by the end of the study, the majority of setts achieved this within the first few days. Failure of cameras or memory cards resulted in two setts being reduced to only one working camera for three of the ten nights

The fit of these data to the general linear mixed models carried out indicated that there were two top models that best explained the variation in bait disappearance between setts (less than a difference of 2 between their AIC scores)(table 2). Of these, the model which offered the best explanation contained both of the activity measures as well as day of study and the interaction between the amount of time spent interacting with bait and the day of study. The second model contained all the variables specified in the full model

(detailed in the methods). The output, from averaging the coefficients of the two top models, are shown in table 3. This shows that the average model coefficients indicated a consistent positive effect of both measures of badger activity on bait disappearance (number of passes not interacting with bait- 95% CI, 0.12- 0.44, relative importance=1; amount of time spent around bait- 95% CI, 0.18- 0.54, relative importance=1)(figures 1 & 2). These results imply that bait disappearance increased as the levels of activity increased. Day of study has a positive effect on the disappearance of bait (95% CI 0.49- 0.76, relative importance=1) indicating that bait disappearance increased as the study progressed. The averaged model coefficients shows that the interaction term of the amount of time spent around the bait and day of study shows a negative effect on the disappearance of bait (95% CI, -0.82- -0.18, relative importance=1). This suggests that the time spent interacting with bait decreased as the study progressed (figure 1). The other term included in one of the top models that did not have any importance in explaining the variation in the data was the interaction term of number of passes not interacting with bait and day of study.

Model	Intercept	Time spent around bait	Number of non-bait interaction passes	Day of study	Time spent around bait x Day of study	Number of non-bait interaction passes x Day of study	d.f	AIC score	Delta	Weight	Marginal $r^2$
16	+	+	+	+	+		7	502.38	0.00	0.68	0.098
32	+	+	+	+	+	+	8	503.81	1.53	0.32	0.098

Table 2. Summary table of the two top models to explain the variation in bait disappearance from the data derived from the 2013 study. Inclusion of a given variable is indicated by the symbol (+), the AIC value and Delta value (the difference in AIC score from top model) are displayed as a measure of model fit. The weight (probability a given model is the best at explaining the data) and the marginal  $r^2$  (estimating the variation that is explained by a particular model) is also displayed.

Variable	Estimate	Std. Error	Z Value	Probability	Relative importance	Confidence interval	
						2.5%	97.5%
Intercept	2.31	0.52	4.42	<0.01	1.00	1.29	3.33
<b>Day of study</b>	0.62	0.07	9.17	<0.01	1.00	0.49	0.76
<b>Number of non-bait interaction passes</b>	0.28	0.08	3.53	<0.01	1.00	0.12	0.44
<b>Time spent around bait</b>	0.36	0.09	3.97	<0.01	1.00	0.18	0.54
<b>Time spent around bait x Day of study</b>	-0.50	0.16	3.05	<0.01	1.00	-0.82	-0.18
Number of non-bait interaction passes x Day of study	-.019	0.18	1.01	0.31	0.32	-0.55	0.18

Table 3. Summary table of the outputs from averaging of the two top models to explain the variation in bait disappearance from the data derived from the 2013 study. The highlighted terms indicate they have a significant impact in explaining the variation associated with the data, as is shown by the values of the 95% confidence interval being consistently on either the negative or positive side of zero.

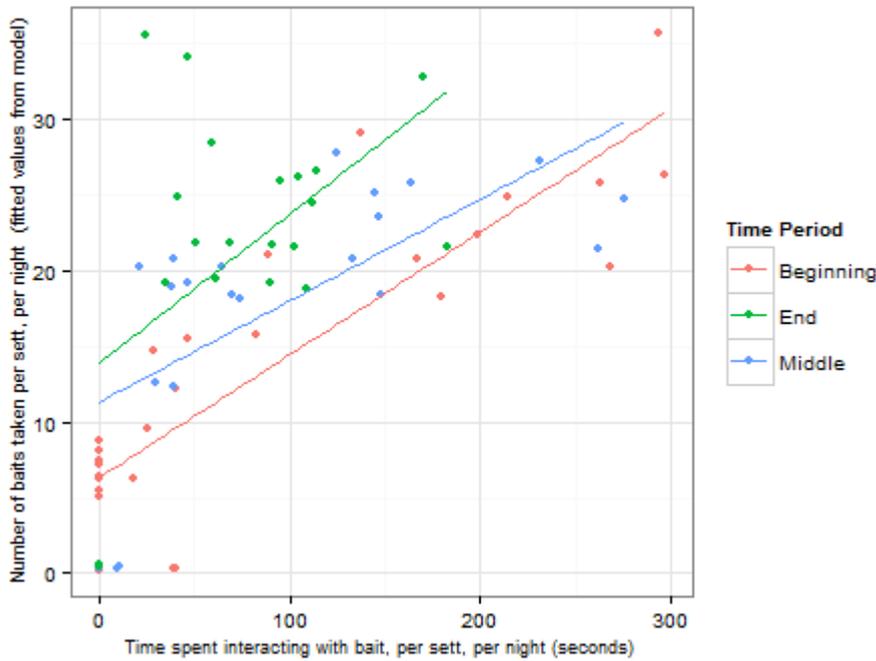


Figure 1. A scatter plot showing the relationship between the fitted values of number of baits taken, per sett, per night (as predicted by the top model), against the amount of time spent interacting with the bait, per sett, per night. Three lines of best fit are shown, each colour corresponding to a different time period.

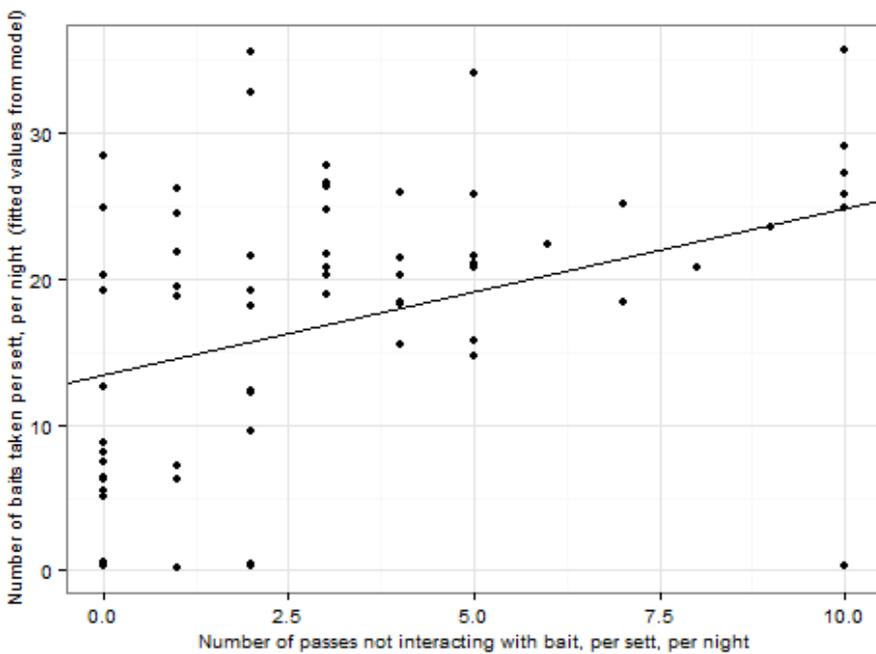


Figure 2. A scatter plot showing the relationship between the fitted values of number of baits taken, per sett, per night (as predicted by the top model), against the number of passes not interacting with bait, per set, per night. Also included is the line of best fit across all study days

### How badger social group demographics influence bait uptake

Uptake data were available from a total of 628 trapped badgers from 116 different social groups, over the three years of study. The total percentage bait uptake averaged over all three years was 65%. Subsequent AIC analysis of all the possible models to fit the data resulted in 13 top models <2 delta AIC points of each other (table 4). None of these models contained the interaction term 'proportion of cubs x proportion of males' or 'Year', but all other terms were represented in one of the models. The top model, as illustrated by the table, contained just the terms, age class, proportion of cubs in a group and proportion of the social group that were IPA positive. Table 5 displays the output from averaging the coefficients of the top general linear mixed models and allows us to interpret the importance and significance of each variable in explaining the variation associated with the data. Age had a consistent positive effect on the likelihood of bait uptake (95% CI, 0.40- 1.51, relative importance=1). Cubs, as can be seen from figure 3, are statistically more likely to be positive for an IPA marker than adults. The proportion of cubs in each social group has a consistently negative effect on the response variable (95% CI -2.35- -0.49, relative importance=1). This suggests that the higher the proportion of cubs in a group, the lower the probability of an individual in that group consuming bait (figure 4). The effect of the proportion of individuals in a group testing positive for IPA is obviously related to the probability of uptake and this is shown by the analysis (95% CI 5.41-7.58, relative importance=1). A consistent negative effect of the interaction term 'group size x proportion of group positive for IPA uptake' on the response variable, was identified (95% CI -3.12- -0.05, relative importance=0.65). This implies that the effect that group size has on the likelihood of an individual badger consuming bait varies depending on the proportion of other members that have eaten bait (figure 5 & 6). The other terms included in one of the top models that did not have any importance in explaining the variation in the response variable were sex, group size, proportion of males in a group, age class and group size, sex and group size, age class and sex and group size.

Model number	Intercept	Group size	Year	Proportion of cubs	Proportion of males	Age	Sex	Proportion of group ipa uptake	Proportion of group ipa uptake x Group size	Proportion of cubs x Proportion of males	Age x Group size	Sex x Group size	Sex x Age x Group size	d.f	AIC score	Delta	Weight	Marginal $r^2$
50	+			+		+		+						5	265.58	0.00	0.13	0.76
52	+			+		+	+	+						6	265.93	0.35	0.11	0.76
572	+	+		+		+	+	+	+					8	266.20	0.62	0.10	0.78
700	+	+		+		+	+	+	+		+			9	266.25	0.67	0.10	0.78
570	+	+		+		+		+	+					7	266.25	0.68	0.10	0.77
698	+	+		+		+		+	+		+			8	266.30	0.73	0.09	0.78
956	+	+		+		+	+	+	+		+	+		10	267.23	1.65	0.06	0.79
828	+	+		+		+	+	+	+			+		9	267.40	1.82	0.05	0.78
3002	+	+		+		+		+	+		+	+	+	10	267.48	1.90	0.05	0.78
58	+	+		+		+		+						6	267.51	1.93	0.05	0.76
3004	+	+		+		+	+	+	+		+	+	+	11	267.54	1.96	0.05	0.79
114	+			+	+	+		+						6	267.56	1.98	0.05	0.76
954	+	+		+		+		+	+		+	+		9	267.56	1.99	0.05	0.78

Table 4. Summary table of the thirteen top models to explain the variation in bait uptake from the data derived from the three previous field trials. Inclusion of a given variable is indicated by the symbol (+), the AIC value and Delta value (the difference in AIC score from top model) are displayed as a measure of model fit. The weight (probability a given model is the best at explaining the data) and the marginal  $r^2$  (estimating the variation that is explained by a particular model) is also displayed.

Variable	Estimate	Std. Error	Z Value	Probability	Relative importance	Confidence interval	
						2.5%	97.5%
Intercept	0.96	0.28	3.388	<0.001	1.00	0.40	1.51
<b>Age (Adult)</b>	2.01	0.52	3.910	<0.001	1.00	1.00	3.03
Sex (Female)	-0.49	0.35	1.42	0.16	0.47	-1.17	0.19
Group size	-0.46	0.50	0.92	0.36	0.70	-1.43	0.52
<b>Proportion of group IPA uptake</b>	6.50	0.55	11.74	<0.001	1.00	5.41	7.58
<b>Proportion of cubs</b>	-1.42	0.47	3.00	<0.01	1.00	-2.35	-0.49
Proportion of males	-0.08	0.34	0.23	0.82	0.05	-0.75	0.59
<b>Proportion of group IPA uptake x Group size</b>	-1.59	0.78	2.03	<0.05	0.65	-3.12	-0.05
Age x Group size	-1.34	0.99	1.36	0.18	0.40	-3.28	0.60
Sex x Group size		0.73	0.56	0.58	0.27	-1.02	1.83
Age x Sex x Group size		1.46	1.38	0.17	0.10	-0.85	4.85

Table 5. Summary table of the outputs from averaging of the thirteen top models to explain the variation in bait uptake from the data derived from the three previous field trials. The highlighted terms indicate they have a significant impact in explaining the variation associated with the data, as is shown by the values of the 95% confidence interval being consistently on either the negative or positive side of zero.

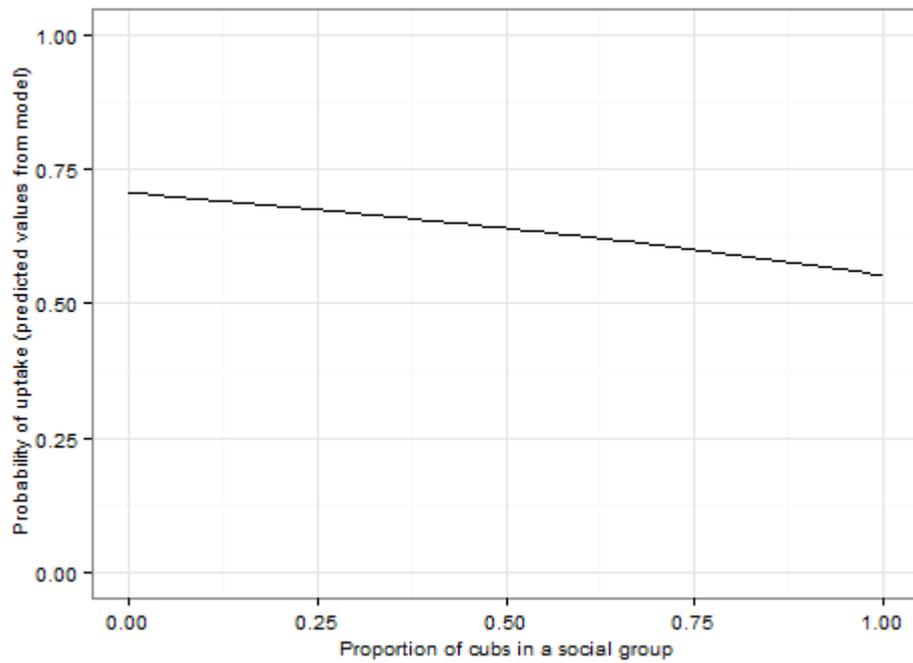


Figure 3. A graph showing the relationship between the probability of uptake (predicted values from the top model) and the proportion of cubs in a social group. Data points cannot be shown because of the nature of predicting values from a binomial model.

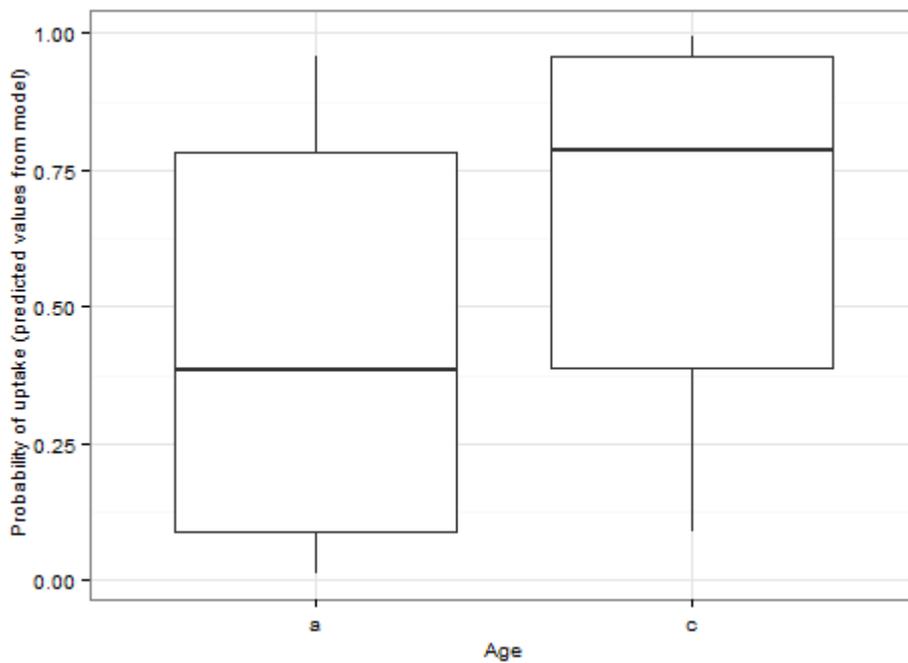


Figure 4. A box plot showing the difference between age classes in probability of uptake (predicted values from the top model).



### 3.4 DISCUSSION

The main findings of this study are that the consumption of baits by badgers is influenced by a number of factors relating not only to the activity of a badger's main sett but also to the age of the badger and the composition of the group to which it belongs. The first part of the study shows that as activity increases so does the number of baits taken (figures 1 and 2). As mentioned previously it seems sensible to infer information about group size from activity data. Setts with more individuals present are likely to be more active, as quantified by the two measures I employed. Therefore this result is consistent with the view expressed in a study by Cagnacci and colleagues [38], from their raw data they found the setts with the largest numbers of individuals consumed the highest proportion of baits. This might suggest that when bait was not taken, for example at the beginning of the study, it was because of low levels of activity observed on those days (figures 1 and 2), rather than badgers being present and avoiding the bait. An initial neophobic response to the slabs and the scent of the humans, who placed them, is one way to explain this observation, as the badgers became accustomed to the novel stimuli this would be likely to fade [39]. A second way to explain this pattern is that it shows altering of a badger's spatial use around the sett because of the baits attractive properties. The interaction between the time spent interacting with bait and day of study is represented in figure 1. The three different colour lines represent different time periods within the ten-day study, it suggests that as the study progressed, time spent interacting with bait, per bait eaten, reduces. This may have been because badgers had become habituated to eating the bait, moving the slab and where the baits were located.

The results of the retrospective analysis from the three previous field trials, show that age and some factors that contribute to the composition of a badger's social group have a significant effect on the likelihood of uptake. Looking at figure 3, age has a clear influence on the uptake of bait, with cubs more likely to test positive for an IPA marker from consuming bait than adults. Palphramand and colleagues [24] found, contrary to this, that adults consumed more buried baits than cubs. Baits were, however, buried away from the vicinity of the main sett and cubs may have been less likely to forage as far away from the main

sett as adults, at the time of year this study was carried out. Cagnacci *et al.* [38] found no difference in age or sex in the uptake of meat, fruit and cereal based bait types, in badgers. The proportion of cubs in a social group was negatively related to the response variable, the higher the proportions of cubs, the less likely individuals in the group are to have eaten bait (figure 4). As cubs are on average more likely to eat bait than the adults, they may consume all, or a high proportion, of the bait deployed, therefore reducing the probability of others encountering and consuming any. The effect of the proportion of the group positive for a biomarker on probability of bait uptake as a factor on its own is not very descriptive. As mentioned in the method, one would expect a relationship between the probability of uptake and the proportion of the group that have taken the bait. However, the significance of the interaction between the proportion of the group that are bait positive and group size, suggests that this relationship differs between group sizes. For example, in two different social groups of badgers, 50% of the individuals in each group are positive for consuming the bait; one group has few members the other has lots. In the larger group 50% represents more individual badgers and therefore, if the number of baits deployed per group is the same, then because more badgers are eating the bait there is likely to be less available, or none available, to the remaining badgers. In management terms this implies that in larger groups, that are eating the bait, the competition for bait is higher and that more badgers are likely to not ingest the bait at all, throughout the period of bait delivery, and therefore are potentially not receiving an oral bTB vaccination. Badger social group sizes are obviously variable not only within but between habitat types [40] and in order to maximise the number of badgers ingesting a candidate or indeed a vaccine containing bait, this result should not be overlooked.

The potential for oral vaccines to have a substantial role in the control of bTB in the UK is considerable [15,18,41). The type of bait used and the formulation of vaccine, to do this, are outside the scope of this study. However, the current candidate bait which was presented in three different ways as part of the 2013 study, in this research, recorded high levels of disappearance at all but one sett. In order to learn more about the behaviour of badgers, both at an individual and at a sett level, towards a candidate bait, increasing the video coverage of the sett and being able to recognise individuals on video footage would aid in this

further investigation. As well as this, the absolute number of individuals in the sett could be identified and the spatial and temporal dynamics, of who eats bait packages and how many, analysed. Further research such as this would aid in designing a cost-effective programme to vaccinate enough badgers with the ultimate aim to reduce bTB in cattle.

The results from this study detailing the effect of different demographic and group composition variables and sett activity levels on the uptake and disappearance of bait should help to inform further studies into formulating an oral bait vaccine deployment programme. In particular it should aid in shaping the procedure to maximise the proportion of badgers that gain access to and consume a candidate bait. Looking at the results of this study as a whole, perhaps the most important observations are those relating to group size and the effect of numbers of cubs on a social group's uptake of bait. This is something that should be considered in plans of future trials, as in large social groups or those with a higher proportion of cubs, more baits are likely to be necessary in order to give each badger the maximum possibility of eating a bait and ultimately of immunisation. Therefore a system might need to be considered of a more dynamic deployment of bait based on certain group size and/or group demographic variables. However as badger numbers inside a sett are difficult to precisely estimate [26], an approach of over-deployment may be more successful. The cost of each approach in terms of resources and in terms of the risk of not effectively vaccinating a population would have to be considered. This research has helped to identify the importance of sett-level demographic variables on the uptake of bait. Understanding the influence of social-group composition and demographics on bait uptake should be considered alongside bait formulation and presentation as important factors in achieving the uptake of bait in a large proportion of badgers.

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## CHAPTER 4: GENERAL DISCUSSION

### Culling-induced changes to badger movement

The analysis of movement data from the radio tracking of individual badgers indicates increased movement as a result of culling. One of the two novel measures employed to quantify perturbation revealed a difference between individuals from culled and un-culled populations. This adds to the findings of other studies that have discovered a difference in the movement of badgers, associated with culling [1-4]. It is now widely regarded that this perturbation of badger social groups can lead to an increase in disease transmission between badgers and between cattle and badgers [5]. The documented increase in TB incidence in cattle herds inside the reactive culling zones and in the 2km wide buffer outside proactive zones, during the RBCT [5], may have been because of this increase in transmission. The negative effects of perturbation limit the use of culling as a management strategy, in the UK, as at best the benefits can be described as 'modest' [6]. The economic costs of culling have been estimated to exceed the benefits derived through a reduction in cattle TB incidence by a factor of 2-3.5 [7]. In the Republic of Ireland results from the large scale Irish Four Areas Trial, which investigated the culling of badgers in four paired study areas, were more positive [8]. In this trial, however, the existence of natural boundaries such as mountains, sea inlets and rivers around the study areas may have reduced the immigration of surrounding badgers thereby minimising the detrimental effects of perturbation [5].

Interpreting the results from the culling of badgers is clearly complex and its effect on bTB incidence in cattle herds is dependent on numerous factors. Reducing the increase in disease transmission that is associated with the disruption to badger social groups seems to be key, if culling is to be used in any sort of management strategy. The outcome from the RBCT and the unpopularity of this approach suggests that culling is not a long-term or cost-effective approach to tackle the widespread bTB problem on the UK mainland.

### How the composition, demographics and activity of badger social groups influence bait uptake

The delivery of a BCG vaccine in an oral bait is generally recognised as the best prospect for the vaccination of badgers over a large area [5]. Many factors are likely to affect the consumption of baits by naive badger populations, in order to formulate an effective bait deployment strategy, these factors need to be researched.

The activity of a badger's main sett as well as its demography and composition influence bait consumption, as detailed in the second piece of research, within this dissertation. This demonstrates the complexity of devising a bait deployment strategy that will achieve high levels of uptake across all badger setts. The results of this research suggest that in order to increase levels of bait uptake, across all social groups, that increasing the number of baits deployed, where social group sizes are larger or there are a higher proportion of cubs, would be necessary. However, reliable estimates for these parameters will not be possible in real-world deployment, therefore a general increase in the number of baits deployed may account for differences in the likelihood of bait uptake in setts of different sizes and age class compositions.

2015 is the earliest anticipated data for a licensed oral vaccine [5]. Much work needs to be done before this, to perfect the bait, vaccine and deployment methodology. After licensing, continued improvement is likely to be necessary in these areas as well as analysing the effect on the epidemiology of bTB in badgers and the resultant effect on the incidence of bTB in cattle herds. The idea of an oral bait vaccine is popular amongst the general public and providing funding is available for its continued research and development, it has the potential to play an important role in reducing bTB in badgers, over a wide area, in years to come.

### Conclusion

The control of bTB in the UK and the Republic of Ireland is clearly complex, with a number of management strategies that either target the disease directly: in the major wildlife reservoir of the European badger and in cattle, or target the transmission of the disease from badgers to cattle and vice versa and from cattle to cattle. This research has increased the knowledge of how to measure movement of badgers as

a result of culling and the influence of certain demographic and social group composition factors in the uptake of a candidate oral vaccine bait. Both should assist in the development of a multi-faceted approach, based on sound scientific evidence that targets the disease in badgers and cattle and aims to reduce transmission between and amongst these two species. As well as this, constant improvements should be made, through research and evaluation, to current practises, with the aim of dramatically reducing the stranglehold of this disease, not only on farmers but on the cost to the taxpayer. The original definition of disease should also not be forgotten this is ‘an impairment of normal functions to an organism due to a disease agent’ [9]. Sometimes the effect disease has on an individual animal’s welfare is overshadowed by the economic loss to the industry with which it is concerned. In reducing the prevalence of bTB in cattle and badgers, the risk of, not only, wild and domestic animals but also man contracting the disease is reduced. In turn reducing the number that would otherwise undergo suffering and an impairment to normal function, that occurs as a result.

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## APPENDIX

### LANTRA Certificate of Training- Cage Trapping and Vaccination of Badgers

In order to satisfy the conditions of the MbyRes and contribute to valuable work within the AHVLA it was necessary to complete a LANTRA accredited training course titled The Cage Trapping and Vaccination of Badgers. The itinerary of the course is detailed below as well as the certificate of completion. In order to pass the training course it was necessary to have been judged as competent by your assigned field trainer/assessor and to pass a written assessment with at least 70%.

### **Cage Trapping and Vaccination of Badgers Course 2012**

#### **COURSE CONTENT & TIMETABLE**

##### ***Monday (Day 1)***

##### **Session 1 Theory (09:30-11:00):**

##### **1. Introduction to Badgers & TB**

- Overview of history of badger involvement in TB problem.
- Initial discovery, culling history
- Why badgers are a good potential TB reservoir for cattle.
- Longevity, abundance, TB prevalence, ecology
- Evidence for badger contribution to TB in cattle.
- Cattle TB rates in response to badger culling studies and policies. RBCT, 4 Areas etc
- What vaccination of badgers can offer in terms of TB control.
- Sustainable, risk reduction, herd immunity, publicly acceptable.

##### **2. Licences and legal requirements**

- Introduce the Protection of Badgers Act. Further legislation to be covered in Vaccination module
- What protection does it confer / what does it prohibit.
- ‘Taking’ badgers, definitions of disturbance and current use of setts
- What can be done under licence.
- Who grants licences, and under what circumstances.
- What are the responsibilities under licence.
- Reporting, annual returns.
- Certificate of Competence

##### **3. Badger Ecology**

- Abundance, distribution, habitat and food preferences.

- Social organisation and territoriality.
- Field signs – setts, latrines, footprints, paths.
- Activity and foraging patterns.

### **11:00-11:15 Coffee Break**

#### **Session 2 Theory (11:15-13:00):**

##### **4. Fieldwork theory**

- Assessing sett activity-what to look for, and difficulties to be aware of. Indications of badgers in residence, relationship (lack of) between sett activity and badger residents, extrinsic factors that affect sett appearance
- Recording sett activity
- Estimating the number of traps to deploy
- Placement and digging in of traps
- Setting traps
- Assessing Health & Welfare. Including dealing with non-target species. Assessing adverse weather conditions
- General Biosecurity
- Dealing with the public

##### **5. Fieldwork Health & Safety**

- General fieldwork Health & Safety
- Trapping specific Health & Safety
- Dealing with wildlife & livestock
- Personal Protective Equipment
- TB specific issues
- Cleaning & Disinfecting vehicles & traps
- Vaccines including needles & sharps
- Control Of Substances Hazardous to Health

### **13:00–14:00 Lunch**

#### **Session 3 Practical (Guide times 14:00-15:00):**

##### **6. Surveying for badger activity, sett checking, placement and setting of traps**

### ***Tuesday (Day 2)***

#### **Session 4 Theory and Classroom Practical (09:30-11:00):**

##### **1. Vaccination**

- Legal requirements
- Cold chain
- Handling vaccine
- Preparation of vaccine
- Injection of vaccine
- Records to be kept

### **11:00 - 11:15 Coffee Break**

## **2. Practical in handling vaccine and syringes**

-Safely handling vials and syringes

-Injecting bespoke silicon pads (commercially produced to simulate animal tissue as used by vet schools.)

**12:15 – 13:00 Lunch**

### **Session 5 Practical (13:00-17:00):**

**3. Pre-baiting and setting traps in real trapping scenario**

### **Session 6 Practical (Guide times 3-4 hours):**

**1. Checking traps, assessing behaviour & welfare, vaccination**

Following early morning trapping operations, there will be time to rest and review training before going out on fieldwork.

### **Session 7 Practical (Guide times 12:00-16:30):**

**2. Pre-baiting and setting traps in real trapping scenario**

## ***Thursday (Day 4)***

### **Session 8: Practical (Guide times 3-4 hours):**

**1. Checking traps, assessing behaviour & welfare, vaccination**

### **Session 9: Assessment (Guide times 1 hour):**

**2. Breakfast followed by written assessment of trapping and vaccination**



# Certificate of Training

**Josh Flatman**

has successfully completed training and assessment in

**Cage Trapping and Vaccination of  
Badgers**

Course Duration : 4 Days  
Date : 02 July 2012  
Instructor : Fiona Rogers

This is Customised provision approved by Lantra Awards

Robert Tabor  
Responsible Officer

Valerie Owen OBE  
Chair

Date of Issue: 08/08/2012

Ref: HC00176691 433501

