

The Effects of Culling on the Badger (*Meles meles*) Population in Ireland.

A thesis submitted to the University of Dublin for the degree of Doctor of
Philosophy.

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Declaration

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“There never was any heart truly great and generous,
that was not also tender and compassionate.”

-Robert Frost

Summary

The European badger (*Meles meles*) is a protected species in Ireland. However, it has also been identified as the main wildlife reservoir for bovine tuberculosis (bTB). Consequently, badgers are culled nationwide as part of the bTB eradication scheme, in an effort to reduce the incidences of the disease in the national cattle herd. The control program carried out by the Department of Agriculture, Food and the Marine (DAFM), provided an unparalleled opportunity to study the effects of long term culling on a protected mammal species. This project combined two main components: one, to investigate the effects of culling at population level, social group level and an individual level and two, to accurately predict parturition to define the most humane closed season.

Surprisingly, and contrary to much of the published literature, repeated culling as found to have a minimal effect on the population dynamics and social group sizes of Irish badgers. The same proportion of females were found to be pregnant, body weight and condition remained constant and bite wounding levels did not increase as had been expected. The age profile of the immigrating/dispersing females was also unexpected, as it was not primarily made up of younger females but instead it was a mix of adults and aged adults.

However, there were some significant differences seen between individuals in repeatedly culled areas and those from undisturbed areas. Females in repeatedly culled areas had significantly fewer blastocysts and those that did breed had significantly smaller litters. This suggests that some aspects of culling, possibly the stress associated with the repeated disruption and reforming of social groups, is negatively affecting badger reproduction. This study also investigated the variation in parturition timing, and whether body weight could be used to predict pregnancy. It also determined that teat width, length and mammary gland depth change significantly with pregnancy and that reproductively active females could be distinguished from their non-breeding counterparts using these criteria early as January.

With fecundity not decreasing as expected, this means that there may be ethical concerns for culling in repeatedly culled areas during the breeding season as lactating females may be culled and their cubs left to starve. This brings into questions the effectiveness of the current closed season, which would appear to be too short, at the wrong time of year and not currently implemented in all areas. A change to this current policy is strongly recommended.

In summary, this project added new information to our understanding of the dynamics of the Irish badger and suggested a means to predict parturition on an annual basis. Furthermore, it provided novel insights into how long term intensive culling can influence a mammal population and the ethical and demographic considerations which must be taken into account before future work is undertaken.

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1. General introduction

The culling of badgers during the bTB eradication program provided an opportunity to conduct a detailed study on changing population dynamics of badgers in Ireland. Badger culling has been ongoing in Ireland since 1989, resulting in a huge amount of data gathered by numerous sources during this time period. To date, very little of these data have been utilised to study the effects of culling on the targeted population. Utilizing both post-mortem examination and historical data resulted in a unique and comprehensive study on the effects of culling on a protected species and possible implications for the future.

The studies described here provide novel insights into the effects of widespread, long-term culling on a mammal population. The main aim of this study is to assess the effects of culling on badgers at a population level, a social group level and an individual level. With the exception of publications by Byrne *et al.*, (2013b) and Whelan (1998) relatively little is known of the long-term effects of repeated culling of badgers in Ireland. Byrne *et al.*, (2013b) assessed the effect of culling on animal abundance and Whelan (1998) used culled badgers to study reproduction in males and females. This current study examines the effects of repeated culling in much more detail, ranging from the effects on the population through to effects on individual animals. An important aspect of this study is to identify the dynamics of a previously undisturbed, ie non-culled, population and those in a repeatedly disturbed area. Only then can the effects of long-term culling be assessed. It is essential to understand how culling has altered the badger population so that these changes can be incorporated into models and projections for future work. The epidemiology of bTB and projections for how to combine culling with effective oral vaccine delivery, depend on understanding the population dynamics of the badgers after culling. Finally, this study will investigate methods to accurately predict parturition dates, as there have been ethical concerns regarding the culling of breeding and lactating females.

The following introduction will present detailed information on the ecology and reproductive biology of the badger, allowing for a thorough understanding of this species. It will also discuss the practice of culling on both the badger and other species, detailing any known effects which culling, particularly repeated culling events, may have on the targeted population. This chapter will also provide information on methods used to predict parturition and determine any methods which may be utilised in Ireland to aid future research. The objectives of this study will be summarised at the end.

1.1 Badger ecology

The Eurasian badger (*Meles spp.*) is a medium sized omnivore that displays both crepuscular and nocturnal lifestyles (Neal and Cheeseman, 1996). It is a member of the Mustelidae family and has a wide geographical distribution that extends across Eurasia from the island of Ireland in the west to Japan in the east. It can be found as far south as Palestine and its northern range extends into the Russian Arctic Circle (Yamaguchi *et al.*, 2006). However recent genetic research has found that the *Meles meles* species as originally defined is in fact comprised of four distinct parapatric species (Marmi *et al.*, 2006; Del Cerro *et al.*, 2010; Abramov and Puzachenko, 2013). The Asian badger (*Meles leucurus*) is found in northwest/central Asia while the Transcaucasian badger (*Meles canescens*) is found in southwest Asia and the mountains of Middle Asia. Japanese badgers (*Meles anakuma*) are found only in Japan. The European badger (*Meles meles*) is found throughout Europe, with its most eastern range now thought to be the Caucasus Mountains in Russia (Marmi *et al.*, 2006; Del Cerro *et al.*, 2010; Abramov and Puzachenko, 2013). Unsurprisingly, given its extensive distribution, it exhibits a high level of adaptability to different habitats and also shows substantial variation in social group formation.

While many mustelids tend to live solitary lives, *Meles meles*, (henceforth referred to as “badgers”) are considered to be social animals and can live in pairs or large groups of individuals (Kruuk and Parish, 1982; Revilla and Palomares, 2002; Page *et al.*, 1994; Johnson *et al.*, 2001). Many animals benefit from social living, especially in areas such as predator avoidance or alloparental care (Alexander, 1974; Johnson *et al.*, 2002b). However, badgers are considered to form spatial groups, as opposed to true social groups (MacDonald, 1983) as there appears to be no direct benefits of social living. Group members do not often forage together or co-operate in offspring rearing (Kruuk, 1989; Woodroffe and MacDonald,

2000; Roper, 2010). The Resource Dispersion Hypothesis (RDH) has often been used to explain group formation and sociality in badgers (Kruuk, 1978; MacDonald, 1983). RDH predicts that where resources, ie setts, food etc, are unevenly distributed, the smallest defensible territory for a pair of animals may also accommodate additional animals without needing co-operation as a reason to form social groups. This hypothesis suggests that territory size and group size are not related, but rather territory size is determined by resource dispersion and social group size is determined by resource abundance (Johnson *et al.*, 2002b; Robertson *et al.*, 2015).

There have been numerous investigations into the ecology and biology of the European badger, but there is a substantial bias caused by the large number of studies performed on a small number of high density populations in Britain. Studies from these British populations have shown that badgers are highly social animals living in groups of up to 30 individuals (Page *et al.*, 1994; Johnson *et al.*, 2001). However, mean social group size for these high density areas is much lower (8.8 adults per social group in Woodchester Park - Page *et al.*, 1994). The average social group size for Britain has been calculated between 5.35-5.9 adults per social group (Roper, 2010; Byrne *et al.*, 2012a).

The traits found in these high density populations are not characteristic of the whole European population. The badger is known to display a highly elastic social system. Social group sizes in some areas have been shown to be small, consisting mainly of male and female pairs or small family groups in low density areas such as Scotland (Kruuk and Parish, 1982), Spain (Revilla and Palomares, 2002), Poland (Kowalczyk *et al.* 2000) and Switzerland (Do Linh San *et al.*, 2007). In Ireland, estimates of mean social group size vary from 1.6 badgers (Stuart, 2010) to 5.9 badgers (Smal, 1995). Ireland has substantially lower badger densities compared to certain parts of Britain, particularly in south of the England. Badger density varies between 0.85 - 5.86 badgers km² in Irish studies (O’Corry-Crowe *et al.*, 1993; Smal, 1995; Eves, 1999; Sleeman *et al.*, 2009b) and 8.4 - 44.33 badgers km² in British studies (Rogers *et al.*, 1997b; MacDonald and Newman, 2002). However, as mentioned above, there is not an even distribution of badgers throughout Britain. Some areas such as Scotland have much lower badger densities (2.2 badgers per km² – Kruuk and Parish, 1987) compared to areas further south. This may also be occurring in Ireland, where culling has been ongoing for a number of years. It is possible that previously undisturbed areas have medium density badger populations compared with lower density populations in repeatedly culled areas.

Badger density across other European countries appears to be similar to the lower densities found in Ireland and Scotland (see Table 1.1), rather than the high density populations of southern Britain.

Table 1.1. Badger densities per km² across Europe.

Country	Badger density per km ²	Source
Albania	0.43	Griffiths and Thomas, 1993; Johnson <i>et al.</i> , 2002a
Austria	0.36	Griffiths and Thomas, 1993
Czech Republic	0.12-0.98	Matyáščík and Bičík, 1999
France	0.15-0.16	Griffiths and Thomas, 1993; Roper, 2010
Kazakhstan	0.52	Kowalczyk <i>et al.</i> , 2000
Latvia	0.3	Zoss, 1992
Poland	0.3	Goszczyński, 1999; Kowalczyk <i>et al.</i> , 2003b
Spain	0.28-0.5	Rodriguez <i>et al.</i> , 1996; Revilla <i>et al.</i> , 1999
Sweden	1.35-3.2	Griffiths and Thomas, 1993; Johnson <i>et al.</i> , 2002a; Roper, 2010

Habitat has also been found to significantly influence the social group size, density and spatial ecology of badgers (Feore and Montgomery, 1999). In parts of Northern Europe, badgers were positively associated with several species of deciduous trees (Van Apeldoorn *et al.*, 1998). In Portugal, badgers were found to primarily favour oak (*Quercus* sp.) woodland with understory vegetation which provided a cooler microclimate in summer months (Rosalino *et al.*, 2008). Similar habitat preferences were seen in Northern Ireland where social groups with strong territorial boundaries favoured areas of parkland with mixed woodland, while subprime areas of moorland had lower densities and exhibited less territorial defence (Feore and Montgomery, 1999). In Ireland, lowland pastoral and woodland habitats have significantly larger social group size compared to areas at higher

altitude (Byrne *et al.*, 2012a), suggesting that these areas are either able to support larger groups or may be examples of specific habitat selection by badgers. Furthermore, while badgers in Ireland have been found in upland areas up to 800m, the majority of social groups are generally found at altitudes below 200m (Gaffney and Sleeman, 2006). In other parts of Europe, badgers can successfully inhabit high alpine valleys (Wandeler and Graf, 1982; cited in Woodroffe, 1995).

Early badger population estimates determined that there are approximately 216,000 - 300,000 badgers in Britain, from which about 34,000 social groups are composed (Clements *et al.*, 1988; Wilson *et al.*, 1997). These estimations were achieved by surveying 1 km squares for the presence of badger setts which if present, were classified into the following categories: main, annexe, subsidiary or outlying. An average social group size was then used to calculate the badger population across Britain (Wilson *et al.*, 1997). Not surprisingly the badger population was not evenly distributed across the country. The majority of animals were located in southern areas with 21.9 - 24.9% in southeast and southwest of England respectively, whereas only 14.0% of badgers were found in Wales and 9.9% in Scotland (Cresswell *et al.*, 1989; Wilson *et al.*, 1997). A more recent survey of social groups found a substantial increase in the number of badger social groups. The current abundance estimation of social groups is 71,600 in Wales and England, with an increase of up to 103% in some areas of central England (Judge *et al.*, 2014). The increase in the abundance of social groups may be explained in several ways. While no current population estimation was given in the survey, it is possible that if social group numbers have increased, so too has the general badger population. If this is the case, it means Britain may have a much larger population of badgers than previously stated, possibly upwards of 400,000 individuals. Conversely, more social groups may not necessarily mean a larger population. It is possible that the disturbance attributed to culling may have fragmented previously stable populations, causing more social groups to form, but with fewer individuals in each social group.

There have been several studies over the last two decades that have attempted to assess the abundance of badgers throughout Ireland. Smal (1995) estimated a population of approximately 200,500 adult animals in Ireland. In 2009, a second population estimate was completed, this time taking into consideration the number of individuals culled in different habitat types, as well as accounting for vacant setts within the test area. This resulted in a much lower population estimate of 84,000 badgers (Sleeman *et al.*, 2009b). Finally, the most

recent population study reused data previously collated by Smal (1995). The data were then corrected to account for land class and the corresponding social group size within those land classes. This method resulted in a predicted population of 148,000 badgers in Ireland (Byrne *et al.*, 2012a).

Population density may affect the degree of territoriality exhibited by badgers. Territorial boundaries are often defined by the presence of boundary latrines. Latrines are collections of dung-pits usually found close to setts (hinterland latrines) or along boundaries (boundary latrines) (Roper, 2010). In high density populations, well defined paths link a number of border latrines, while in low density populations scent marking only occurs close to the setts and territorial defence may cease altogether (Feore and Montgomery, 1999; Revilla and Palomares, 2002; Roper, 2010). Males maintain boundary latrines more than females which suggests a possible function of such boundaries may be to act as a form of mate guarding and as a deterrent to neighbour males (Roper *et al.*, 1993; Stewart *et al.*, 2002). Latrine activity shows seasonal variation, with a peak occurring in spring and a smaller one in autumn (Roper *et al.*, 1993). This corresponds with the two main mating periods of badgers (Cresswell *et al.*, 1992; Page *et al.*, 1994).

Badger territories have been found to range in size from 14ha in areas of high density badger populations (Whelan, 1998) to 1500ha in low density populations (Kruuk and Parish, 1987). Territory size was also found to increase after a cull induced reduction in population density and social group abundance (O’Corry-Crowe *et al.*, 1993; Sleeman and Mulcahy, 2005). Additionally, there is evidence to suggest that in very low density populations badgers may become non-territorial (Revilla and Palomares, 2002). As there is a strong linear relationship between badger density and territory size, with social groups in high density populations having smaller territories, it is unsurprising that Ireland, with its low-medium density population has large, flexible territories (Roper, 2010; Byrne *et al.*, 2012a).

A consequence of territoriality may be episodes of aggressive behaviours such as bite wounding. Previous studies found a higher frequency of bite wounding in higher density populations and larger social groups (MacDonald *et al.*, 2004; Delahay *et al.*, 2006b; Byrne *et al.*, 2012a). Cresswell *et al.*, (1992) found that badgers in a high density population in Gloucestershire displayed elevated occurrences of bite wounding in both sexes, with bite wounding present in 15 - 70% of males and 10 - 40% of females. Levels of bite wounding

varied by month, with the greatest incidence of wounds occurring in conjunction with mating and parturition periods (Cresswell *et al.*, 1992). Other British studies have found lower frequencies of bite wounding, with approximately 8% of individuals in an undisturbed populations showing signs of injuries (Delahay *et al.*, 2006b). As Ireland has lower population densities and smaller social group sizes, it would be highly expected that bite wounding frequency is less common than the levels found in Gloucestershire. In previous studies, bite wounding was found in 4 - 6% of the Irish badger population (Murphy *et al.*, 2010). Stuart (2010) also found very low levels of bite wounding injuries, with less than 1% of badgers presenting with wounds. These data suggest that this aggressive behaviour is uncommon in the Irish population, especially when compared to higher density populations found in Britain.

Across Europe, the body weight of badgers varies significantly between countries. Comparisons of annual mean body weights of badgers suggest that both Irish and Spanish badgers have significantly lower body weights compared to British badgers (Revilla *et al.*, 1999; Roper, 2010). It is likely that this weight difference may be a result of these populations being morphologically distinct, rather than the difference being solely linked to density variation. A study of cranial measurements in 1997, found that Irish badgers had significantly smaller cranial measurements than British badgers (Lynch *et al.*, 1997). This indicated that in some cases lower body weight may be a result of smaller body composition and not density related.

Diet may also play an important role in badger densities, as areas rich in food resources can support greater numbers of animals compared to those with limited or patchy resource availability. In Britain, badgers forage primarily in pasture where they feed predominantly on earthworms (*Lumbricina*) (Kruuk, 1978). However, other dietary studies conducted across Europe have found that earthworms contribute very little to the overall ingested biomass compared to other, more readily available food sources such as insects and fruits (Roper, 2010; Byrne *et al.*, 2012a). A recent investigation into the diet of the Irish badger has revealed that earthworms make up only a small percentage of the ingested biomass bulk (3 - 4%) (Cleary *et al.*, 2009). Furthermore, a study in County Kilkenny found no significant correlation between badger density and earthworm biomass (Muldowney *et al.*, 2003). In Ireland, plant material occurred in greater volume and frequency than animal material in both gut contents and faecal material from Irish badgers, most of which was grass, leaves

and wood (Cleary *et al.*, 2009; Cleary *et al.*, 2011). It is probable that the ingested plant materials are an incidental consumption while foraging for the true food sources. Larvae of the crane fly (*Tipulids*) and yellow under-wing moth, frogs and bees are the primary digestible prey items eaten by the badger (Cleary *et al.*, 2009). However, badger diet was found to be highly seasonal and dependent on the availability of prey species. During autumn and winter months noctuid larvae were preferentially consumed, while tipulid larvae were favoured during spring (Cleary *et al.*, 2009; Cleary *et al.*, 2011).

1.2 Badger reproduction

One of the most likely things to change in a culled population, and one of the most important things to know in order to predict its recovery, is the reproductive success of the recovering population. In spite of geographical differences in diet and social group organisation there are common biological processes that happen across all European badger populations.

Female badgers exhibit a phenomenon known as delayed implantation. Delayed implantation can be classified in one of two ways: lactational, also known as facultative diapause, and seasonal also known as obligate diapause (Sandell, 1990). Facultative diapause occurs when a female is fertilised while still lactating. The suckling of the offspring results in the newly fertilised embryos entering into diapause (Daniel, 1970). Female badgers are obligate implanters. This practice means that implantation of the embryo occurs at a specific time of year for all females, usually during December/January (Canivenc & Bonnin, 1979).

Delayed implantation has been recorded in 47 mammalian species, although the mechanics of delayed implantation differs between groups (Sandell, 1990). There are several mammal families that display similar implantation processes to those found in Mustelid family. These include bears, seals, honey and pygmy possums (*Tarsipes rostratus* and *Burramys parvus*) and the feathertail glider (*Acrobates pygmaeus*). Delayed implantation occurs when the fertilised egg does not attach to the uterine wall and undergoes a period of diapause where there is no noticeable cell division (Sandell, 1990). After a period of delay of up to ten months during which the blastocyst remains free in the uterus, it implants in the uterine wall and the development of the embryo begins again (Stuart, 2010). In badgers, delayed implantation is characterised by a gradual increase in blastocyst size before implantation

happens (Renfree and Shaw, 2000). Studies have found significant growth in blastocyst diameter during winter months prior to implantation, allowing for an approximate time of fertilisation to be calculated from the measurements (Ahlund, 1980; Stuart, 2010). Superfetation is a process by which pregnant females may still ovulate and become fertilised. This rare phenomenon has been found to occur occasionally in badgers (Sweden-Ahlund, 1980; Britain - Cresswell *et al.*, 1992; Ireland - Stuart, 2010; Corner *et al.*, 2015), and allows for multiple fertilization opportunities within a single oestrus cycle.

Canivenc and Bonnin (1979), Mead (1993), and Woodroffe (1995) have argued that the timing of implantation is determined by factors such as photoperiod and by body condition. Decreasing day length has been found to act as an implantation trigger in badgers, with most females implanting between mid-December and early January (Canivenc and Bonnin, 1979; Ahlund, 1980; Whelan, 1998; Stuart, 2010). Additionally, body condition can affect implantation timing with heavier females implanting earlier in winter compared to females with poor body condition, who can fail to produce blastocysts at all (Cresswell *et al.*, 1992; Woodroffe and MacDonald, 1995a; Delahay *et al.*, 2006a).

In badgers, the main oestrus cycle of female badgers takes place early in the year, between February and April, with a second, smaller cycle later in the year during autumn months (Cresswell *et al.*, 1992; Page *et al.*, 1994; Stuart, 2010). Parturition date varies with geographical location but generally occurs earlier in southerly, warmer climates (Stuart, 2010). Parturition dates can range from early January in warm regions of Spain through to early April in parts of Russia (Neal and Cheesman, 1996; Revilla *et al.*, 1999). In Britain and Ireland, badgers have been found to give birth between January and March, with a peak in early February (Whelan and Hayden, 1993; Woodroffe, 1995; Whelan, 1998; Roper, 2010; Stuart, 2010; Corner *et al.*, 2015). Females predominately have litters made up of 2 - 3 cubs, with an even sex ratio distribution (Whelan and Hayden, 1993; Page *et al.*, 1994; Whelan, 1998; Dugdale *et al.*, 2003; Stuart, 2010). Table 1.2 illustrates the range of key phases in the reproductive cycle of sexually mature female badgers. This timeline is primarily based on literature from Ireland and Britain, as the timing of some of these phases can vary with geographical location.

After a period of diapause, implantation can occur any time in a period between November and January (Whelan, 1998; Roper, 2010; Stuart, 2010). Gestation is 7 - 8 weeks in length

(Roper, 2010; Stuart, 2010; Corner *et al.*, 2015). In Britain and Ireland parturition occurs between January and March, with a peak of births occurring in February (Whelan and Hayden, 1993; Woodroffe, 1995; Whelan, 1998; Roper, 2010; Corner *et al.*, 2015). Cubs are dependent on their mother for a minimum of 12 weeks and are fully independent by 15 weeks, which results in lactating females being present in the population from January through June (Neal and Cheesman, 1996, Roper, 2010). The current literature suggests there are two main mating periods; spring and autumn, although mating behaviour has been observed throughout the year.

Table 1.2 Seasonal time-frame of the female reproductive cycle in badgers from Ireland and Britain.

	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Implantation¹												
Gestation²												
Parturition³												
Lactation⁴												
Main Mating periods⁵												

¹ Whelan, 1998; Roper, 2010; Stuart, 2010.

² Roper, 2010; Stuart, 2010; Corner *et al.*, 2015.

³ Whelan and Hayden, 1993; Woodroffe, 1995; Whelan, 1998; Roper, 2010; Corner *et al.*, 2015.

⁴ Neal and Cheesman, 1996; Roper, 2010.

⁵ Cresswell *et al.*, 1992; Page *et al.*, 1994; Stuart, 2010.

For both male and female badgers, sexual maturity is reached in the second year of life, between 12 and 24 months (Ahlund, 1980, Whelan and Hayden, 1993; Stuart, 2010). Matings have been observed to happen all year around (Neal and Cheesman, 1996) and matings with multiple partners can result in 16 - 25% of litters being of mixed paternity (Carpenter *et al.*, 2005; Dugdale *et al.*, 2007). Females may choose multiple mating partners

for several reasons such as assurance of fertilization, reduction of infanticide by males or even genetic benefits such as encouraging sperm competition in an effort to maximise the quality of genetic material (Dugdale *et al.*, 2007). Mixed paternity litters can also be a result of superfetation.

In high density populations in Britain, badgers have low reproductive success with less than 45% of females successfully producing cubs in any individual year (Cresswell *et al.*, 1992). Reproductive failure occurs in all stages of the reproduction cycle, although the greatest losses are during the implantation process (Cresswell *et al.*, 1992; Page *et al.*, 1994). In contrast, in both Sweden and Ireland, where social group sizes are smaller than in Britain and densities are lower, reproductive success is much higher with upwards of 65% of females breeding annually (Ahlund, 1980; Whelan, 1998; Stuart, 2010; Corner *et al.*, 2015). In Ireland, Stuart (2010) and Corner *et al.*, (2015) suggested that reproductive failures mostly occur as a failure to fertilize. The differences in reproductive failure stages between Ireland and Britain may be due to the differences in social structure. In Britain, the lack of reproductive success may be caused by breeding suppression between the dominant and subordinate females within high density social groups (Woodroffe and MacDonald, 1995b). In Ireland, where population densities are lower, the low fertilization rates are most likely caused by a lack of successful matings or by loss of blastocysts before implantation (Stuart, 2010). As a result, in Britain in high population densities, the removal of dominant animals through culling, may encourage additional reproductive success. However, in Ireland, it is possible that badger populations in culled areas will be slower to recover as social structure disruption caused by culling may contribute to lower fertilization rates. If this is the case, population recovery will be slower than might previously have been expected.

1.2.1 Definition of terms

As badgers use delayed implantation as a means of reproduction, specific terms are used to describe the different states of reproduction in female badgers. In this study the term pregnant refers to females carrying a conceptus - fertilised ova through to foetuses. Therefore, if a female badger successfully mates, i.e. has been successful fertilised, she can be classified as pregnant. The term gravid refers to females carrying embryos or foetuses i.e. only pregnant females with successful implantation of the fertilised ova. “Embryo” refers to early stage development after fertilization has occurred, while “foetus” refers to later

developmental stages – when the body of the growing foetus is fully formed. These definitions have been based on those provided by Corner *et al.*, (2015).

Other descriptive terms have also been used in this thesis, particularly in Chapter 6. Reproductively active females are defined as females which are/have been pregnant, gravid or lactating within one breeding season. Reproductively inactive females are those which show no detectable signs of having been pregnant, gravid or lactated within the breeding season, despite being sexually mature.

1.3 Culling animals to prevent disease

Worldwide there are numerous culling initiatives used to prevent or reduce the spread of zoonotic diseases. Dog culling occurs in Brazil in an effort to control visceral leishmaniasis (Reithinger and Davies, 2002; Costa, 2011). In many areas of the world, culling of poultry and wild bird populations has occurred in an attempt to reduce the spread of avian influenza (Capua and Alexander, 2004). Several terrestrial species such as wild boar (*Sus scrofa*), African buffalo (*Syncerus caffer*) and badgers have been recognized as a reservoir of bTB in countries such as New Zealand, South Africa and across Europe. (O’Keeffe, 2006; Corner, 2006; Cross *et al.*, 2009; Mentaberre *et al.*, 2014). Because of this, numerous different culling programmes have been initiated to prevent and/or limit the spread of the disease between wild and domestic animals, although the success of these programs varies.

It is vitally important to identify and fully understand the effects of long term culling. Without knowing exactly how culling affects the population, especially long - term culling, it is impossible to accurately predict the full impacts on a targeted population. Currently, there is very little within the current body of literature that goes beyond simple density reduction or reductions in disease transmission. There is a worrying lack of understanding of both behaviour and physiological changes which may occur as a result of culling. This is particularly important when protected species such as the badger are the main targets of such culls.

1.3.1 Culling as a response to bTB in mammals

As previously mentioned, in many countries different animal species may act as a wildlife reservoir for bTB and despite ongoing control programs the disease does persist in national cattle herds. Local depopulation of the wildlife reservoirs may be carried out by a variety of different methods, such as poisoning, shooting or snaring.

1.3.2 Possums

The common brushtailed possum (*Trichosurus vulpecula*) is an invasive alien species in New Zealand. It acts as a reservoir for bTB and localised culling of this species has been used to address the problem in some areas (Roberts, 1996; Ji *et al.*, 2005), as possums are considered to be invasive and conservation pests. Shooting, trapping and poisoning are the main methods used for culling. In recent times however, there has been a move away from traditional culling methods, towards the implementation of a vaccine program (Tompkins *et al.*, 2009). Modelling studies have predicted that proactive culling which results in a lowering of the population density to less than 45% is the most effective way to prevent bTB establishing in an uninfected population; while a vaccination programme may be a more efficient approach in already infected populations (Roberts, 1996). However, possum culling alone has resulted in a significant reduction in bTB reactor rates in the surrounding cattle herds (Tompkins *et al.*, 2009) and appears to be a cheap and efficient bTB control method.

1.3.3 Wild boar

Over the last decade wild boar (*Sus scrofa*) and feral pig populations have been expanding in distribution and abundance across Europe. This is likely to be due to decreased hunting pressure as rural populations fall due to human migration to more urbanised areas (Gortázar *et al.*, 2012). In New Zealand, feral pigs have a higher frequency of bTB compared to other species such as cattle, deer or possums and can have a prevalence of >90% in some areas (Nugent *et al.*, 2003). While there is little evidence that suggests epidemiologically significant transmission from live pigs to livestock (Corner, 2006), infected carcasses may pose a transmission risk to scavengers (Nugent *et al.*, 2003). This in turn may add to the transmission of the disease back to livestock. Poisons are commonly used in New Zealand as they achieve high kill rates, although they fail to provide full local eradication as a single

method of culling (Nugent *et al.*, 2003). Wild boar culling also occurs throughout Europe in an effort to reduce the disease in cattle (Gortázar *et al.*, 2012; Mentaberre *et al.*, 2014). In recent times bTB in feral pigs has been detected in southern Britain, adding to the challenges for disease control in livestock in areas where this species' abundance and range is increasing (Gortázar *et al.*, 2012). However, there is contradictory evidence on the effectiveness of intensive culling in wild boar, as the reduction in the prevalence can be as much as 21-48% or as little as none at all (Mentaberre *et al.*, 2014).

1.3.4 Deer

As seen in wild boar, deer populations have expanded rapidly across Europe in recent times due to changes to management, such as supplementary feeding over winter months and translocations for hunting purposes (Corner, 2006; Gortázar *et al.*, 2012). bTB infections have been found in a number of cervid species, with red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) being significant routes of transmission to cattle in Britain (Delahay *et al.*, 2007). In Michigan, USA, Whitetail deer (*Odocoileus virginianus*) are thought to be responsible for the re-emergence of bTB in cattle after its successful eradication (Corner, 2006). bTB infected cervids have been found throughout Europe in Austria, Germany, Portugal, Spain and Britain (Gortázar *et al.*, 2012). While France was officially declared to be bTB free in 2000, bTB has been found in its red deer populations (Gortázar *et al.*, 2012). At present about 30% of its deer population has been found carry the disease. This is also a growing area of interest in Ireland. A recent study conducted in Co Wicklow found that up to 18% of deer tested positive for bTB, which has prompted a proposal that deer culling is required in bTB hotspot areas (O'Keeffe, 2015). With the disease being found in both farmed and wild deer herds, this had significant implications for the spread of bTB into surrounding cattle herds.

1.3.5. Badgers

Badgers act as a wildlife reservoir for bTB throughout Europe, with France, Poland, Spain, Ireland, UK and Switzerland all reporting incidences of bTB positive badgers (Gortázar *et al.*, 2012). However, badger culling as a means to reduce the disease incidence in cattle herds is most common in Ireland and Britain. During the 1950s bovine tuberculosis (*Mycobacterium bovis*) levels in Irish cattle had risen to extremely high levels, with 17% of

animals and over 80% of herds infected (O’Keeffe, 2006). In an effort to eliminate this disease from the national cattle herd, there was a voluntary test and slaughter scheme for cattle implemented in Ireland in 1954, although this didn’t become compulsory or nationwide until 1962 (O’Keeffe, 2006; Byrne *et al.*, 2012a). While there was an initial rapid decrease in the number of positive test cases (O’Keeffe, 2006), this has not continued and bTB continued to occur throughout the national herd (O’Keeffe, 2006), with Ireland, along with Britain, having some of the highest incidences of bTB throughout Europe (Ní Bhuachalla *et al.*, 2015).

The first bTB infected badger was discovered in Ireland in 1974 and subsequently several studies found evidence that in areas where badgers were found to have latent bTB infections there was also an increased incidence of bTB in cattle (Dolan, 1993; Martin *et al.*, 1997). In Ireland, badgers have protected status under the Wildlife Act 1976 and the Berne Convention on the Conservation of European Wildlife and Natural Habitats. However, under license DAFM initiated a nationwide strategy of culling badgers in targeted areas to form a basis of temporary disease control (Byrne *et al.*, 2012a). From 1989 - 2004, two field trials were carried out to assess the effect badger culling had on bTB prevalence in the surrounding cattle herds. The East Offaly Project (EOP) ran from 1989 - 1994 and the Four Areas Project (FAP) ran from 1997 - 2002. The studies used a mix of both reactive (targeting setts in areas in a circle of radius two kilometers around the herd after a positive bTB herd test) and proactive culling (targeting setts in general areas of high bTB prevalence in cattle herds). It was found that intensive proactive badger removal led to a decrease in bTB levels in cattle herds in the surrounding areas (Eves, 1999; Griffin *et al.*, 2005).

A similar culling study, the Randomized Badger Culling Trial (RBCT) was conducted in Britain from 1998-2005. This study found a reduction in the prevalence of bTB in cattle herds in proactively culled areas (Bourne *et al.*, 2006). However, there was also a 22% increase in herd breakdown in areas adjacent to proactively culled areas and a 27% increase in bTB prevalence in cattle herds in reactively culled areas (Donnelly *et al.*, 2003; Donnelly *et al.*, 2006). Despite the RBCT ending early due to the increase in reactivity, additional pilot studies for further culling have been undertaken in West Gloucestershire and West Somerset (Ares and Hawkins, 2014; Independent panel, 2014; Ares and Thorpe, 2015).

The Irish badger culling program which began in the late 1980's, aimed to significantly reduce badger density in local, targeted areas, from ≥ 2 badgers per km² to less than 0.5 badgers per km² (Ní Bhuachalla *et al.*, 2015). This threshold was based on research which suggested that badger densities of more than one badger per km² would allow for bTB persistence in surrounding cattle herd (Anderson and Trehwella, 1985; Hayden, 1993). This lowered density is not evenly distributed across the country; instead it is limited to local bTB hotspot areas where the risk of badger-cattle transmission is at its highest. However, it not yet apparent whether continued culling will be viable in the long term and what other effects it is having on the badger population. Nonetheless, Hayden (1993) does state that recovery within 5 years after culling ceases is likely, if migration and movement occurs naturally in the population, if there is some survival of cubs from targeted social groups, and if there are contiguous social groups within the hotspot areas.

As cage trapping has been found to be less efficient than other methods (Byrne *et al.*, 2012a), stopped free - running snares (referred to as stopped restraints in Ireland) are the preferred method of capture in Ireland. Stopped restraints are multi - strand steel loops, approximately 143cm long with a stop on the wire to prevent it closing further than 28cm (Anon, 1996; Griffin *et al.*, 2005). As the animal's head enters the wire loop, the forward momentum activates the snare which tightens around the neck or torso of the individual, restraining the animal. The stopping mechanism prevents strangulation of the animal. The stopped restraints are placed close to active setts for 11 consecutive nights and are checked each morning. Captured animals are killed with a 0.22 calibre rifle (Griffin *et al.*, 2005). Targeted setts may be re-culled if bTB reoccurs in the surrounding cattle herds (DAFM, *pers. comm.*)

Badger culling in Ireland has been classified as a medium - term strategy and will be supported by the introduction of a badger vaccine. The vaccine program aims to reduce badger to cattle transmission of bTB (O'Keeffe, 2006; Byrne *et al.*, 2012a). Vaccine trials are currently ongoing and while the implementation of the vaccine program is projected to begin presently, no formal dates has been decided (Byrne *et al.*, 2013a).

Prior to 2005, badger culling was conducted in all months of the year. Since then, culling during the first three months of the year had been restricted to only those areas undergoing repeated culling events. The 3 month 'closed season' was implemented due to concerns that lactating sows in established social groups were being killed, leaving their dependant cubs

to starve (L. Corner, *pers. comm.*). As culling can occur within this time period when deemed necessary by DAFM, this appears to be a voluntary closed season as opposed to a legal requirement. Despite repeated attempts, more information regarding the particulars of the licensing agreement was not forthcoming.

1.4 Impacts of culling on a population

Culling and other intensive control programs can result in significant changes to the population dynamics of the species (Tuytens *et al.*, 2000a; Tuytens *et al.*, 2000b; Coulson *et al.*, 2004; Sadler and Montgomery, 2004). It is important to recognise that the changes brought about by such control programs can have long-term consequences and may affect the target population for several generations after these operations have ceased (Coulson *et al.*, 2004). For many species culling results in significantly reduced abundance and density within selected areas. In instances of human-wildlife conflict, such as those seen with cougars (*Puma concolor*) in USA, studies have found that control programs resulted in a 60-80% decline in population density after culling (Stoner *et al.*, 2006)

1.4.1 Changes to reproductive success

The fecundity of the species concerned can affect population dynamics in targeted areas. Species with high fecundity, such as birds and small mammals, will repopulate culled areas more quickly than species with low fecundity, such as larger mammalian species. Many larger mammalian species require immigrant based recolonisation to re-establish social groups within culled areas (Lindenmayer *et al.*, 2005). Some studies have found that fecundity increased after culling events, as seen in common brushtail possums (Ji *et al.*, 2005). However, this is not always the case as studies on female red deer found no evidence of increased reproductive success following culling (Putman *et al.*, 2005). Indeed, some badger populations that have been greatly depleted by large scale culling were found to have reduced fecundity rates and may take years to recover to their pre-removal densities (Tuytens *et al.*, 2000b; Sadler and Montgomery, 2004). Before the present study it was not known how culling affects the fecundity of the Irish badger. It is possible that a similar situation may happen in Ireland, leading to a very low reproductive success in repeatedly culled areas. However, it should also be noted that some studies have found that badgers are able to withstand some degree of disturbance to the population. Badger reproductive output

was found to be unaffected despite disturbance from hunting pressures in Germany (Keuling, 2011). Therefore, it may also be possible that badger culling in Ireland will have little effect on local fecundity.

1.4.2 Behavioural and physical changes

In cases where there is disruption to abundance and territoriality in a targeted population, it is probable that other behaviours may also be disrupted after culling has taken place. For instance, possums in reduced density populations have been found to use more denning sites after removal programs took place (Ji *et al.*, 2005). In feral horses (*Equus caballus*) significant behavioural changes were found after culling: stallions showed evidence of decreased herding behaviour, but harem-tending behaviour increased by almost 200%. Female group fidelity also increased significantly despite social perturbation caused to social groups (Ransom *et al.*, 2014). Aggressive behaviours have been found to increase in highly fragmented elephant social groups as a result of poaching in Tanzania (Foley *et al.*, 2001).

Bite wounding has been reported in both disturbed and undisturbed badger populations although, it has been found to be more common in high density populations (Cresswell *et al.*, 1992; MacDonald *et al.*, 2004). In general, males are more likely to have a higher incidence of bite wounds and wounds of greater severity than females (Cresswell *et al.*, 1992). During recolonisation of culled areas female badgers were found to have slightly higher frequency of bite wounding compared with females from uncultured areas (Delahay *et al.*, 2006b). This suggests that aggressive interactions could potentially increase post-culling, possibly due to reforming social groups and establishing dominant/subordinate hierarchy. However, over time bite wounding was found to decrease significantly in areas undergoing culling (Delahay *et al.*, 2006b). Therefore, given the extra availability of resources due to the decrease in badger numbers and a possible decrease in territorial behaviour in culled areas, it is possible that aggressive behaviours, such as bite wounding levels in Irish badgers may also decrease in culled areas.

When a population is successfully culled, territories can be left vacant or severely under populated, providing additional opportunities for other animals and/or species surrounding the targeted areas to colonise the vacant space or make use of the additional availability of resources. When hooded crows (*Corvus cornix*) were culled on Rathlin Island, Northern

Ireland, this resulted in an expansion of common ravens (*Corvus corax*) into territories which they had previously been excluded from, either by direct or indirect means (Bodey *et al.*, 2009). In the case of badgers culling may reduce competition for resources such as food which may result in heavier animals, with better body condition, after culling has taken place. In Britain, badgers in previously culled areas were heavier and in better condition than those in undisturbed areas (Tuytens *et al.*, 2000b). Badger body weight has also been found to correlate with habitat, with strongly positive associations between body weight and agricultural grassland (Delahay *et al.*, 2006a). Therefore, if access to these habitat types is opened up due to culling, it is possible that there will be an increase in mean badger body weight as density reduces. However, it is also possible that mean body weight may not change. At low-medium densities as found in Ireland, badgers may already have access to enough food to maintain an ideal weight, it may be that the extra accessibility to food resources may not cause an increase in body weight.

1.4.3 The rate of population recovery after culling

The rate of population recovery is an important factor in any culling program, especially as it can vary between species. Clearly the reproduction rate of the species is highly relevant to its recovery rate, but even allowing for that, species recovery can take much longer than expected or need some level of intervention to recover sufficiently. Depopulated bush rat (*Rattus fuscipes*) populations took over two years to recover to preculling levels (Lindenmayer *et al.*, 2005). In badgers, populations can take longer to recover, with some previously undisturbed high density populations in Woodchester Park taking 9-10 years to return to pre-cull levels (Cheesman *et al.*, 1993; Smith *et al.*, 2001).

Unsurprisingly, culled areas are often repopulated from animals dispersing into the area from surrounding areas. Research on population recovery in seabird populations, found that distance from a source population was the most influential variable affecting recolonisation, (Buxton *et al.*, 2014). This was also the case in areas that have undergone intensive control programs for grey squirrels (*Sciurus carolinensis*). Population recovery varied between 4 weeks and 2 months and was dependant on both the extent of the cull and the distance to the nearest undisturbed area (Lawton and Rochford, 2007). If a similar trend is seen in Irish badger populations and there are a number of culled areas adjacent to each other, this would suggest that population and social group recovery may be slower in those areas furthest away

from source populations, or those surrounded by other targeted areas with already lowered population densities. However, data from the RBCT suggested that social perturbation increased as a result of culling (Woodroffe *et al.*, 2005b; Bourne, 2007; Woodroffe *et al.*, 2008). Therefore, increased movement and ranging behaviour within a disturbed population may result in a rapid initial recolonisation of vacant areas even if the return to a normal stable population is slow (Cheeseman *et al.*, 1993; Tuytens *et al.*, 2000a).

1.5 Impacts of culling on social groups and individuals

The effects of culling at a population level have been reported in a number of studies, however there is a lack of peer reviewed literature regarding how such eradication programs affect the social groups and individuals of the targeted species within the culled areas.

1.5.1 Rate of social group recovery after culling

As stated previously, intensive culling reduces the number of individuals within a population, which in turn will result in either a reduction in the number of social groups, or in a reduction in the size of each social group. Social group size in Ireland is similar to that found at low and medium densities in Europe and is lower than the group sizes seen in high density populations in Britain (Kruuk and Parish, 1982; Rogers *et al.*, 1997b; MacDonald and Newman, 2002). Irish studies have found that social group size can vary from small (2 badgers) to medium sized groups (5-6 badgers) (Stuart, 2010; Smal, 1995). As social groups may comprise breeding pairs and their offspring (Roper, 2010), it is possible that the territories left vacant as a result of culling provide opportunities for young adults to leave their natal territories and form their own social groups. If this is the case, it is likely that the reforming social groups may be smaller than those previously found there.

1.5.2 Changes to social groups and individuals

Animal populations, particularly ones recovering from disturbance, may be influenced by a number of both intrinsic and extrinsic factors. Intrinsic factors such as territoriality and reproductive suppression along with extrinsic factors such as environmental factors and food availability can significantly affect population (Wolff, 1997) and thus social group size and

group formation. Dispersal of juveniles from natal groups is often the main cause of migration in mammals (Wolff, 1997) and can be caused by a number of factors such as reproductive suppression, avoidance or resource competition (Beier, 1995; Woodroffe *et al.*, 1995b; Woodroffe *et al.*, 1995c; Wolff, 1997). Previous studies on recovering or disturbed populations have found that culled areas have a younger population structure (cougars - Beier, 1995; Stoner *et al.*, 2006; badgers - Tuytens *et al.*, 2000b; grey squirrels-Lawton and Rochford, 2007). If younger animals are more likely to establish themselves in vacant territories, it is possible that badger culling in Ireland may result in a disruption of the age profile of social groups, with younger individuals being more common in culled areas. The sex profile of such social groups may also be altered, as there is evidence from both Ireland and Britain, that young female badgers are frequent recolonisers of culled areas (Britain - Tuytens *et al.*, 2000b; Ireland - Sleeman *et al.*, 2009a). This may adversely affect the reproductive success in culled areas, if these animals are either too young to breed or miss out on mating opportunities due to a lack of sexually mature males in the recolonised area.

There are other sources of reproductive failure other than lack of mates, as evidenced by studies which investigated the effects of population disturbance on fecundity. Studies on African elephant (*Loxodonta africana*) populations found a lower infant-mother ratio in groups affected by poaching, which suggested that the disturbance to the population resulted in reduced fecundity within affected social groups (Foley *et al.*, 2001). Cougar fecundity levels have also been found to react negatively to population control and hunting pressures. No compensatory reproduction was seen in disturbed areas over the duration of the study period (Stoner *et al.*, 2006). Disturbance has also been found to affect fecundity in badgers. In Britain, badgers in repeatedly culled areas were less likely to reproduce compared to those in previously undisturbed areas (Tuytens *et al.*, 2000b). Therefore, it is possible that conflict arising from the establishment of new territories or stresses caused by forming new social groups may have an adverse effect on the reproductive success of the individuals in repeatedly culled areas. Consequently, it is possible that areas which have undergone intense and repeated culling may have very low fecundity rates.

Conversely, there is also evidence to suggest that breeding success may increase in areas after culling has taken place. In north east Spain, average litter size of red foxes (*Vulpes vulpes*) were higher and the proportion of reproductively inactive females were lower in areas undergoing intense disturbance (Gortázar *et al.*, 2003). This suggests that animals

recolonising culled areas had higher reproductive success in areas of lower density populations, with less competition for resources. Therefore, it is also possible that this may occur in the Irish badger population and culled areas may experience a rise in productivity. Because the literature contains opposing results obtained in different studies, it is not possible to predict the effect of culling on the badgers in this study.

1.6 Culling and cub welfare

There are ethical concerns regarding when culling occurs throughout the breeding season and actively breeding individuals are targeted. To reduce the risk of lactating females being culled, and thus dependent offspring dying of starvation, closed seasons have been implemented for a number of species. In south east Norway Eurasian beaver (*Castor fiber*) hunting is illegal after April 30th, as parturition for this species is thought to occur in late April/early May (Parker and Rosell, 2001). Female brown bears (*Ursus arctos*) in Sweden are afforded legal protection from hunting when they have young at foot as females are essential for the offspring's survival (Bischof *et al.*, 2009). During badger culling in Britain, a 3 month, legally enforced, closed season of February 1st to April 30th was implemented due to welfare concerns (Woodroffe *et al.*, 2005a; Bourne, 2007). This is slightly later than the current Irish closed season which sees a cessation of culling in previously undisturbed areas from January 1st through March 31th (O'Keeffe, 2006). Furthermore, the Irish closed season is only a voluntary and a partial ceasing of culling, which is imposed by DAFM only in previously undisturbed areas. As it is thought that females in repeatedly culled areas are less likely to have dependant cubs at the time of culling, year round culling has been deemed to be suitable in previously culled areas. This exclusion is based on findings in Britain that suggest females in culled areas are less likely to reproduce successfully due to a lack of mating opportunities or not being sexually mature (Tuytens *et al.*, 2000b). Culling is also acceptable in previously undisturbed areas within the closed season if bTB is considered to be a substantial risk to the surrounding cattle herds (DAFM, *pers. comm.*). However, as previous Irish studies have found evidence of young females successfully breeding (Stuart, 2010; Corner *et al.*, 2015), even if the culled areas are recolonised by young females, it is possible that there are females breeding in repeatedly culled areas in Ireland. If this is the case, this will lead to welfare concerns regarding cub survival if lactating females are culled. Therefore, it is important to ascertain whether females in repeatedly culled areas do reproduce successfully.

Since the culling programme in Ireland looks set to continue, it is essential that the closed season takes place at the most appropriate time of year and at all setts where dependent cubs are likely. When determining the most opportune time for this closed season to take place it is important to establish whether parturition occurs at the same time each year. If this is not the case then it will be necessary to establish what factors influence the timing of parturition dates to allow prediction of the best closed season in any given year.

There are numerous ways of estimating parturition dates. Hormones play an important role in the reproductive cycle and control specific stages such as ovulation, implantation and eventually birth (Perry, 1971; Mead, 1980; Stuart, 2010). Therefore, identifying specific hormonal patterns through the examination of blood samples can accurately predict parturition dates (Concannon, 2000; Luvoni and Beccaglia, 2006). In canids, it has been found that ovulation takes place 2 days after a surge in lutealizing hormone levels and that there is an associated rise in progesterone levels which are needed to maintain the pregnancy (Perry, 1971; Concannon, 2000). Studies have suggested that an oestrogen surge is needed for successful implantation in rodents, while other species such as rabbits, may only need progesterone to successfully implant (Perry, 1971). As badgers exhibit delayed implantation, hormones have a very important role in the reproductive cycle. Progesterone is essential to maintain the blastocyst during its period of diapause (Mead, 1980). The main progesterone peak occurs from December to February and is linked to implantation and gestation (Stuart, 2010). However, Perry (1971) found that implantation could not be prematurely induced with the administration of progesterone and concluded that other factors, such as photoperiod and body condition, were needed for implantation to occur. Therefore, as other stimuli are needed for successful implantation, monitoring of hormone levels in badgers may not be an accurate method of predicting parturition and would anyway be very difficult to implement in the field.

Diagnostic sonography or medical ultrasound is a common and accurate way of predicting parturition dates in many species including badgers (canids - Kutzler *et al.*, 2003; Luvoni and Beccaglia, 2006; Kim *et al.*, 2007; equids - Turner *et al.*, 2006; cervids - Lenz *et al.*, 1993; badgers - Woodroffe, 1995; humans - Fitzgerald and Drumm, 1977). Several different measurements may be used to predict birth dates in mammals. Body length (crown-rump) and diameter of the foetus are commonly used measurements (Woodroffe, 1995; Kutzler *et*

al., 2003; Kim *et al.*, 2007), although other dimensions such as foetal eye size can also be used to estimate foetal age (Turner *et al.*, 2006). In cases involving canid pregnancy, sonography may accurately predict parturition dates with an accuracy of ± 3 days in 75-100% of instances, although accuracy of such predictions drops if the scanning is carried out closer to parturition (Kutzler *et al.*, 2003; Kim *et al.*, 2007). While ultrasound measurements have been used to predict parturition dates of badgers in British studies (Woodroffe, 1995), foetal length obtained at the time of post mortem examination has been used in Ireland. The crown-rump length, used in conjunction with a predicted growth velocity, can estimate the days since implantation, and thus the predicted parturition dates (Whelan, 1998; Stuart, 2010). Therefore, foetal measurements, obtained either by ultrasound scanning or by direct measurement during post mortem, is a useful and accurate method for predicting parturition in badgers. However, ultrasound is often not practical, particularly where assessment needs to be carried out in the field, especially if animals need to be anesthetized for the procedure. Furthermore, a large sample group would be needed to obtain enough foetal measurements to predict a range of parturition dates.

Other physical parameters can also be used to assess breeding status. Numerous studies have found a relationship between pregnancy and teat length (small mammals - McCravy and Rose, 1992; badgers - Cresswell *et al.*, 1992; Dugdale *et al.*, 2011a; wolves - Mech *et al.*, 1993; fishers - Frost *et al.*, 1999; fur seals - McKenzie *et al.*, 2007). While teat length cannot be used to predict parturition timing, it can be used as indicator that the female is currently pregnant or possibly had a successful pregnancy. Females which have successfully implanted and produced offspring have significantly longer teats compared to those that haven't (Frost *et al.*, 1999; Mech *et al.*, 1993; McKenzie *et al.*, 2007 Dugdale *et al.*, 2011a) and this measurement can indicate when females within an area are actively lactating. This suggests that assessing teat size may be a quick and accurate method of determining a female's reproductive status.

Body weight and condition can also influence reproductive success in some species. Primates, fur seals and caribou are species whose reproductive cycle may be affected by maternal body condition and weight (Mori, 1979; Bercovitch, 1987; Cameron *et al.*, 1994; Guinet *et al.*, 1998). There is evidence to suggest that a minimum weight threshold is needed for reproductive cycling to begin (Mori, 1979; Bercovitch, 1987). Previous studies have found that female badgers with poor body condition during winter months have less

reproductive success (Woodroffe, 1995, Whelan, 1998) and that those with better body condition implant earlier and thus give birth earlier (Woodroffe, 1995). However, it is not yet known if the implantation dates, and thus parturition, of badgers can be predicted by female body weight, as it can in other species (Cameron *et al.*, 1994).

1.7 Summary of this study and its aims:

The Irish badger cull presents a novel situation to study the effects of long term culling on a protected species from population level through to the effects on individuals. This study aims to add to the current knowledge of Irish badger population dynamics, behaviour and morphology by examining the changes brought about by widespread culling as a result of DAFM's bTB eradication program. To do this I investigated aspects of the population from 1998, the year that widespread culling began in numerous Irish counties, and therefore a snapshot of an undisturbed population. Data from this year was compared with data from subsequent years and identified aspects of the population that were changing in response to culling. Mean body weight, fecundity, bite wounding occurrence and bTB prevalence were assessed to investigate whether culling affected these parameters.

I also determined whether there were differences between social groups in previously undisturbed areas and areas that have been repeatedly culled. I investigated the size of social groups before and after culling and determined if social group recovery time can be predicted within the time span of the records available. The age profile and sex profile of the social groups of culled badgers were also examined.

Furthermore, I examined the impact of repeated culling of the population on the female badgers recolonising culled areas, with particular emphasis on body condition and reproductive success of individuals within those areas. Finally, I contrasted and compared several methods of predicting parturition dates on an annual basis, with a view to moving the established closed season to its most opportune time to prevent lactating females being culled. Therefore, the aims of this study are to:

- Investigate how widespread culling has affected the dynamics of the Irish badger population (Chapter 3).

- ☛ Explore culling induced changes to social groups in undisturbed areas and repeatedly culled areas (Chapter 4).
- ☛ Determine individual response to repeated culling, particularly reproductive success in disturbed areas (Chapter 5).
- ☛ Contrast and compare different ways of predicting annual parturition date range, through both lethal and non-lethal methods (Chapter 6).
- ☛ Advise the DAFM on the best timing and duration of the closed culling season (Chapter 6 & 7).

Chapter 2: Methods and Materials

The material and data utilised in this study originated from several different sources. Tissue samples were gathered from post mortem examinations carried out by a veterinary pathologist, at the Irish Equine Centre (IEC). Dr Fogarty performed the post mortem examinations of the sampled badgers and extracted the gross tissue samples requested by the author. Further processing of these samples (see below) was solely carried out by the author. In addition, the author carried out extensive data mining of the DAFM historical records, stored on card filing systems, from which all information about the previous years' population dynamics were sourced and digitised by the author to build a data set which could be effectively analysed. This data set was then interrogated to provide a detailed picture of the effects of culling over the 10-year timespan.

2.1 Study population

This project examined female badgers culled as part of the nationwide programme for the control and eradication of bovine tuberculosis (bTB) in Ireland. The aim of this control programme was to remove social groups and sufficiently reduce badger density in the targeted areas and thus the transmission of bTB between badgers and cattle. In cases where there was good reason to believe badgers to be the source of bTB infections in surrounding cattle herds ie. areas with no possible source from cattle movements, the Department of Agriculture, Fisheries and the Marine (DAFM) were granted special licences by the National Parks and Wildlife Service (NPWS) to cull badgers in designated areas. Metal stopped restraints were the preferred method of capture in Ireland as they have been found to have a greater capture success compared to cage trapping. However, as the stopped restraints have a minimum stopping size, they are biased against capturing younger and/or smaller animals, which will have excluded most young and particularly small animals from the sample. Thus the data only refer to adult badgers, and may also have some degree of size bias. The restraints were placed close to active sett openings for 11 consecutive nights by DAFM employees and were checked each morning. Setts continued to be assessed for badger

activity on an annual basis and were re-culled if there was evidence of animal activity within the sett. Setts could be re-culled within 12 months if bTB recurred in the surrounding cattle herds. Captured animals were killed with a 0.22 calibre rifle and stored in individual plastic bags, tagged with an ID number to await collection and transportation to the processing centre.

The culled animals were sent to the Irish Equine Centre (IEC), Naas, Co Kildare, where post mortem examination occurred. The IEC is an independent organisation providing laboratory testing services. Depending on the county in which the culling took place, the animals may have been dead for several days before processing occurred. It was not possible to select badgers from specific counties to ensure an equal geographical spread. Therefore, the animals used in the study were taken as a random sample from the animals which were culled and processed during the duration of this study. As a result, there was some over-representation of counties that had ongoing bTB related culling at the time this study took place. Counties such as Cork, Tipperary and Leitrim contributed large numbers of samples to this study, while other counties such as Wicklow, Monaghan and Kildare were under-represented (Table 2.1). Furthermore, there were counties which had no samples included in this study. These were Donegal, Dublin, Louth, Mayo and Westmeath.

Table 2.1 The number of female badgers taken from each county in the study.

County	Number of badgers	County	Number of badgers
Carlow	9	Longford	14
Cavan	23	Meath	18
Clare	21	Monaghan	7
Cork	56	Offaly	14
Galway	23	Roscommon	10
Kerry	12	Sligo	24
Kildare	6	Tipperary	43
Kilkenny	15	Waterford	22
Laois	12	Wexford	16
Leitrim	28	Wicklow	8
Limerick	21	Total	402

2.2 Post mortem examination procedure

For this study, post mortem examinations began at the beginning of January 2007 and ran through until end of May 2008, during which a total of 402 female badgers were examined. The post mortem examinations were conducted by Dr Fogarty. Prior to the post mortem examination being carried out, the total weight (kg) of the badger was recorded using digital scales. After weighing, the badger was placed on its front on a wooden bench. Measuring tape was used to measure the total length (cm) of the animal from nose tip to the base of the tail. The hind foot length (cm) from heel to toe (excluding the claws) was also taken at this time. The whole body was then assessed for the presence of bite wounds. An incision was made through the subcutaneous fat to the underlying muscles. A plastic ruler was inserted into the incision and used to measure the depth of the subcutaneous fat. The animal was then turned over onto its back for further examination.

A Y-shaped incision was made in the carcass, from the shoulders down to the lower abdomen. The lungs and major lymph nodes were examined for any gross lesions or signs of infection. Any areas of concern were sampled and removed for further processing. Once the examination for visible bTB was concluded samples for this current study were taken. A kidney and its surrounding fat were removed. The entire reproductive tract was extracted. This included the uterine horn, ovaries, cervix and vagina. It was essential that the cervix was intact during extraction as it prevented any material being lost from the uterus prior to examination. The mammary glands were visually assessed for signs of active secretions. The mammary gland was then removed, and a sample of blood was also taken from the thoracic cavity for future hormonal analysis. However, it was later found that the sample was too degraded for use.

The lower jaw and femur were removed. The jaw was removed by either sawing or cutting through the lower jaw bones to the rear of the hind teeth. During the first part of the study, the femur was removed, with the aim to determine whether epiphyseal fusion could be linked to specific age in badgers. Later it was decided to focus solely on tooth wear (Hancox, 1988) as a method of aging so no further examination of the femur bones was undertaken. An ear punch was also taken for future DNA analysis and these are currently being used in another ongoing study. This concluded Dr Fogarty's role in the process.

DAFM information sheets provided the DAFM badger code as well as county of capture, sex and capture date. Post mortem record sheets were used by the author to note the date of post mortem and the unique IEC number in addition to the measurements of each female. As no central database existed at the time, it was decided that both the IEC number and the DAFM number would be recorded as it was not yet certain whether further information would be needed from these sources pertaining to individual animals. All females examined in this study were also issued with a corresponding Trinity College Dublin (TCD) badger number. All samples were labelled with the corresponding TCD badger number and name of relevant tissue.

2.3 Gross analysis of the female reproductive tract

It was necessary to carry out a gross analysis of the reproductive tract to investigate annual parturition trends and reproductive differences between undisturbed groups and newly established groups. Once the post mortem examination process was concluded, the samples were further processed by the author in an adjoining laboratory.

Firstly, the uterus was flushed to remove any unimplanted blastocysts or embryos. To do this, the reproductive tract was laid out completely flat, ensuring that there were no twists in the uterine horns. An incision was made in the vagina just before the cervix. A catheter was inserted into the opening of the cervix and a 10 ml syringe was attached to the catheter (Fig. 2.1.1). Between 5-10ml of saline was pumped into the uterus, expanding the horns. A piece of fat was removed from elsewhere in the body and wrapped around the base of one of the uterine horns to stop the forceps damaging the horn and each horn was clamped with the forceps while the other was flushed. An incision was made near the ovary using a sharp scalpel. More saline was pumped through the horn into an underlying weigh boat. The liquid from the uterine horns was transferred to a storage jar which was labelled with the date and TCD badger number. The procedure was repeated for the other uterine horn. The material was examined later for blastocysts and unimplanted embryos.

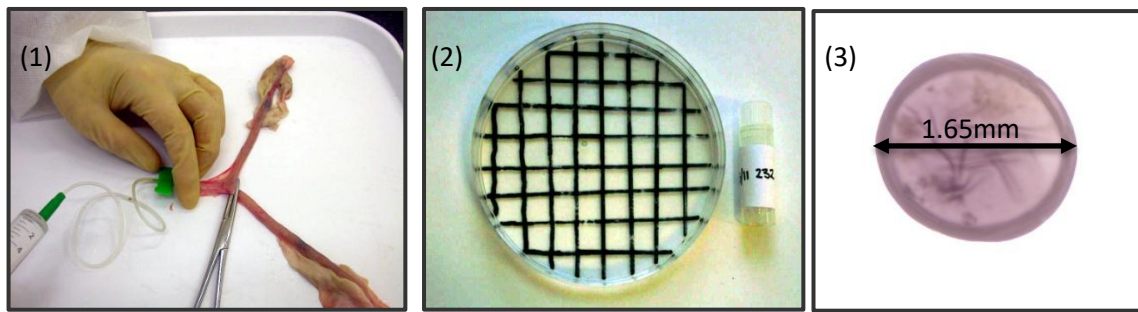


Figure 2.1 (1) Flushing of the uterus. (2) Gridded Petri dish containing fluid from uterine horn. (3) Diameter of blastocyst measured at widest point ($\times 125$). (*Photos courtesy of L. Stuart*)

After the flushing procedure was carried out the ovaries were removed and stored in formalin. Each uterine horn was cut open length wise using a pair of scissors and was examined for the presence of embryos, implantation sites and placental scars. Placental scars are darkened marks that develop on the wall of uterus. They indicate where placentas from implanted embryos attach to the uterus. In the months after parturition they are vivid black bands that are easily recognisable, but they fade over time, turning a light grey and become less defined in the course of a year. Thus, the number of placental scars can be used as a record of the number of embryos implanted during the most recent breeding season. The number and locations of the placental scars were recorded, as was any other evidence of implantation.

If the badger was pregnant at the time of the post mortem examination, then it was not always possible to flush the uterus. At these times the foetuses were removed, and the embryonic sack and placenta were discarded. The foetuses were weighed individually and were measured (mm) from crown to rump using digital callipers. Sex was determined where possible. The back, left leg of each foetus was removed and stored in a vial that was labelled with the mother's TCD number and the foetus number. These were frozen for possible later use in DNA studies on paternity in badgers.

To estimate the implantation dates of the foetuses, the average weight of foetuses from each female was calculated and used with the formula below (Whelan 1998). It was then possible to estimate the birth date of the foetuses from their implantation dates.

$$t_0 = t_g - \frac{\sqrt[3]{W}}{a}$$

Where t_0 is the number of days till parturition,

t_g is gestation length taken as 52 days

W is average foetal weight (g)

a is the growth parameter of 0.1295 (calculated for badgers by Frazer and Huggett, 1974)

2.4 Further analysis of the tissues

After gross analysis, the samples were brought back to Trinity College to allow for more detailed analysis by the author. The fluid collected from flushing the uterus was placed in a gridded Petri dish (Fig. 2.3.2) and examined under a microscope for the presence of blastocysts. If any were found the diameter of each was measured using a graticule in the microscope eyepiece at x125 magnification (Fig. 2.3.3) and recorded.

Body condition measurements were assessed using two methods. The first method used body weight and body length to calculate a body condition index. This method was chosen as these measurements were already being recorded as part of this study. Therefore, body condition could be calculated without the need for additional data collection. An index of body condition was determined by the formula used by Tuytens *et al.*, (2002):

$$\text{Body condition index} = \log \left\{ \frac{\text{body weight}(kg)}{\text{body length}(cm)} \right\}$$

The kidneys and surrounding fat were also used to determine body condition of female badgers. Each kidney and its peri-renal fat were weighed separately and the ratio of fat weight to kidney weight was converted to a percentage kidney fat index. This is known as ‘Riney’s Kidney Fat Index’. This was done for all females. The following equation was used to calculate the index (Riney, 1955).

$$\text{Riney's Kidney Fat Index} = \frac{\text{perirenal fat weight (g)}}{\text{kidney weight (g)}} \times 100$$

The mammary glands were processed by measuring the length of the teat, from the base of the teat flush to the skin to the tip of the teat. A further two measurements were taken, one across the short width at the tip and one across the longer width along the base of the teat. These were then averaged to get the mean width of the teat. The mammary gland tissue depth was assessed by lying the tissue sample out flat, teat side up, and cutting downwards through the sample from outer skin side through to the base of the dissected mammary gland, as close as possible to the base of the teat to give a clean, straight edge. This allowed for measurements to be taken from the top of the epidermis to the base of the mammary tissue.

2.5 Aging

The badger jaws were brought back to TCD for further processing. As the jaws had been stored in formalin, each jaw had to be washed to ensure it was safe to handle. The formalin was poured off and disposed of according to standard protocol. The jaws were flushed through with fresh water to remove any residual formalin. The jaws were aged using a method reviewed by Hancox (1988) and used by Cleary (2009) and Stuart (2010) in previous Irish studies. It has been suggested that tooth wear can be a simple and relatively accurate way to give an indication of the age of the individual (Hancox, 1988). However, this method only allowed for the placement of an individual into broad age classes of juvenile (<1 year), yearling (1-2 years), adult (2-4 years) and aged adults (> 4 years). Table 2.2 lists the categories and descriptions of tooth wear features used to separate badgers into age categories. See also fig. 2.2.

Table 2.2 Description of tooth wear features used to separate badgers into one of the four possible age categories (adapted from Stuart, 2010).

Teeth	Juvenile <1 year	Yearling 1-2 years	Adult 2-4 years	Aged >4 years
Incisors	No visible dentine	Dentine visible on small areas on outer incisors	Large, well defined areas of dentine visible on all incisors	Large area of dentine visible and teeth are flush with gums or missing entirely
Canines	Sharp and pointed	Points slightly blunt and small area of dentine visible	Blunt with dentine visible and wear on inside of tooth	Very blunt with dentine visible, teeth flush or missing
Premolars	Incomplete eruption and very sharp	Fully erupted but slightly blunt with small area of dentine visible	Blunt with dentine visible	Very blunt with large areas of dentine visible, teeth flush or missing
Molars	All cusps present and sharp	Wear visible on some cusps	Wear on all cusps, occasionally with whole sections worn	Heavy wear evident, teeth hollow or missing
Overall Colour	Very white	Some signs of yellowing	Yellow coloration	Dark yellow/black (decay)

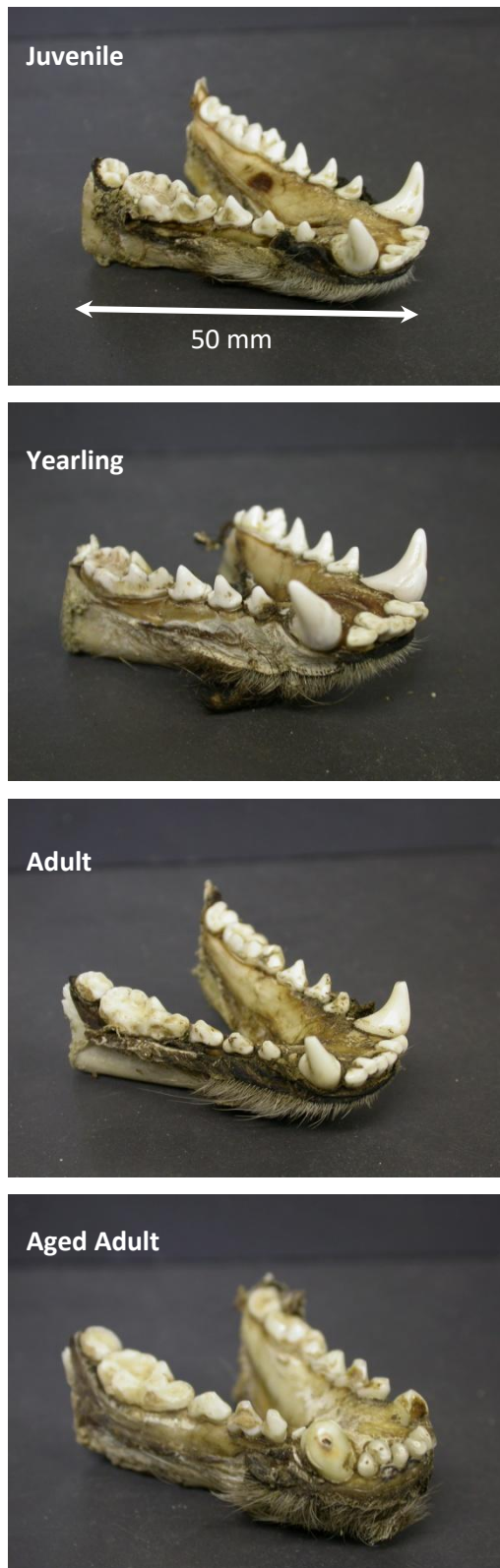


Figure 2.2 Mandibles showing progression of tooth wear through the 4 age categories.

2.6 Data mining

The use of data recorded over several years is an important aspect of this study. Records kept by the IEC and DAFM start as early as 1995, although procedures for information recording and post mortem examination did not become standardised until 1998. It was decided that the records from 1998, 1999, 2001, 2005, 2006, 2007 and 2008 would be used in this study to provide an understanding of how culling has affected the Irish badger population. Data from 1998 were used as the source of information on Irish badgers before culling began as there had been no large scale culling conducted in Ireland before then. The capture methods and post mortem examination methods from 1998 were the same as those used at the end of the study so the information collected was directly comparable to the most recent records.

2.6.1 IEC records

When each badger was trapped and killed, DAFM employees were required to provide written documentation on the DAFM tag number, county of origin, sett number and location, as well as date of capture. This record was then sent, in the form of a record sheet/card, with the carcass to the IEC where further information was added during post mortem examination. At the IEC each badger was given a reference number and further information on weight, sex, age, pregnancy status and bite wounding level were added to existing record sheet. Furthermore, if samples were sent for bTB testing this information was also recorded by noting the location and tissue sampled. Laboratory tests for the presence of bTB were later attached to the original documentation by IEC staff. This documentation was filed and stored in the IEC. It was found that the paper records contained information that had never before been analysed. Each record card was transcribed by the author into digital form into an Excel file. Information on county and date of capture, DAFM and IEC tag numbers, sett number, weight, sex, age, pregnancy status, number of embryos/foetuses, bite wounding and bTB diagnosis was taken from each card. This was a very slow process due to the nature of the documents as often handwriting was difficult to decipher. The resulting excel sheets were transformed into a database. Due to the length of time this process took and the time constraints of the project it was decided to only include data from 1998, 1999, 2001, 2005. Other years were not available or were missing data from the months of interest. The 4 years chosen were deemed to have sufficient number of samples and span enough time to allow

for possible culling induced changes to appear. In total, information from 2882 individual badgers (1420 females and 1462 males) was extracted from the written documents (Table 2.3).

Table 2.3. The data mined records from each country from 1998, 1999, 2001 and 2005.

County	1998	1999	2001	2005
Carlow	1	28	0	0
Clare	16	119	30	12
Cork	144	127	99	48
Donegal	105	136	34	47
Dublin	0	8	0	0
Galway	35	30	93	46
Kerry	1	19	11	5
Kildare	28	38	4	14
Kilkenny	0	10	0	0
Laois	19	2	0	0
Limerick	0	11	7	0
Longford	71	68	48	41
Mayo	10	25	140	55
Meath	0	0	69	0
Monaghan	8	8	16	55
Offaly	127	100	22	7
Roscommon	52	0	82	13
Sligo	8	0	0	2
Tipperary	66	106	10	12
Waterford	0	1	1	1
Westmeath	28	0	0	17
Wicklow	7	99	108	72
Total	726	935	774	447

From 1998 through to 2005 it was also possible to discern from the record cards which badgers tested positive for bTB. In 1998 – 1999 bTB status was assessed during the initial post mortem examination carried out by Dr Fogarty, by the presence or absence of gross lesions. From 2000 onwards a more sensitive method of testing was introduced, bacteriological culturing, which was carried out by the Central Veterinary Research Laboratory, Celbridge, Co Kildare. The tissue samples were processed and later incubated on plates for a minimum of 8 weeks. When bacterial growth was visible, smears were prepared and stained. Growth of *M. bovis* generally occurred within 3 – 6 weeks of incubation. This change in bTB testing means that the bTB prevalence pre-2000 and post-2000 are not directly comparable.

2.6.2 DAFM records

In 2010 various requests for additional information were made to DAFM by the author regarding all the animals which underwent post mortem examination during 2007 and 2008. DAFM's data files provided information on 3553 badgers culled within those dates including the 402 females which were examined for reproductive status by the author described in section 2.3. These 2007 and 2008 data included county of origin, sex, sett number and the number of animals killed per culling event. The data relevant to the animals in this study was extracted from several different file sources using the DAFM badger number and sett number. A unified database using information on the rest of the culled animals was created by the author and allowed for analysis of social group size and of population recovery post culling. It was also used to assign animals to a cull event and extract information on original social group size during the initial culls and social group size at time of the subsequent culling events. Using the capture date data for each separate culling operation it was possible to estimate the time between multiple cull events.

Finally, as there are several data sources and different sample sizes used in this study, Table 2.4 gives a breakdown of the origin and sample size of animals used in each chapter. Not all the data collected in this study could be used in the analysis, therefore this table provides a clear breakdown of the total animal numbers used for each chapter and the source of these data.

Table 2.4 A summary of data sources used in this study for each chapter.

	Chapter 3	Chapter 4	Chapter 5	Chapter 6
Post mortem examination and processing		299 from 2007 103 from 2008	299 from 2007 103 from 2008	185 females (from 2007/2008) including 14 gravid females (2007) and 11 gravid females (2008)
IEC records	1420 – females 1462 – males (1998/1999/ 2001/2005)			221 gravid females (1998/1999/ 2001/2005)
DAFM records		Additional info re: social group size and culling event from 2007/2008.	Add. info on culling events of post mortem examined females from 2007 and 2008.	
Stuart, 2010				132 females with blastocysts and 30 gravid females (2005/2006)

Chapter 3: The Effects of Culling on the Irish Badger Population

Abstract

Long term disturbance of a mammal population results in significant changes to the population dynamics of the species (Tuytens *et al.*, 2000a; Tuytens *et al.*, 2000b; Sadler and Montgomery, 2004). This chapter investigated the cumulative effects of long term culling on the Irish badger population to identify difference between populations in repeatedly targeted areas compared to those in previously undisturbed areas.

The study found that body weight varied significantly by year. Both male and female badgers culled in 2001 were significantly lighter than those culled in earlier years, but this was more likely due to varying climatic impacts rather than culling affects. Unsurprisingly, gravid females were significantly heavier than non-gravid females. Gravid females were recorded from the first week in January until mid-February. The timing of gravidity in sows appears to vary from year to year within a window of time stretching at least between the beginning of January and the middle of February. However, contrary to what was expected the annual percentage of females found to be gravid did not significantly change. Females from both previously undisturbed and those in repeatedly culled areas both reproduced successfully with no significant reduction seen in fecundity. Furthermore, the average litter size did not change during the cull, with most females having litters comprising 2 or 3 cubs, although litters of 1 and 4 cubs were also recorded.

Bite wounding was found infrequently and disappeared completely during 2005. Both males and females showed similar levels of bite wounding, again all of which was unexpected given findings in the published literature. There is strong evidence to suggest a significant link between bite wounding and bTB infection indicating that badgers with bite wounds have a much higher prevalence of bTB than those without bite wounds. No change in bTB levels was detected over the duration of the study. However, as the sensitivity of detection levels has increased, this may be obscuring a decline in bTB levels.

These findings suggest that the Irish badger population is responding unexpectedly to long term culling compared to those described in other population studies and so provides novel insights to the population's response to culling.

3.1 Introduction

Zoonotic diseases can be detrimental to both public health and economic stability of a country and therefore the control and/or reduction in transmission rates of such infections is often the primary aim of culling events. In Ireland, total localized removal, through reactive culling of badger social groups, is being used as a disease control measure (O'Keeffe, 2006). The bTB eradication program in Ireland uses cattle as sentinels for disease in badgers. A bTB breakdown in a cattle herd triggers a veterinary epidemiological investigation of the potential source and where alternative sources of infection are excluded, badgers are culled from the surrounding area (Griffin *et al.*, 2005; O'Keeffe, 2006; Corner *et al.*, 2008). It is possible that the resulting change to density and population structure as a result of the ongoing culling program may change disease dynamics. In a study by Corner *et al.*, (2008) a decrease in the population density in an Irish badger population was associated with a decrease in the prevalence of *M. bovis* which was attributed to a reduced likelihood of contact between animals.

Many species of mammal such as deer (Dodd, 1984; Palmer *et al.*, 2000; Corner, 2006; Gortázar *et al.*, 2012), boar (*Sus scrofa*) (Gortázar *et al.*, 2012; Mentaberre *et al.*, 2014), cats (*Felis catus*), ferrets (*Mustela furo*) and stoats (*Mustela erminea*) (Ragg and Waldrup, 1995) have been found to carry bTB. Deer have been identified as a possible source of bTB infection as they are a candidate for transmission to cattle given the possible interactions between both species (Dodd, 1984; Palmer *et al.*, 2000; Palmer *et al.*, 2004; Corner, 2006). Palmer *et al.*, 2004 found evidence of bTB transmission between cattle and deer through indirect contact and the sharing of food and holding pens. While this is a growing area of interest, currently there are few studies which examine the incidence of deer transmitted bTB in Ireland. Instead, research to date has focused on the badger as the main wildlife reservoir for tuberculosis in cattle across Ireland and Britain. This has resulted in the species becoming the target of both large scale proactive and reactive culling in an attempt to reduce the prevalence of tuberculosis in cattle herds.

Where badger removal has taken place on a large scale, the population dynamics of the remaining badgers has changed considerably (Woodroffe *et al.*, 2005b; Woodroffe *et al.*, 2008; Corner *et al.*, 2008). Proactive culling led to a 73% reduction of latrine density and a decreased frequency of badger road kills in the culled areas (Woodroffe *et al.*, 2008). This was caused by a significant reduction in badger density in these areas, also evidenced by badger captures dropping by up to 78% with repeated culling over a 6 year period (Byrne *et al.*, 2012a).

The process and timescale of population recovery to its original level is dependent on several factors, including the scale and the intensity of the culling (Tuytens *et al.*, 2000b) and the fecundity of the remaining population. Populations that have been greatly depleted by large scale culling were found to have reduced fecundity rates (Tuytens *et al.*, 2000b; Sadlier and Montgomery, 2004) and may take years to recover to pre-removal densities. The original density of the population influences the social structure and therefore the recovery dynamics. In Britain, high density populations can take up to ten years to recover, although lower density populations can do so in half the time (Carter *et al.*, 2007).

There are many influencing factors on implantation and reproductive success, such as shortening photoperiod and maternal body condition (Canivenc and Bonnin, 1979; Woodroffe, 1995). However, there is also evidence to suggest that competition between females can result in reproductive suppression within larger social groups (Woodroffe and MacDonald, 1995b) in high density populations. However, as female/female competition is linked to resource availability (Woodroffe and MacDonald, 1995b), it is possible that areas with greater territory quality will have less female/female competition. This may result in a higher proportion of females who breed successfully. In lower density populations, several females from the same social group have been recorded to reproduce successfully (Sweden - Ahlund, 1980; Ireland - Stuart, 2010; Corner *et al.*, 2015). Therefore, higher territory quality and less dense populations may lead to a higher proportion of females that breed (Da Silva *et al.*, 1993; Woodroffe and MacDonald, 1995b), resulting in faster population recovery times. Repopulation of culled areas has only been studied in high density populations (Tuytens *et al.*, 2000b). In that study a badger removal operation in North Nibley, Gloucestershire, Britain showed marked effects on the sex and age ratio of the recovering population. Young females, thought to be from neighbouring social groups, were the first to recolonise the area (Tuytens *et al.*, 2000b).

The changes brought about by large scale culling may also affect territorial behaviour. Previous studies have found an increase in aggressive interactions detected by increased incidence of bite wounding as a result of culling (Vicente *et al.*, 2007). It is possible that this increase in aggressive behaviours is due to the social disturbance caused by culling. Bite wounding injuries may increase as animals occupy and defend new territories. In high density populations, an increase in the movement of animals between social groups was observed following a removal operation (Tuytens *et al.*, 2000b) and the home-ranges of the remaining badgers increased in size (Woodroffe *et al.*, 2005b). The Randomised Badger Culling Trial (RBCT) in Britain resulted in 34-43% of animals being removed from targeted social groups and as a result home ranges expanded by 43.5% and the overlap of individual territories increased by 73% (Riordan *et al.*, 2011). As badgers exhibit a degree of territoriality, particularly in high density populations (Kruuk, 1978; Tuytens *et al.*, 2000a), this increased movement into neighbouring areas may result in additional conflict between animals. Studies conducted on a disturbed badger population in southwest Britain found evidence that female badgers suffered more bite wounds during recolonisation of a culled area, compared to those in an undisturbed population (Delahay *et al.*, 2006b).

The findings described above were observed in high density populations in Britain which have badger densities ranging from 8.4 badgers per km² to 44.33 badgers per km² in some areas (Kruuk and Parish, 1982; Rogers *et al.*, 1997b; MacDonald and Newman, 2002). Badger density in Ireland is much lower, ranging from 0.85 badgers per km² to 5.86 badgers per km² (O’Corry-Crowe *et al.*, 1993; Smal, 1995; Eves, 1999; Sleeman *et al.*, 2009b). It cannot be assumed that the responses to culling will be the same in the lower density Irish badger populations as in the high density populations in Britain. A study on a badger population in Co. Cork, Ireland in the late 1980’s which looked at the effects of the population density changing from 2.9 to 0.5 badgers per km² (Sleeman and Mulcahy, 2005), found high levels of elasticity in some badger territories. Territoriality was less important as density decreased and ceased to be important at a density of less than one badger per km². Therefore, it is possible that the disturbance of badger populations in Ireland may have different effects than those reported in studies in Britain. With a lower population density the occurrence of bite wounding and other aggressive behaviours would be much less frequent to begin with in lower density populations in Ireland than those in higher density British populations. It is also possible that levels of aggressive behaviour and territoriality

in repeatedly targeted areas may reduce or even disappear as local populations fall below one badger per km².

In undisturbed populations of badgers, body weight is negatively associated with population density and social group size. (Tuytens *et al.*, 2000b). In other words, badgers are larger where they are living at lower densities. This trend is particularly strong in female badgers (Tuytens *et al.*, 2000b). This may indicate a cost of living in large social groups, especially for females. A lower population density may open resources to the remaining animals through reduced competition and could even result in greater reproductive success. Previous studies have found that females with better body condition implant earlier, while those in poor condition may not produce blastocysts at all (Cresswell *et al.*, 1992; Woodroffe and MacDonald, 1995a; Delahay *et al.*, 2006a). Therefore, extra resource availability after culling may increase fecundity. Research on badgers in Spain found that as territory quality increased so did the number of reproductively active females (Da Silva *et al.*, 1993). This has also been reported in other species, for example common brushtail possums (*Trichosurus vulpecula*) in New Zealand, had a higher reproductive success and less seasonal fluctuation of body condition after localised culling (Ji *et al.*, 2005). Albon *et al.*, (1987) also found density dependent fecundity in deer populations, whereby reproductive success was lower as density increased. This suggests that a reduction in population density may improve the fecundity of reproductively active females, at least in some species.

As the literature suggests, there are many effects of culling. Some studies have some unexpected positive outcomes associated with culling such as an increase in fecundity due to a reduction in population density and greater availability of resources to the remaining/recolonising animals (Da Silva *et al.*, 1993; Woodroffe and MacDonald, 1995b). However, the predominant effects of continuous culling would appear to be having negative impacts on the target populations. Disturbing animal populations can result in changes in territoriality and ranging behaviour, which may affect disease transmission (Tuytens *et al.*, 2000b; Woodroffe *et al.*, 2005b; Delahay *et al.*, 2006b). Long term culling can also delay population recovery and leads to younger age profiles of the recolonising individuals (Tuytens *et al.*, 2000b; Carter *et al.*, 2007). What specific effects culls have had on the Irish badger population is currently unknown. With so little known about the population dynamics of badgers in Ireland, it is important to compare patterns in undisturbed and culled

populations. Only then can the effects of long term culling be assessed. Therefore, the aims of this study are:

- To compare the body weight of animals in culled and undisturbed areas as animals in culled areas in Britain have been found to be heavier and have better body condition as it is thought that they face less competition for resources (Tuytens *et al.*, 2000a).
- To identify reproduction and fecundity changes attributable to culling at a population level as previous studies have found a significant reduction in fecundity in disturbed populations (Tuytens *et al.*, 2000b; Sadler and Montgomery, 2004).
- To assess levels of aggressive behaviours in undisturbed and culled populations. In other badger populations, aggressive behaviours such as bite wounding are a common occurrence (Cresswell *et al.*, 1992), and therefore, it is possible that high levels of population disturbance may result in additional aggressive encounters between animals in targeted populations.
- To ascertain whether bTB levels in badgers have altered in response to culling. Bite wounding has been shown to be a transmission route of bTB in other populations (Corner *et al.*, 2008) so any change in frequency may affect bTB levels in the badger population.

3.2 Methods

The badgers used in this study originated from counties where culling had been continuously undertaken from 1998 - 2005. Data from 1998, 1999, 2001 and 2005 were used as other years were not available. The objective of the study was to evaluate the effect of continued culling on the population structure. The 11 counties used in the present study were: Clare, Cork, Donegal, Galway, Kerry, Kildare, Longford, Mayo, Offaly, Tipperary and Wicklow (Fig. 3.1). These counties were selected for use as they provided the largest sample sizes across the years of interest in the study. Table 3.1 provides a breakdown of the number of badgers used in this study



Figure 3.1 Irish counties from which badgers were sampled in this study.

Table 3.1. The county breakdown of culled badger numbers.

County	1998	1999	2001	2005
Clare	16	119	30	12
Cork	144	127	99	48
Donegal	105	136	34	47
Galway	35	30	93	46
Kerry	1	19	11	5
Kildare	28	38	4	14
Longford	71	68	48	41
Mayo	10	25	140	55
Offaly	127	100	22	7
Tipperary	66	106	10	12
Wicklow	7	99	108	72
Total	610	867	599	359

Records maintained by the Irish Equine Centre (IEC) and the Department of Agriculture, Food and the Marine (DAFM) commenced in 1998, when procedures for capture, information recording and post mortem examination were standardised. Badger culling did take place before this date, however, it was confined to selected areas undergoing investigation during two field trials, the East Offaly project and the Four Areas Project (Offaly, Monaghan, Kilkenny and Donegal). Therefore, 1998 was the year in which widespread culling began throughout the country. The data were obtained for individual badgers recorded by DAFM Wildlife Unit staff when each badger was captured and by IEC personnel following post-mortem examinations. The information consisted of:

- ☛ Capture date.
- ☛ County and capture area.
- ☛ Sex and weight of the animal.
- ☛ Age class (adult, yearling and cub).
- ☛ bTB status, which was ascertained by the presence or absence of visible lesions in 1998/1999 and by bacteriological culturing in 2000 and 2005.

- ☛ Pregnancy status, which was determined by the presence of embryos/foetuses during post mortem examination.
- ☛ Presence/absence and location of bite wounding were visually assessed by the veterinary surgeon.

Only adult badgers were used in the analysis as there were insufficient numbers of cubs and yearlings to provide meaningful results.

The data from the I.E.C (used in this chapter only) was limited as it was not possible to ascertain which setts were previously undisturbed or undergoing repeated culling in the later years. In 1998 all setts were undisturbed as culling had recently commenced, but thereafter some undisturbed setts will be present in the data set, with increasing numbers of previously culled setts in later years. In order to reduce the number of first time culled setts in the later years, every effort was made to choose badgers from areas with the same area code ($\sim 2\text{km}^2$), if it was not possible to use the same sett as in the original culled areas in 1998/1999. As the average territory size in Ireland has been found to range between 1.84km^2 and 2.5km^2 (Gaughran *et al.*, 2018), it was hoped that this would reduce the number of first time culled setts as much as possible. However, it is possible that previously uncultured setts may have been included in the data set even of 2005. The following results must be interpreted against the uncertainty of exactly how many undisturbed setts remain in later years and can therefore only be taken as indicative of the changes over time present in a culled population, not a direct comparison of disturbed vs. undisturbed populations. In the absence of more precise data, it is of merit to consider the long term changes evident across the whole population during the 7 year timespan available.

3.2.1 Analysis

Data were analysed using IBM SPSS 22.0 for Windows. The distribution of continuous data was checked and in all cases was found to be normally distributed thus allowing parametric statistical testing to be used. Descriptive statistics for categorical variables are presented as percentages. Chi-squared tests and Fisher Exact Tests were used where appropriate and multi-sample comparisons used ANOVA with Bonferroni *post hoc* tests. The threshold for statistical significance was set at $p = 0.05$.

3.3 Results

Table 3.2 shows the breakdown of the study population. Data for the full 12 months of 1998 and 1999 were available, whereas 2001 and 2005 only had data from 2-3 months. As noted earlier, the original sample size was 2,882 animals, however, due to missing variables, not all recorded individuals could be used in this analysis. This gave a sample size of 2,435 badgers used in this study: 1180 females and 1255 males.

Table 3.2 The breakdown of the study population.

	1998	1999	2001	2005	Total
Available data (months)	12 Jan-Dec	12 Jan-Dec	3 Jan-March	2 Jan-Feb	
Females	297	436	282	165	1180
Males	313	431	317	194	1255
Total	610	867	599	359	2435

3.3.1 Body weight

The weight of gravid females was examined over a 6 week period in each year, from January 1st to mid-February. This time period was chosen so as to include the gravid females found in each year (Fig. 3.2). A total of 174 females were found to be gravid within this time period (1998 N = 31, 1999 N = 30, 2001 N = 70, 2005 N = 43). Similar numbers of females were culled in 1998 and 1999, however this number increased during 2001 and 2005 (Table 3.3).

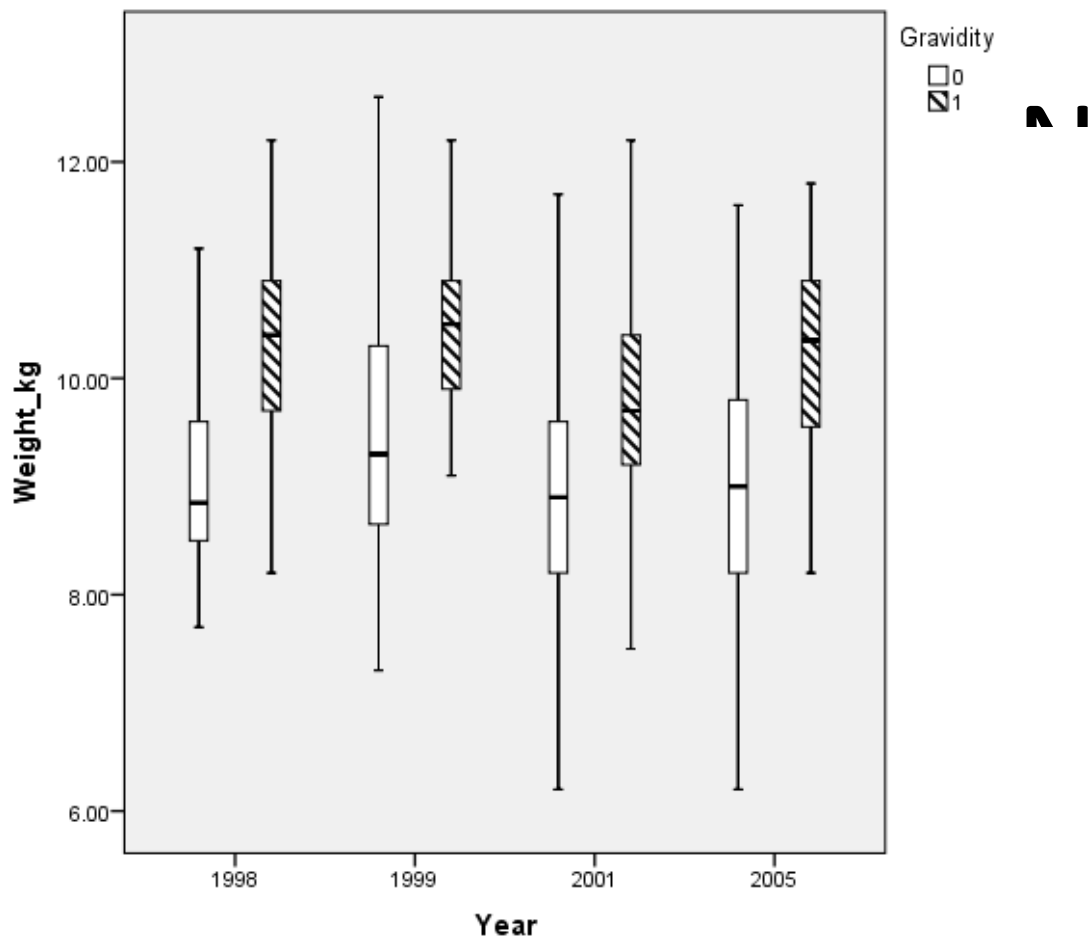
Table 3.3 Summary statistics showing the weight (in Kg) of gravid and non-gravid females in 1998, 1999, 2001 and 2005.

Year	Pregnancy Status	Mean	Std. Deviation	N
1998	Non-gravid	9.24	1.28	54
	Gravid	10.37	0.95	31
	Total	9.65	1.29	85
1999	Non-gravid	9.22	1.19	45
	Gravid	10.48	1.02	30
	Total	9.72	1.28	75
2001	Non-gravid	8.82	1.15	140
	Gravid	9.83	1.08	70
	Total	9.16	1.23	210
2005	Non-gravid	8.96	1.12	112
	Gravid	10.27	0.96	43
	Total	9.38	1.24	155

Both year and gravidity status had a significant effect on the weight of female badgers, with gravid females being significantly heavier than non-gravid females (two-way ANOVA: year: $F = 5.489$ $df = 3$; $p < 0.001$; gravidity: $F = 108.758$; $df = 1$; $p < 0.001$). Females in 2001 were significantly lighter than those culled in 1998 and 1999. (Bonferroni post hoc test = 2001 vs. 1998 ($p = 0.004$) and 1999 ($p=0.001$)).

Table 3.4 Descriptive statistics for male weights (Kg) in 1998, 1999, 2001 and 2005

Year	Mean	Std. Deviation	N
1998	10.52	1.54	87
1999	10.65	1.44	95
2001	10.13	1.39	253
2005	10.44	1.42	185



Gravid N = 31 30 70 43
Non gravid N= 54 45 140 112

Figure 3.2 The median, interquartile ranges, and upper and lower ranges of weight distribution in non-gravid and gravid females in the first 6 weeks of 1998, 1999, 2001 and 2005.

The mean body weight of male badgers was also assessed over the same 6 week period in each year. This time frame was chosen to make the four years comparable for analysis (Fig. 3.3). More males were culled than females during the same time period. Although mean body weight variation was slight between years, there was a significant variation seen, with males from 2001 being significantly lighter than males from 1999 (ANOVA: $F = 4.662$; $df = 3$; $p < 0.007$; Bonferroni post hoc test = 1999 vs 2001 $p = 0.015$). This is similar to the pattern seen in female badgers (Fig. 3.2).

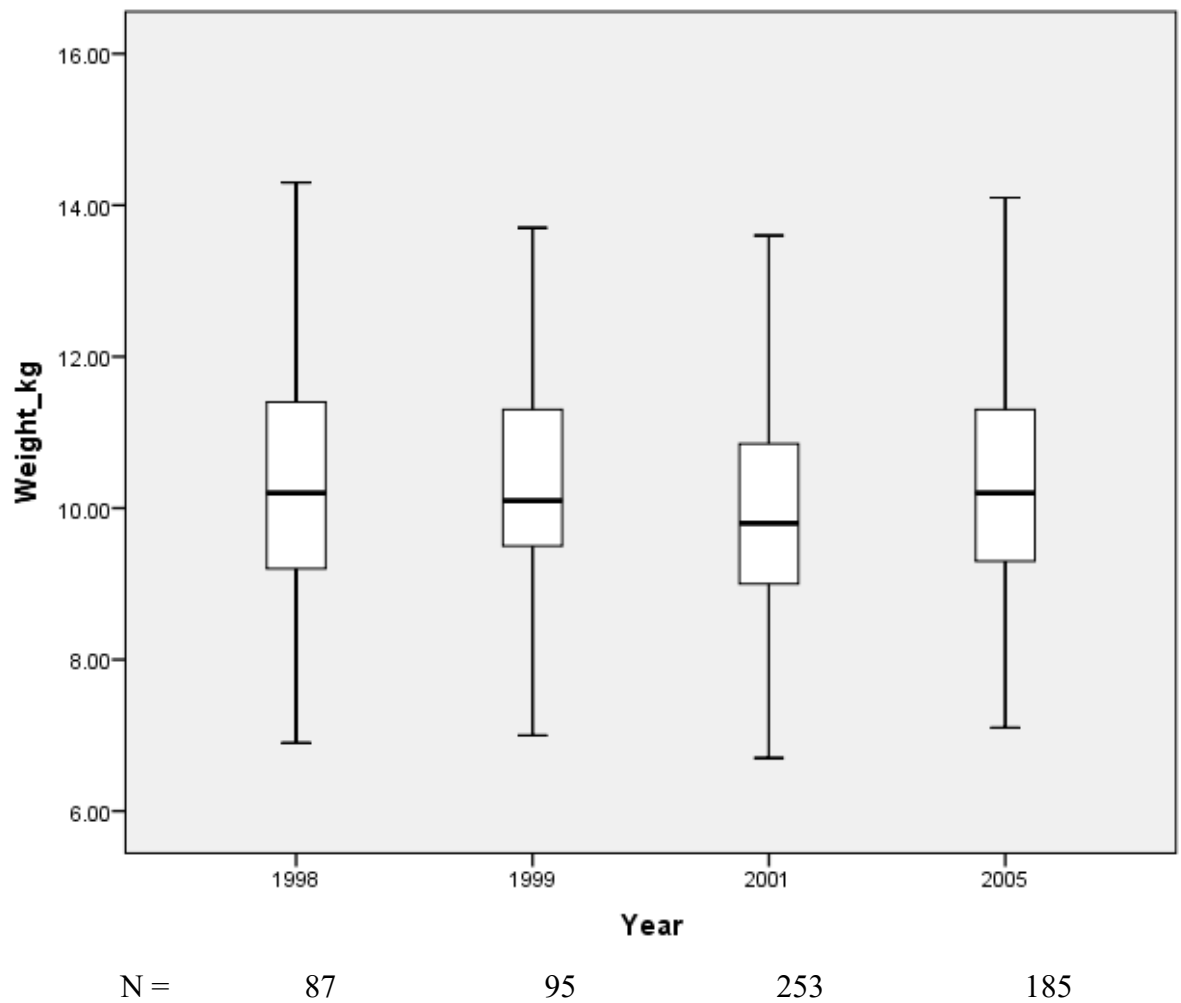
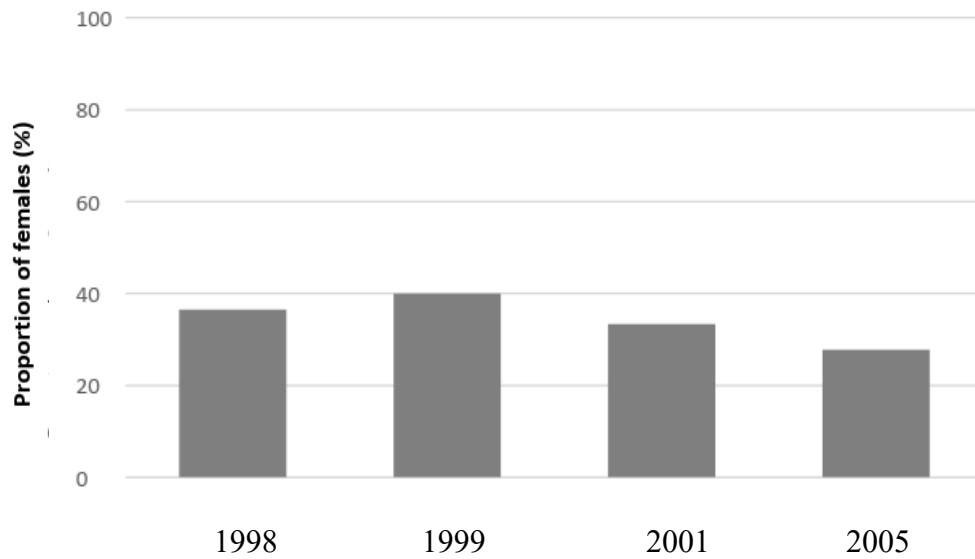


Figure 3.3 The median, interquartile ranges, upper and lower ranges of weight distribution in male badgers in the first 6 weeks of 1998, 1999, 2001 and 2005.

3.3.2 Fecundity

Over the number of years included in this study there was no significant variation between years in the proportion of gravid females in areas undergoing repeated culling ($\chi^2 = 4.06$ df = 3, $p = 0.255$) (Fig 3.4).



Gravid N	=	31	30	70	43
Non gravid N=		54	45	140	112

Figure 3.4 The annual percentage of female badgers found to be gravid.

Litter size, the number of embryos/foetuses found during post mortem examination of gravid females, was also assessed. Litter size ranged from one to four embryos/foetuses. The mean ranged between 2.32 (2001) and 2.55 embryos/foetuses (2005). Litters of two and three were the most frequent (Fig. 3.5). Overall there was no variation by year in litter size ($\chi^2 = 8.529$ df = 9 p > 0.05).

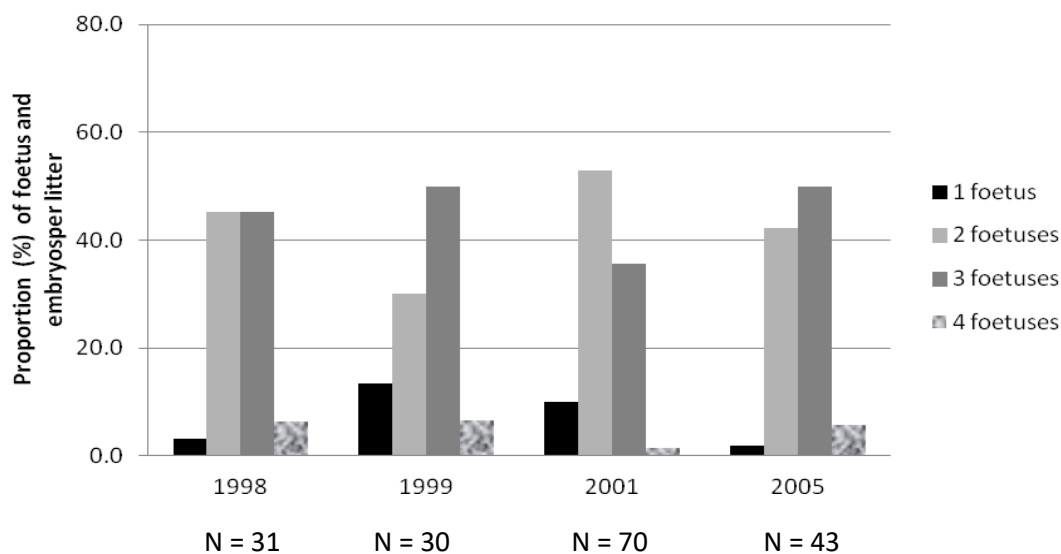


Figure 3.5 The distribution of litter size in gravid females.

3.3.4 Timing of breeding

In both 1998 and 2001 the highest proportion of females that were gravid was found in mid to late January and showed the same rapid drop off in February, although only 2001 had evidence of gravid females after the 11th of February (Fig. 3.6). It must be noted that no females were culled in the first week of January in 1998 which may have resulted in pregnant animals being missed during this week.

In 1999 a higher percentage of gravid females were found in mid to late January (72-77%) and consequently fewer were seen at other times of the selected timescale, indicating a later breeding season. A different pattern was observed in 2005, where the percentage of gravid female badgers was higher than other years in the beginning of January and continued rising until it reached a peak between January 15th and January 21st, followed by a sharp decrease each week until mid-February. This may suggest an earlier breeding season in this year. Thus the timing of gravidity in sows appears to vary from year to year within a window of time stretching at least between the beginning of January and the middle of February.

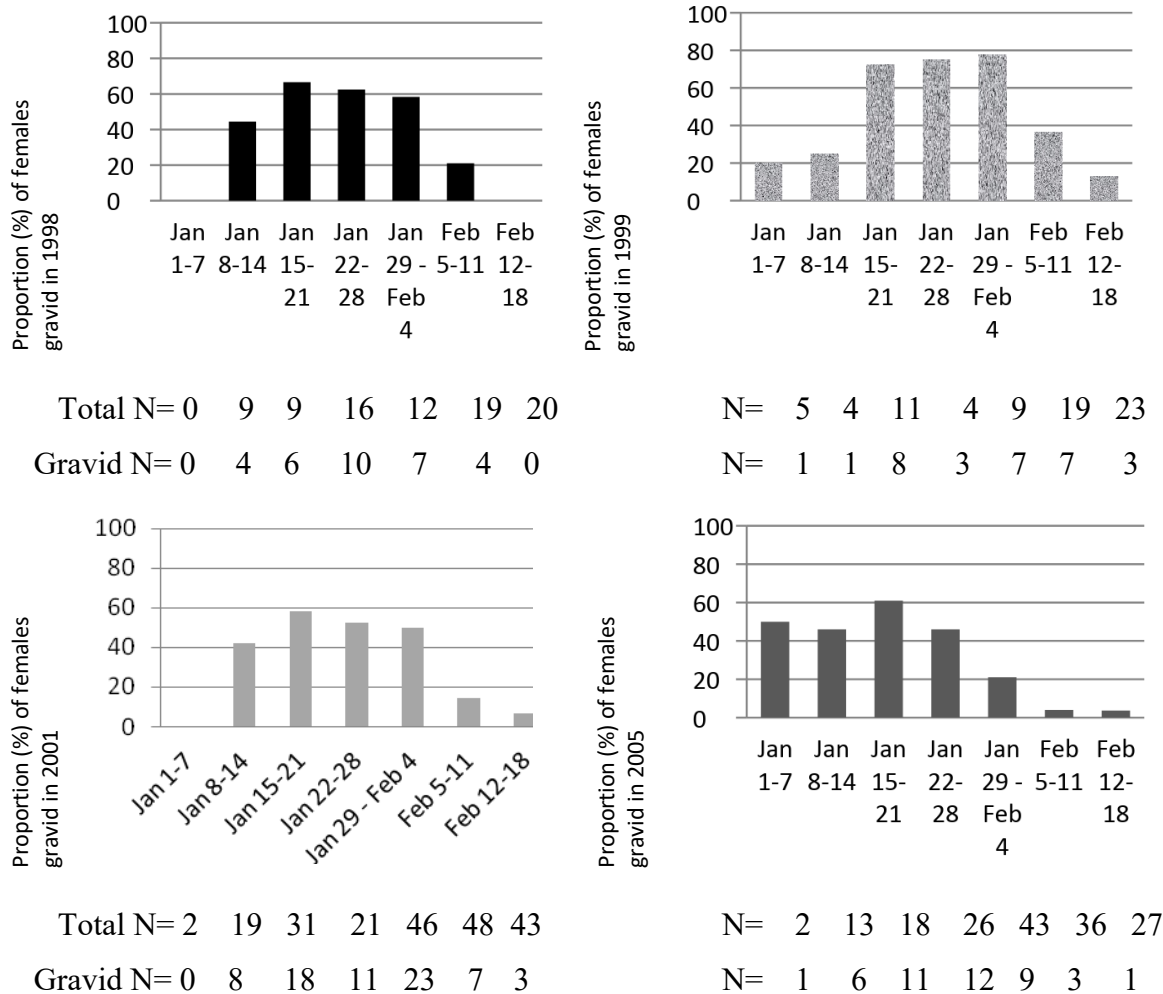


Figure 3.6 The annual breakdown of the proportion (%) of female badgers found to be gravid in 1998, 1999, 2001 and 2005.

3.3.4 Bite wounding

The complete annual prevalence of bite wounding was only available for 1998 and 1999 (Table 3.5) with badgers from 2001 and 2005 only providing information during the first 2-3 months of each year. Bite wounding was found in 1998, 1999 and 2001, but not in 2005. As bite wounding was assessed in all months in 1998 and 1999, these years were used to investigate how the frequency of bite wounding changed through the year. The proportion of badgers carrying a bite wound per month ranged from 0 to 10%. Bite wounds were seen in all seasons and occurred in 10 out of 24 months in 1998 and 1999 in male badgers, and 7 out of 24 months for female badgers during the same time period.

Table 3.5 Proportion (%) of the male and female adult badger population with evidence of bite wounding during 1998 and 1999 (all percentages rounded to two decimal places).

	Males		Females	
	1998	1999	1998	1999
January	1/41 = 2.44%	0/39 = 0.00%	0/41 = 0.00%	0/26 = 0.00%
February	2/55 = 3.64%	1/78 = 1.28%	0/53 = 0.00%	3/73 = 4.11%
March	0/24 = 0.00%	1/97 = 1.03%	0/31 = 0.00%	0/81 = 0.00%
April	2/78 = 2.56%	0/20 = 0.00%	1/43 = 2.33%	0/39 = 0.00%
May	1/16 = 6.25%	0/43 = 0.00%	0/19 = 0.00%	2/49 = 4.08%
June	0/10 = 0.00%	1/10 = 10%	1/10 = 10%	0/7 = 0.00%
July	0/4 = 0.00%	0/4 = 0.00%	0/1 = 0.00%	0/5 = 0.00%
August	0/12 = 0.00%	0/4 = 0.00%	0/4 = 0.00%	0/4 = 0.00%
September	0/15 = 0.00%	1/37 = 2.70%	0/16 = 0.00%	0/30 = 0.00%
October	1/28 = 3.57%	0/35 = 0.00%	1/25 = 4.00%	1/49 = 2.04%
November	0/13 = 0.00%	1/42 = 2.38%	0/15 = 0.00%	0/39 = 0.00%
December	0/17 = 0.00%	0/22 = 0.00%	0/39 = 0.00%	1/34 = 2.94%
Overall % w/bite wounds	2.24	1.16	0.67	1.38

Table 3.6 illustrates the overall frequency of bite wounding of male and female badgers in January and February in 1998, 1999, 2001 and 2005. The prevalence of bite wounding in both male and female badgers was low to begin with and did not change significantly after culling (males: $\chi^2 = 5.761$ df = 1 $p > 0.05$; females: $\chi^2 = 6.53$ df = 1 $p > 0.05$).

Table 3.6 Overall % of badgers with bite wounds during January and February of 1998, 1999, 2001 and 2005

	1998	1999	2001	2005
Males	3/96 = 3.13%	1/117 = 0.85%	3/280 = 1.07%	0/194 = 0.00%
Females	0/94 = 0.00%	3/99 = 3.03%	4/251 = 1.59%	0/165 = 0.00%
Overall % w/bite wounds	1.58	1.85	1.32	0

3.3.4 Tuberculosis in badgers

Table 3.7 and 3.8 describe the prevalence of bTB found in animals in this study. As there was a change in bTB detection methodology post 2000, it was not possible to compare and contrast bTB rates all four years together. 1998 and 1999 were directly comparable to each other, as were 2001 and 2005. Badgers from 1998 had the highest prevalence of bTB with 18.21% of males and 17.51% of females found to have bTB. Over all male badgers had the highest incidence of bTB in December 1998 (47.06%) while female badgers had the highest incidence of bTB in June 1998 (30%). While bTB detection appeared lower in both males and females in 1999, this apparent decline in bTB prevalence was not significant (males: $\chi^2 = 0.4969$ df = 1 $p > 0.05$; females: $\chi^2 = 0.0356$, df = 1, $p > 0.05$). BTB was found in both sexes in all months except for July 1998 in male badgers, and July - August 1998, and June – August 1999 in female badgers.

Table 3.7 Proportion (%) of the male and female adult badger population diagnosed with bTB during 1998 and 1999 (all percentages rounded to two decimal places).

	Males		Females	
	1998	1999	1998	1999
January	6/41 = 14.63%	6/39 = 15.38%	7/41 = 17.07%	5/26 = 19.23%
February	7/55 = 12.73%	7/78 = 8.97%	7/53 = 13.21%	9/73 = 12.33%
March	5/24 = 20.83%	20/97 = 20.62%	8/31 = 25.81%	18/81 = 22.22%
April	12/78 = 15.34%	3/20 = 15.00%	10/43 = 23.26%	6/39 = 15.38%
May	5/16 = 31.25%	5/43 = 11.63%	3/19 = 15.79%	10/49 = 20.41%
June	3/10 = 30.00%	3/10 = 30.00%	3/10 = 30.00%	0/7 = 0.00%
July	0/4 = 0.00%	1/4 = 25.00%	0/1 = 0.00%	0/5 = 0.00%
August	1/12 = 8.33%	1/4 = 25.00%	0/4 = 0.00%	0/4 = 0.00%
September	2/15 = 13.33%	6/37 = 16.22%	2/16 = 12.50%	4/30 = 13.33%
October	5/28 = 17.86%	9/35 = 25.71%	4/25 = 16.00%	10/49 = 20.41%
November	3/13 = 23.08%	6/42 = 14.29%	2/15 = 13.33%	8/39 = 20.51%
December	8/17 = 47.06%	3/22 = 13.64%	6/39 = 16.67%	4/34 = 11.76%
Overall % w/bTB	18.21	16.24	17.51	16.97

Prior to 2000, badgers had been considered bTB positive only if the animal presented with visible lesions. However, this method has been shown to considerably underestimate disease

levels. Since 2000, bacteriological culturing, a much more sensitive procedure, has been used for bTB detection. Table 3.6 illustrates the bTB prevalence in male and female badgers in January and February in 2001 and 2005. While there was an apparent reduction in bTB prevalence between the two years, these changes did not reach statistical significance in either male or female badgers (males: $\chi^2 = 3.168$ df = 1 $p > 0.05$; females: $\chi^2 = 1.342$ df = 1 $p > 0.05$).

Table 3.8 Proportion (%) of the male and female adult badger population diagnosed with bTB in January and February of 2001 and 2005.

	Males		Females	
	2001	2005	2001	2005
January	16/106= 15.09%	6/87 = 6.90%	19/84 = 22.62%	5/67 = 7.46%
February	34/174= 19.54%	17/107 =15.89%	20/167 =11.98%	14/98 =14.29%
Overall % w/bTB	17.86	11.86	15.54	11.52

Even though bite wounding incidences were uncommon in male and female badgers, it was found that the majority of animals with bite wounds were also found to be carrying bTB (Fig. 3.7). The prevalence of bTB among badgers carrying a bite wound ranged from 57% to 100% in males, and from 85.7% to 100% in females, across the years of the study. As there was no evidence of bite wounding found in 2005 animals from this year were excluded from this analysis. In comparison, the prevalence of bTB in badgers not carrying a bite wound was very much lower, ranging between 11.86% and 18.21% for males, and 11.52%

and 17.51% for females (Fig. 3.8). These data strongly suggesting a significant link between bTB transmission and bite wounding ($\chi^2 = 72.55$, $df = 1$, $p < 0.001$).

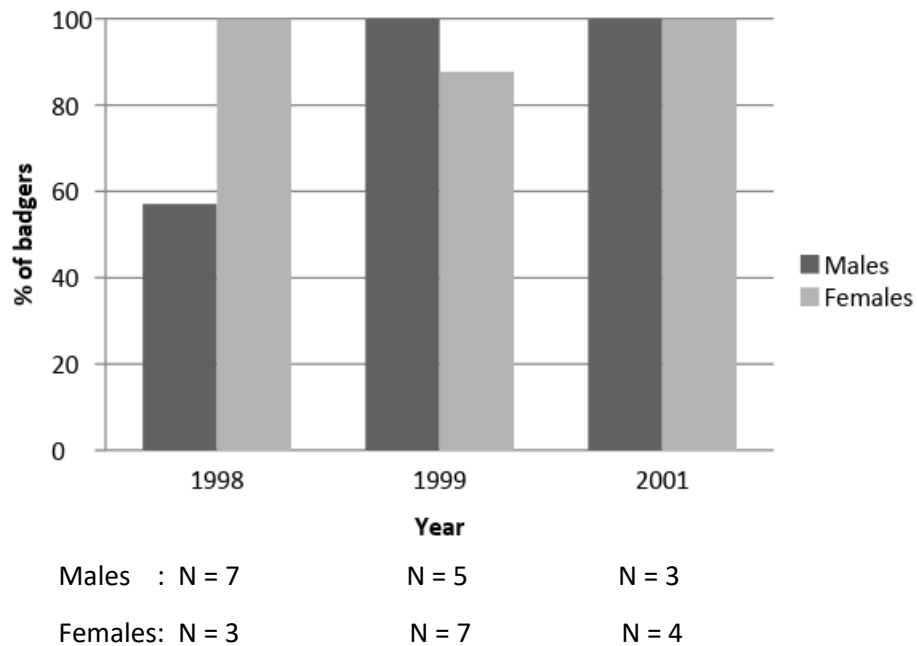


Figure 3.7 Animals with evidence of bite wounding that also tested positive for bTB.

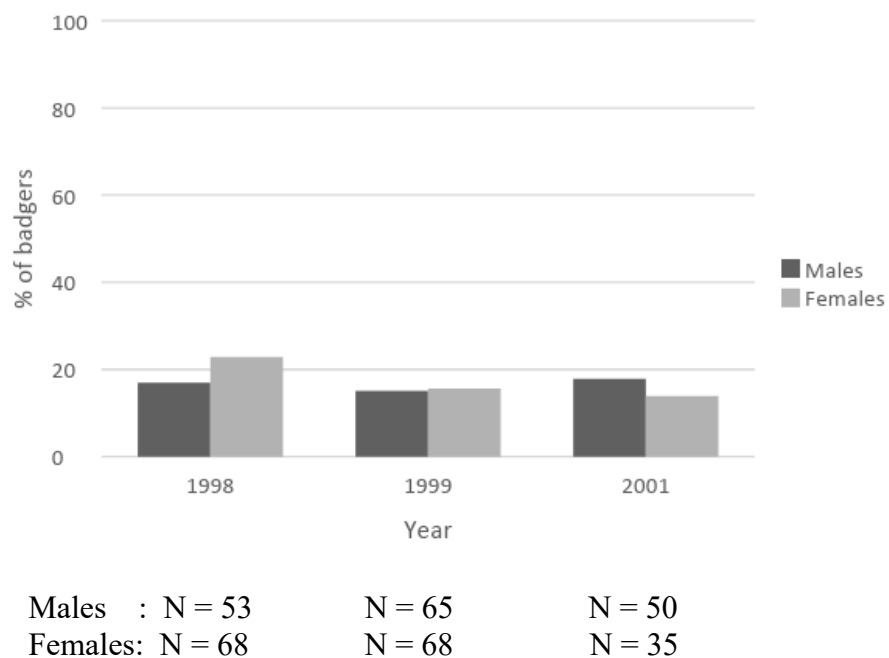


Figure 3.8 Animals with no bite wounding that tested positive for bTB.

3.4 Discussion

The limitations of this data set, i.e not knowing how many previously culled and unculted setts occur in each year must be borne in mind when considering the trends that this chapter presents. In addition, any effects of year are confounded with the increasing number of previously culled setts over time. While 2001 was a wetter year the general trends across the data set as disturbance increased were expected to have changed consistently. What is most interesting about this data set is that contrary to the expectations raised by the literature, no major changes in badger populations were found across the entire timeframe of the study.

3.4.1 Body weight

Where culling had caused a significant reduction in localised badger abundance in Ireland (Corner *et al.*, 2008; Byrne *et al.*, 2013b), it was possible that there might have been an advantage to the remaining animals due to the increase in resources available, which could have increased body condition and fecundity. Such an effect has been seen in other animal populations such as the common brushtail possum (Ji *et al.*, 2005). However, this study did not find an increase in Irish badger body weight in response to culling. Both year and gravidity were found to have a significant effect on female body weight, with gravid females being heavier in general, and females from 2001 being lighter than those culled in earlier years. It must be noted however, that as females were weighed before post mortem examination took place, gravid female weight also included unborn cub weight. Cubs can weigh between 75 - 135g at birth (Roper, 2010). Therefore, a litter of cubs, plus placental material, amniotic fluid weight in addition to the development of mammary tissues and elongated teats may increase the weights of gravid females. This may be the reason why gravid females were significantly heavier than non-gravid females.

Male badgers culled in 2001 were significantly lighter than those culled in 1999, by 2005 male mean body weight had increased and was back in line with that seen in the early years of the study. Therefore, it seems likely that the variation seen in body weight was not directly linked to the effects of culling, but rather other factors. Mild, dry winters have been linked to heavier body weight in badgers (MacDonald and Newman, 2002). The average monthly rainfall for the autumn/winter preceding the spring of 2001 was greater than the autumn/winter preceding the other 3 years. An average monthly rainfall ranging between

120.8 - 180.4mls was recorded in the months preceding 2001 compared with 61.6 - 121.8mls (1998), 96 - 135.3mls (1999) and 82.7 - 146.1mls (2005) (Met Eireann, 2018). Met Eireann also recorded severe flooding in the east and southeast in November 2000, which is where many of the badgers in this study came from. Therefore, it is possible that the lower mean body weights in 2001 for both male and female badgers may be due to difficulty in foraging or higher metabolic requirements caused by cold and wet weather of late 2000. Consequently, this study found no effect of culling on the average body weight of the badger population which outweighed the effect of natural inter-annual variation. It is possible that annual environmental fluctuations of factors such as temperature and rainfall may be more influential in weight changes by affecting the foraging ability of animals (MacDonald *et al.*, 2010).

3.4.2 Fecundity

Previous studies carried out in Ireland (Stuart, 2010; Corner *et al.*, 2015), Britain (Cresswell *et al.*, 1992) and Sweden (Ahlund, 1980) have found that between 65% and 95% of adult females had viable blastocysts. Female badgers in Sweden have a high reproductive success rate, with almost all animals found with blastocysts going on to implant (Ahlund, 1980). Conversely, other studies in Britain found a high loss of embryos throughout the different stages of the reproductive cycle. Many female badgers which were known to be pregnant did not successfully reproduce, with up to 60% failing to implant (Roper, 2010). Furthermore, even gravid females had high losses, with 35% failing to give birth to live young (Woodroffe and MacDonald, 1995a; Roper, 2010), resulting in only 14% of females having a successful pregnancy in any particular year. In Ireland, the majority of females go on to implant and on average 67.5% of all adult females culled had embryos/foetuses during January and February, suggesting high reproductive success (Stuart, 2010; Corner *et al.*, 2015).

In the early years of the cull, in 1998 and 1999, 36.47 - 40% of females were found to have embryos/foetuses, which we can assume to be the percentage when this population was relatively undisturbed. This is lower than the figures of 67.5% quoted by Stuart (2010) and Corner *et al.*, (2015). Given that these data come from IEC records one possibility for this lower figure could be that earlier stages of pregnancy may have been missed during the brief post mortem examination where other procedures and sampling were carried out. Stuart

(2010) was solely focusing on the reproductive cycle of badgers and therefore would have afforded more time to a thorough examination of each female and may have been more successful at detecting early pregnancy. While it was not possible to detect the levels of fertilization and blastocysts for the badgers in this data set, if there were high levels of fertilization similar to those observed by Stuart (2010) then these results may suggest a substantial loss of embryos/foetuses between fertilisation and birth, similar to those seen in high density populations in Britain.

Previous studies conducted in the Britain found that large scale culling greatly reduced fecundity (Tuytens *et al.*, 2000b; Sadler and Montgomery, 2004). With young females being the primary colonisers of culled areas, Tuytens *et al.*, (2000b) concluded that the reduction in fecundity could be attributed to lack of mating opportunities due to the sex bias of recolonising females or possibly due to those females being sexually immature. However, this study clearly established the females are still reproducing at similar rates, regardless of the cull. It also suggested that reproduction appeared to be unaffected by repeated culling.

The average litter size in this study (mean 2.32 - 2.55 embryos/foetuses) was similar to that found in badgers across Europe when comparable methods of detection were used (e.g. Anderson and Trewhalla (1985) 2.7 cubs per litter). Studies on protected badger populations in the Netherlands found that while population growth occurred, litter size did not significantly increase over 19 years of observation, with a mean litter size of 2.3 cubs (Van Apeldoorn *et al.*, 2006). In Oxfordshire, England, studies on a high density uncultured population using ultrasound scanning found a smaller average litter size of 1.8 cubs (Woodroffe and MacDonald, 1995a). This lower litter size may be due to the resource competition likely to be present in high density populations, and thus may not be a common occurrence across the rest of the badger's geographical range.

3.4.3 Timing of breeding

There are several factors which influence implantation and parturition in badgers. Photoperiod, geographical factors such as latitude and altitude have been found to influence implantation (Canivenc and Bonnin, 1979; Wandeler and Graf, 1982 cited in Woodroffe, 1995; Neal and Cheesman, 1996). In general, badgers in warmer, southerly locations tend to cub earlier in the season. For example, badgers in Spain and the south of France are gravid

in December and early January (Neal and Cheesman, 1996; Revilla *et al.*, 1999). In Britain gravid females are mostly seen in January and February, although births have been recorded in late March (Cresswell *et al.*, 1992; Woodroffe, 1995; Yamaguchi *et al.*, 2006). In Finland, gravid females are found in February, but the majority are gravid until later in March (Kauhala, 1995 cited in MacDonald and Newman, 2002). The time period in which gravid females were captured in Ireland seems to correspond with that seen in Britain, although pregnancies do seem to be concentrated into a shorter timeframe than is seen in Britain and elsewhere. It is possible this is due to a more predictable oceanic climate compared to the somewhat continental climate experienced in Britain. This study found that although there was some minor variation between years, all gravid females captured were found between January and mid-February, with the highest percentage seen in late January. The peak in breeding moved slightly by a few weeks within this period, however, it fell outside the scope of this study to investigate the factors causing such shifts.

3.4.4 Bite wounding

Incidence of bite wounding between 1998 and 2005, at between 1 and 2%, was much less frequent than in Britain, where bite wounding can range from over 40% in high density populations (Cresswell *et al.*, 1992) to 7.5% in lower density, disturbed populations (Delahay *et al.*, 2006b). Previous studies in both Britain and Ireland observed that males were more likely to have moderate or severe wounds compared to females and cubs, possibly due to competition for breeding opportunities and aggressive territorial behaviour. (Britain - Cresswell *et al.*, 1992; Tuytens *et al.*, 2000b; Delahay *et al.*, 2006b; Ireland - Corner *et al.*, 2012). However, in disturbed populations females were more likely to have bite wounds than males, which has been attributed to disputes occurring between recolonising females (Delahay *et al.*, 2006b). While the presence of bite wounding was found to be very low in this current study, there was no evidence to suggest an association with sex. Bite wounding was found equally in both males and females.

There were no obvious annual patterns in bite wounding, nor did presence of bite wounding differ greatly between years, although it reduced from low levels to complete absence from 2005 onwards. Again, this differed from other studies where levels of bite wounding varied significantly between season and year (Cresswell *et al.*, 1992; Delahay *et al.*, 2006b). This low level of bite wounding may suggest that competition and aggression between animals

in Ireland was less severe (both before and after culling took place) than that found in Britain, where higher densities may be linked to aggressive reproductive and territorial behaviour, especially in male badgers (MacDonald *et al.*, 2004).

There did, however, appear to be a significant relationship between bite wounding and bTB. Of the 15 male badgers with bite wounds, 12 (80%) also tested positive for bTB, while 13 out of 14 (92.9%) bitten females also had bTB. This contrasts with the rest of the unbitten badgers in this study, where prevalence of bTB ranged between 11.52 - 17.51% in females and 11.86 - 18.21% in males. Previous studies in both Britain and Ireland have also suggested a link between bite wounding and bTB transmission. Corner *et al.*, (2012) found that although there was a low prevalence of bite wounds, they were a significant route of infection for bTB in badgers, so it may be that those animals with bite wounds are more likely to be infected with bTB. Conversely, English studies have suggested that animals with advanced disease are more likely to be bitten due to poor health, thus being more vulnerable to aggression. (Jenkins *et al.*, 2008; Jenkins *et al.*, 2012). Although it is outside the scope of this study to expand on the infection dynamics, the data support the existence of a strong correlation between bTB and bite wounding.

3.4.5 Tuberculosis in badgers

There are contradictory theories about the effect culling may have on bTB levels in the badger population. It is possible that changes to the badger population caused by a dramatic reduction in badger abundance in an area may lead to a decrease in the prevalence of *M. bovis* in the badger population. If the density of badgers in an area is lowered, this could reduce the chance of infection of susceptible individuals due to less frequent contact between animals (Carter *et al.*, 2007; Corner *et al.*, 2008). However, in Britain it is thought that badger culling increases the level of infection, probably because the social disruption caused by culling may intensify direct contact and disease transmission between the remaining badgers. These individuals move out of the area being culled, spreading the disease. In addition, the stress caused by the removal operations and subsequent social disruption may cause immunosuppression in the surviving population and further aid the spread of the disease (Woodroffe *et al.*, 2009; Riordan *et al.*, 2011).

Unfortunately, the change in methodology regarding bTB detection meant a direct comparison between all 4 years of this study was impossible. The original method was based on the presence of visible lesions and as Murphy *et al.*, (2009) have found that up to 66.7% of infected animals have no visible lesions it could be argued that previous estimations were underestimated by this much. It is probable that the prevalence of bTB has fallen in the badger population considerably during the culling period but that this result is hidden with the increase of sensitivity in the methods of detection. A study focussed on assessing the prevalence of bTB and adjusting for these sensitivity changes would be needed to describe the effects of the cull on bTB prevalence effectively.

3.5 Conclusion

In areas which have undergone extensive culling, the proportion of reproductively active females appears to be unchanged. This was very unexpected as studies in Britain have found that culling caused a significant reduction in fecundity. With females in repeatedly culled areas breeding this raises concerns at the lack of a closed season in those areas and for young cubs which may starve if their mothers are culled before they are weaned. Bite wounding was found at very low levels throughout the study, insufficient to detect any seasonal trends. However, there does appear to be a strong association between bite wounding and bTB. Other parameters such as body weight, litter size and pregnancy period have remained unchanged over the duration of the cull.

Chapter 4: Culling Induced changes in social groups

Abstract

This chapter investigates the effect of culling on social group size, recolonisation times and the age and sex profile of the incoming badgers.

There was no significant reduction seen in social group sizes following culling. Average social group size was found to be small with most groups containing an average of 5.5 animals during a first cull and an average of 5.0 animals during subsequent culls. The time period in which social group size begins to return to normal in culled areas was impossible to determine within the scope of this study. However, after a rapid initial influx to culled areas, it is clear that most social groups remain at a reduced size more than two years after culling. Significantly more females than males were culled in 2007 during first, second and third culls suggesting a possible sex bias in trapping methods or locations of the stopped restraints. No difference was found in age profile of female badgers from areas undergoing a first cull or subsequent culls which was unexpected given that previous studies have found that recolonisation of culled areas is primarily undertaken by younger females (Tuytens *et al.*, 2000b).

Investigating the effects of culling on social groups will be important for use in future vaccine programs as it will be important to know who is repopulating culled areas and the length of time this recovery takes. If a minimum of 30% of the population must be vaccinated to ensure a reduction in bTB (Anzar *et al.*, 2018), then understanding the recovery process of targeted populations will be necessary to ensure that sufficient vaccination levels are met.

4.1 Introduction

In Ireland, badgers are mainly culled by stopped restraint. Previous studies suggest that stopped restraints are effective for trapping adult animals (Do Linh San, *et al.*, 2003; Monoz-

Igualada *et al.*, 2008; Sleeman *et al.*, 2009b) but are poorly designed to catch younger and smaller animals (Do Linh San, *et al.*, 2003; Byrne *et al.*, 2012b). Therefore, it is possible that the culled badgers will show an age bias, as younger and/or smaller badgers such as cubs or yearlings may be small enough to escape the stopped restraints. However, any adults caught should represent other ages and sexes equally. Stopped restraints are multi-strand steel loops, with a stop on the wire to prevent it closing tighter than 28cm. They are placed close to active setts for 11 consecutive nights and are checked each morning. Captured animals are killed with a 0.22 calibre rifle (Griffin *et al.*, 2005). More than one trapping session may be carried out within a year if badger activity is still found in the area and occasionally a culling event may continue for another full cycle of 11 nights if animals are still being captured at the end of the initial 11 day cull period.

After culling in Britain, social group size decreases in the surrounding uncultured territories as vacant territories provide more opportunities for animals to leave their natal group (Woodroffe and MacDonald, 1995b). As female/female reproductive suppression has been reported between dominant and subordinate animals (Woodroffe and MacDonald, 1995b), younger females may choose to establish their own social groups in areas left vacant after culling. It has been reported that most females move out of their natal groups in coalitions of 2 - 3 females of varying ages (Woodroffe *et al.*, 1995c). This may explain why British studies have found that animals undertaking the initial recolonisation of culled areas were almost exclusively adult and yearling females (Tuytens *et al.*, 2000b). Previous studies in Britain found a phase of rapid initial low level immigration into culled areas (Cheeseman *et al.*, 1993; Tuytens *et al.*, 2000a). However, despite badgers moving into the culled areas soon after culling had taken place social groups could take up to 10 years to recover to the original population density (Smith *et al.*, 2001). Previously disturbed populations, with an already lowered density, were found to recover more quickly than previously undisturbed populations (Tuytens *et al.*, 2000a).

Recolonisation after culling patterns in Ireland was found to be similar to that seen in Britain. Badgers were found to move into culled areas as early as 5 days after major disturbance (Gaughran *et al.*, 2018). However, once again, total population recovery was slow and a return to preculling densities was estimated to take a minimum of 5 years after the last culling event (O’Corry-Crowe *et al.*, 1993). When recolonisation did take place, it was found that the immigrant badgers were moving in from surrounding areas (Eves, 1999). One Irish

study found that the population of badgers in repeatedly culled areas was predominately female (Sleeman *et al.*, 2009a). However, as there is only one study of Irish badger populations that suggests this (Sleeman *et al.*, 2009a) and no evidence of reproductive suppression in females by dominant females in Ireland (Stuart, 2010), the motivation to recolonise an empty sett may be different in Ireland than in the British populations given the overall lower density and lack of competition for resources. Therefore, the recolonisers of culled areas may not be as female and age biased as seen in Britain (Tuytens *et al.*, 2000b) where the main recolonisers of vacant setts are females.

In Britain, animals have been found to live in a variety of social group sizes, with up to 27 individuals in a single social group and an average social group size of 8.8 animals (Rogers *et al.*, 1997b). Studies found that badger culling resulted in an increase in social group size and in the overlap between neighbouring territories (Cheeseman *et al.*, 1993; Tuytens *et al.*, 2000a; Woodroffe *et al.*, 2005b). In Ireland the social group sizes have been found to be smaller than in English populations, with many social groups made up of singletons or pairs of animals (Stuart, 2010) and the average social group size ranging from 2.9 animals (Sleeman *et al.*, 2009b) to 5.9 animals (Smal, 1995). It is not yet clear how culling may affect social group size in Ireland.

As culling has been shown to significantly influence movement, ranging behaviour and badger density in other studies (Cheeseman *et al.*, 1993; Woodroffe *et al.*, 2005; MacDonald *et al.*, 2008a; Riordan *et al.*, 2011), the effects of culling on the population dynamics of specific setts and social groups will be examined. To do so, the differences between setts and social groups that have repeatedly been targeted will be compared to those being culled for the first time. This will determine whether culling in Ireland affects badgers at a social group level. Therefore, the aims of this chapter will be to:

- Determine the age of badgers recolonising culled areas. The literature suggests that the majority of recolonising badgers are young females who are seeking to escape their natal groups and establish their own territories (Tuytens *et al.*, 2000b).
- Investigate whether repeated culling affects social group size. In Britain, culling was seen to increase social group size due to the effects of perturbation (Cheeseman *et al.*, 1993; Tuytens *et al.*, 2000a; Woodroffe *et al.*, 2005b), however as Irish badgers

already live at lower densities, it is possible that culling might not overtly affect social group size.

- 8● Examine social group recovery patterns over multiple culls. Recolonisation of areas after culling in Ireland was found to be similar to that seen in Britain. Badgers may move into areas very rapidly, however, total population recovery is thought to take several years (O’Corry-Crowe *et al.*, 1993).
- 8● Determine the sex ratio of badgers culled in different culling events. Previous research in Britain found that more males than females were removed during culling (Tuytens *et al.*, 2000b), although that has not always been the case with Irish studies which have found high number of female badgers culled (Corner *et al.*, 2008; Murphy *et al.*, 2009; Stuart, 2010)

4.2 Methods

Information from post mortem examinations carried out on 370 female badgers out of a possible 402 animals were used in this study. The 32 animals were omitted as it was not possible to assign them to a specific culling event. Additional information provided by DAFM in the form of electronic spreadsheets allowed for data on another 3,553 male and female badgers caught during 2007 to be included in this study. This information included:

- Date of the initial cull of each sett.
- Original social group size and social group size during subsequent culls.
- Time span between subsequent culls.
- Sex of the animals culled in each consecutive cull.

The female badgers that underwent post mortem examinations were a subset of the DAFM badgers. Using extra data supplied by DAFM, it was possible to assign the culled animals to a specific culled event, enabling us to accurately compare previously undisturbed setts and repeatedly culled out setts. The age range of these animals, as determined by toothwear based on the method used by Hancox, (1988) (detailed methods described in Chapter 2) was also used in this study.

Variance of all data were checked for normality and the threshold for statistical significance was set at $p = 0.05$. Chi-squared tests and Fisher Exact Tests were used where appropriate. Descriptive statistics for categorical variables are presented as percentages.

4.3 Results

From the females which underwent post mortem during this study, 368 jaws were suitable for aging. The other jaws were deemed to have been too damaged or to be unsuitable for the procedure. Using toothwear assessment following the method of Hancox (1998), the ages of female badger killed in different culling events was determined. This allowed for a comparison between the age profiles of the recolonising animals and those previously found in undisturbed areas. The females were assigned to one of four age groups, juveniles, yearling, adult and aged adult, based on their pattern of tooth wear (Table 4.1). There was no difference in the age profile of culled female badgers between 2007 and 2008 (Fishers Exact Test: $p = 0.1282$).

Table 4.1 The age profile of female badgers sampled in this study as determined by toothwear.

	2007 (Jan - Dec excl. Oct)	2008 (Jan - May)	Overall Percentage (N=368)
Juvenile 0 to < 1 years	1.10% (N=3)	1.05% (N=1)	1.08% (N=4)
Yearling +1 to < 2 years	4.02% (N=11)	7.37% (N=7)	4.89% (N=18)
Adult > 2 to < 4 years	70.33% (N=192)	76.84% (N=73)	72.01% (N=265)
Aged Adult 4+ years	24.54% (N=67)	14.74% (N=14)	22.01% (N=81)

4.3.1 Ages of culled badgers

It should be noted that the results of this age profile are not representative of the overall population, but rather the sample that was culled using stopped restraints. Therefore, this is a comparison of age profiles after different culling events. There was very little variation in the age profile of badgers from different cull events (Fig. 4.1). Adults made up the majority

of the sample size of this study, followed by aged adults, yearlings then juveniles. The age profile of the female badgers caught in the first cull was assumed to be the undisturbed age ratio of this sample, although of course lacking the younger and smaller badgers unable to be caught in the restraints. The profile did not change through the different culling events (Fisher Exact test: $p = 0.3111$ – Juveniles were excluded from this analysis due to low sample size). Yearlings were absent from all 2nd cull events, although this may be due to these culls taking place later in the year, thus making it difficult to distinguish between yearlings and young adults.

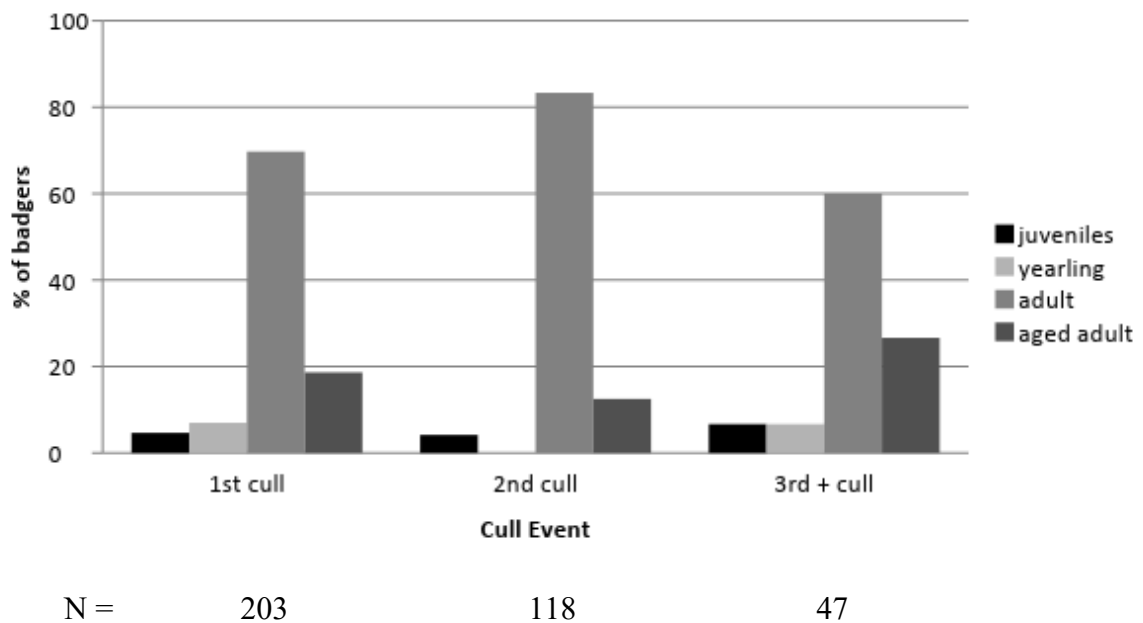


Figure 4.1 The age of female badgers culled in 2007 and 2008, determined by tooth wear.

4.3.2 Social group size

The data received from DAFM provided cull event and social group information on an additional 3553 male and female badgers, from 160 different social groups (Table 4.2). Of these 160 social groups, 72 social groups were undergoing a cull for the first time, 45 additional social groups had undergone both a 1st and a 2nd cull. Furthermore, there was information on a 1st and 3rd cull for 33 social groups and information on a 1st and 4th cull for 10 social groups. Unfortunately, data were not available for each culling event for all setts used in this study. For example, the 1st and 3rd cull setts did not have data for their 2nd culls so are not the same setts as recorded a 1st and 2nd cull. The same is true for the data from the

4th culls, as data from their 2nd and 3rd cull was missing. The information on these 160 social groups was used to calculate an average social group size from those culled and to investigate whether social group size was affected by repeated culling.

Table 4.2 The breakdown of social group size for each cull event.

	1st cull only	1st and 2nd cull		1st and 3rd cull		1st and 4th + cull	
Number of social groups	72	45		33		10	
	1 st cull	1 st cull	2 nd cull	1st cull	3 rd cull	1st cull	4 th + cull
Average social group size	5.2	5.3	4.2	6.9	6.1	5.4	4.2
Largest social group size	15	15	14	16	25	11	6
Smallest social group size or single individual	1	1	1	2	1	3	2

The data show that the number of animals ranged between 1 and 16 for first culls and 1 to 25 for subsequent culls. The group size range was 5 to 7 animals with an average of 5.5 animals per social group during a first cull and 4 to 6 animals, with an average of 5.0 badgers per social group after subsequent culls. This falls within the average range of social group sizes previously found in Ireland (2.9 animals (Sleeman *et al.*, 2009b) to 5.9 animals (Small, 1995)). Overall there was no significant change in the average social group size after repeated culling, however in every case the later cull had a lower average than the earlier one. Of the 160 social groups which had information on the size of social groups during a first cull 16.88% (N=27) were deemed to be ‘large’ social groups of more than 8 individuals. The proportion of large social groups in subsequent culls dropped to 11.36% (N=10).

Of the 45 setts identified as having information on social group size during both the initial and second badger culls (Figure 4.2), 15 were reculled within 6 months, 17 were reculled 7 – 12 months after the first cull, 9 were reculled at 13 – 18 months, none were reculled between 19 – 24 months and 4 were reculled more than 2 years after the initial cull. Setts were classified as having fewer animals, having equal numbers of animals or having more animals in the social group at the second cull than were trapped during the first culling event.

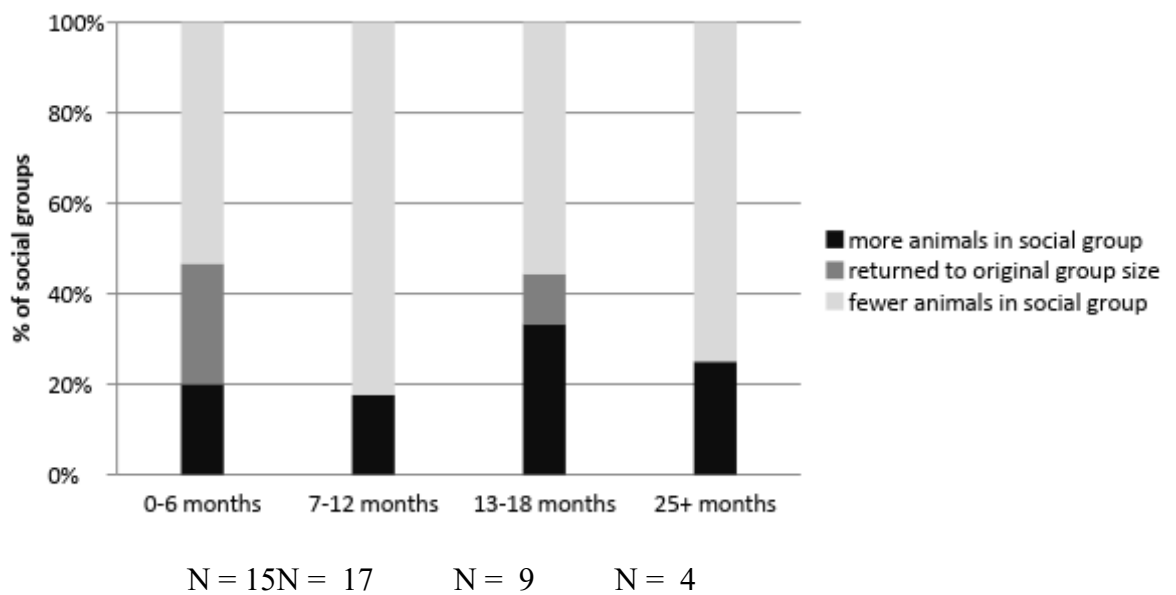


Figure 4.2 Percentage of social groups with fewer animals, original number of animals or more animals in social groups by the time of a second cull.

For this analysis, only data from 1st and 2nd culls were used. The data clearly show that the majority of social groups culled a second time had fewer animals than when initially culled. If a second cull took place between 0 - 6 months after the first cull, 53.3% of social groups culled were smaller than their original size, 26.7% had returned to their original size while 20% of social groups were larger than that found during the initial cull. This suggests a very quick initial recovery from social group removal. However, if the second cull was between 7 and 12 months after the initial cull, 82.4% of social groups were found to be smaller than the original size.

4.3.3 Sex ratios of culled badgers

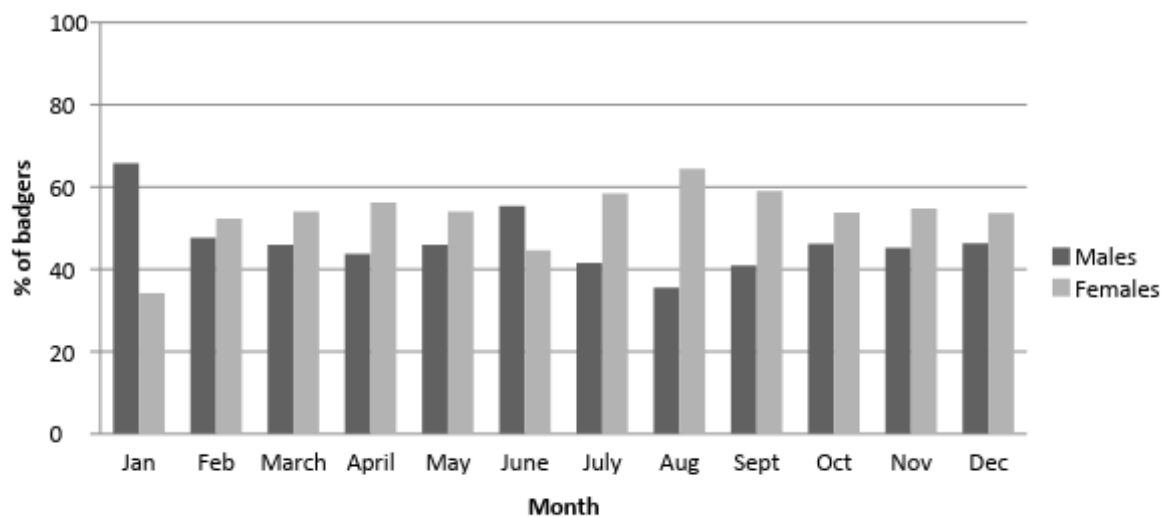
Using the DAFM database, information on 1,634 males and 1,919 females was used to investigate the sex ratios of the culled badgers (Table 4.3). The female to male sex ratio for the study population was 1.2:1, which differed significantly from a sex ratio of 1:1 (Chi-squared test: $\chi^2 = 22.7$; df = 1; $p < 0.001$). Significantly more females than males were culled during the 1st through 3rd culls (1st cull - Chi-squared test: $\chi^2 = 20.2$; df = 1; $p < 0.001$; 2nd cull - Chi-squared test: $\chi^2 = 16.6$; df = 1; $p < 0.001$; 3rd cull - Chi-squared test: $\chi^2 = 7.8$; df = 1; p

= 0.032). During the 4th and subsequent culls, there was no statistically significant difference in the number of each sex culled ($\chi^2 = 1.5$; df = 1; $p > 0.05$).

Table 4.3 The breakdown of male and female badgers culled by DAFM throughout the twelve months of 2007.

	1 st cull	2 nd cull	3 rd cull	4 th + cull	Total
Males	46.09% (N = 767)	45.53% (N = 474)	44.26% (N = 262)	51.17% (N = 131)	45.98% (N = 1634)
Females	53.91% (N = 897)	54.47% (N = 567)	55.74% (N = 330)	48.83% (N = 125)	54.02% (N = 1919)
Total	1664	1041	592	256	3553

If the number of captured badgers is broken down by month, in ten out of the twelve months of 2007, more females than males were culled (Fig. 4.3). The only two months in exception to this were January and June. In January almost twice as many males (65.8%) were culled as females (34.2%), while in June 55.4% of badgers culled were males. In July, August and September there were much higher percentages of females caught compared to males.



Male N =	25	198	348	262	194	67	17	11	43	178	196	95
Female N =	13	217	409	336	228	54	24	20	63	207	238	110

Figure 4.3 The monthly percentage of male and female badgers caught during all culling events in 2007.

4.4 Discussion

4.4.1 Age range of culled badgers

The majority of females caught were adults (2-5 years old), followed by aged adults (>5 years). Yearlings were culled in previously undisturbed areas but were absent from some of the subsequent cull events. This may have been due to these culls taking place later in the year, thus making it difficult to distinguish between yearlings and young adults. Upon examination of the age profile of the sample there is an extreme bias against 0 - 2 year old animals, with this age group only accounting for 5.97% of captured animals. This unexpected result may be due to a number of factors such as the population reproducing insufficiently, the aging techniques being inaccurate, or the method being biased against younger animals.

As the aging techniques utilised in this current research have also been used in numerous other studies (Whelan, 1998; Stuart, 2010, Byrne *et al.*, 2012b) it is unlikely that this method is causing a bias in the age profile. Also, the lack of young animals is unlikely solely due to a reduction in reproduction as fewer juveniles and yearlings were caught in areas undergoing a first cull, not just those areas undergoing a subsequent cull with the associated lowered fecundity as seen in the last chapter. In Ireland stopped restraints are the main capture method. Previous studies have suggested that stopped restraints are more effective compared to cage trapping, particularly in adult animals (Do Linh San, *et al.*, 2003; Monoz-Igualada *et al.*, 2008; Sleeman *et al.*, 2009b). As these have a minimum stopping distance of 28cm (Anon, 1996), this makes them poorly designed to catch younger and smaller animals (Do Linh San, *et al.*, 2003; Byrne *et al.*, 2012b). Recent Irish studies have found that cubs are significantly more likely to be caught in cage traps compared to stopped restraints (Byrne *et al.*, 2012b). Therefore, it is highly likely that the lack of younger animals in this sample size can be attributed to the trapping method being biased against younger, smaller animals.

Furthermore, badgers are yearlings only for a year, whereas they are classed as adult for at least 2 years and aged adults in the years after this. Even with some natural loss within the population, this would mean that there are more adult badgers within the Irish population and therefore, these are more likely to be culled in greater numbers. It also must be noted that despite best efforts not all animals from the targeted areas will be culled. Previous

studies estimated Irish trapping rate at 85% (Sleeman, *et al.*, 2009b). However, recent studies have estimated a much lower success rate using the current trapping method with Byrne *et al.*, (2012b) estimating an annual capture rate of 58%. Therefore, if there are more adult animals within the population to begin with, it's unsurprising that adults are culled in greater numbers. Furthermore, if over 40% of the badger population escapes capture, with younger and smaller animals less likely to be culled due to stopped restraint size bias, this may also account for the unexpectedly high proportion of adults found to be culled in this study. This means that the age ratios reported in this study are not indicative of the population in general.

Despite the bias in the capturing method, conclusions can be drawn about which badgers recolonise culled areas. As this study found similar age distributions in both first cull areas and in the previously culled areas, this suggests that culled areas were recolonised by a subset of the surrounding population, drawn from at least the two age groups effectively sampled by stopped restraints. This is unexpected given what has been observed in other countries, where recolonisation is predominately by younger females (Tuytens *et al.*, 2000b). The presence of aged adults in the recolonizing population demonstrates that recolonization is not restricted to young females. As the majority of subsequent culls took place within 2 years of the initial cull, the time between culls was not sufficient for any remaining young females to mature to aged adults. It therefore appears that in these study areas across Ireland, recolonization was not restricted to or dominated by younger females, which was unexpected given research from Britain (Tuytens *et al.*, 2000b).

4.4.2 Social group size

The data suggest that the majority of badgers in Ireland were living in family groups of between 4 and 7 animals, with the lower end of this range being in areas undergoing repeated culling. These averages are within the higher range of social group size found in previous Irish studies (O'Corry-Crowe *et al.*, 1993; Smal, 1995; Whelan, 1998), and larger than that seen in more recent research (Table 4.4). It is possible that these social groups are made up of a main breeding pair and undispersed offspring from previous years, however it is also possible that coalitions of related females may have formed their own social groups when taking over territories (Woodroffe *et al.*, 1995c). The social group size ranged from 1 to 16 animals in previously undisturbed areas and from 1 to 25 animals in repeatedly culled areas.

This large variation in social group size was unexpected. While the vast majority of social groups were small and similar to those found in previous Irish studies (Whelan, 1998; Stuart, 2010), the larger group sizes were similar to those seen in high density populations in Britain, which have up to 27 individuals per social group (Rogers *et al.*, 1997b). This finding suggests that in Ireland, while most badgers may live in a low to medium density situation, others may experience locally high density in their large social groups and that there might be more variation in local population densities than previously thought.

Table 4.4 The average social group size for badgers throughout the island of Ireland.

Average social group size	Reference
5.8	O’Corry-Crowe, Eves and Hayden (1993)
5.9	Smal, 1995
4.7	Whelan, 1998
2.3	Feore and Montgomery, 1999
3	Eves, 1999
3.6	Sadler and Montgomery, 2004
3.9	Sleeman, 2009b
1.6	Stuart, 2010
Average social group size of this study	
In first culls: 5.5	
In subsequent culls: 5.0	

This study found no significant change in the average social group size after repeated culling. This supports the Resource Dispersion Hypothesis (RDH) which suggests that social group size is influenced by resource abundance (Johnson *et al.*, 2002b; Robertson *et al.*, 2015). Therefore, it is possible that social group size remains similar despite repeated culling, as new animals are moving in to areas capable of supporting additional animals.

This study failed to find a clear pattern of post-culling social group size recovery to original levels. During the second culls the majority of social groups remained smaller than those found throughout the initial cull. This correlates with a study in Gloucestershire, UK that found a disturbed badger population returned to its pre-removal density after 3 years (Tuytens *et al.*, 2000b), although this was after only a single culling event. Other disturbed

populations have taken 9-10 years to recover to high density populations after multiple social group removal (Smith *et al.*, 2001). The time frame of the present study was too short to be certain when recovery was stable, and therefore it was not possible to explore the mechanisms of social group size recovery. It must also be noted that other factors such as the original population density and the spatial extent of culling in surrounding social groups will need to be considered when examining population recovery in the future as these will inevitably affect the source population, and subsequently the rate of recovery.

Badger culling has been shown to severely impact spatial organization of badger populations by affecting home range and territory size (Tuytens *et al.*, 2000a). Many studies have reported significantly increased movements between groups and wider ranging behaviour after badger removal (Tuytens *et al.*, 2000b; Riordan *et al.*, 2011), as well as severe disruption to the badgers' social organization (Tuytens *et al.*, 2000a). The home ranges of surviving animals were also found to increase by over 40% in response to culling (Riordan *et al.*, 2011) and overlapped neighbouring territories (Cheeseman *et al.*, 1993; Tuytens *et al.*, 2000a; Woodroffe *et al.*, 2005b). Furthermore, Woodroffe *et al.*, (2005) found that culling had a graduated effect on social disruption, with greater changes to the social group range size being found close to the disturbed areas and smaller changes further from the disturbance. Therefore, it is possible that repeated disruption of a reduced population might have been the reason for the slow return to the original social group size in culled areas.

4.4.3 Sex ratio of culled badgers

The data from this study found that in addition to an age biased capturing method, significantly more female badgers were trapped in 2007 compared to males. Significantly more females were caught during first, second and third culls. During the 4th or more culls the sex ratio of culled badgers had changed to closer to a 1:1 ratio. This corresponds with other Irish studies which have also shown a female led bias during culling (Whelan, 1998; Corner *et al.*, 2008; Murphy *et al.*, 2009; Stuart, 2010) although it did differ from studies in Britain where more males than females were removed during culling (Tuytens *et al.*, 2000b). Several factors may affect the sex ratio of the capture. As males have been shown to have significantly larger range sizes compared to females and often use a number of setts in multiple social groups (Tuytens *et al.*, 2000a; MacWhite *et al.*, *in prep*), it is possible that males moved out of areas being targeted for culling more so than females, resulting in higher

numbers of females being culled. Furthermore, as the catch-effort was concentrated near to main setts (DAFM, *pers. comm.*), again it is possible that males avoided the stopped restraints more frequently than females if they were away from the main sett due to ranging over larger territories. In Britain, Cheeseman *et al.*, (1988) found evidence that males moved out of an area after a disturbance in the population whereas females were the first to recolonise vacant areas (Tuytens *et al.*, 2000b). Additionally, male badgers have been shown to have a higher prevalence of bTB both in this study and by Corner *et al.*, (2008). As infected badgers forage over a greater proportion of their home range, away from their main sett (Garnett *et al.*, 2005), this may give them a greater chance of avoiding capture during a culling event as most traps are set close to sett openings (DAFM *pers. comm.*).

The bias in the sex ratio of animals caught changed through the year. The peak in culled male numbers was seen in January where almost twice as many males were caught as females. The increase in males culled in January could simply be caused by fewer females undertaking excursions due to pregnancy. Females spending more time below ground as their pregnancy advances could reduce the numbers of females caught in restraints, resulting in the percentage of males to increase. The increase in culled male numbers could also be due to seasonal changes in the male mating system. From January to February there is an increase in testicular and epididymal weight and size, and in the weight of the baculum (Marie-Claude, 1972). Therefore, it is likely that males begin to make mating excursions during January and visit more neighbouring territories than normal, going into neighbouring setts.

As there was a bias towards female badgers being culled in the first three culling events of an area and as there was no bias in cub sex ratios (see Chapter 5), this may have an unexpected impact on recovery rate of the badger population in repeatedly culled areas. With fewer females in each area, there would be a decrease in population recruitment which may lead to an area becoming depopulated more rapidly and increase the time needed for the badger population to recover.

4.5 Conclusions

The results from this study found that the vast majority of social groups in Ireland were relatively small and made up of between 4 and 7 animals. The time frame of this study was too short to accurately predict social group recovery time. While there was evidence of large social groups within the Irish badger population, some upwards of 20 individuals, this is probably natural variation within the population and there was no evidence that group size was influenced by the cull.

The age range of culled badgers was similar in all culling events, however there was a considerable lack of younger animals in all culls. This suggests a bias against culling younger animals, possibly due to the trapping method. The resulting age profiles of different cull events suggest a cross section of the age ranges of the surrounding population moved into vacant areas after culling had taken place.

Significantly more females than males were caught during culling across all culling events in most months. This sex bias was present in the first cull as well as the later ones and so was probably not due to more females moving into cleared areas. Instead, it may have been due to biases in the catching method such as restraint placement and different ranging habits between sexes. January and June were the only months in which more males were culled than females. It is important to recognize the significant bias towards trapping adults and larger animals with the current system and culling more females will adversely affect the recovery rates of repeatedly culled areas.

Chapter 5: Individual responses to the disturbance caused by culling activities

Abstract

This chapter will examine specific culling areas and individuals within social groups to determine whether the pattern of culling induced changes is different between individuals in repeatedly culled areas compared to those being disturbed for the first time.

No evidence of bite wounding was found on any female during post mortem examinations. Females from repeatedly culled areas were heavier in January, than those in undisturbed areas. This trend reversed in Autumn, where females from undisturbed areas were significantly heavier in September compared to females in repeatedly culled areas. Body weight, subcutaneous fat depth (SFD) and kidney fat indices (KFI) of female badgers from both undisturbed and previously culled areas fluctuated on a monthly basis. Females were significantly heavier and carried more fat in January and December compared to summer and autumn months. However, this pattern was not affected by the disturbance caused by culling. Blastocyst numbers were found to differ significantly between disturbed and undisturbed areas and varied significantly from month to month. Females from different culling events were equally likely to have at least one placental scar and maximum potential litter size decreased significantly after repeated culling events. There was no difference in the sex ratio of foetuses when categorised by culling event.

While it appears that badgers are physiologically able to withstand the disturbance associated with repeated culling (as seen in Chapter 3), there does appear to be some affect when it comes to reproductive success of individuals in repeatedly targeted areas. With lower reproductive success in these areas, population recovery will be slower than previously thought and may impact the results of future vaccination programs if not taken into account in those models.

5.1 Introduction

It is possible that culling induced changes may be evident in the physical condition and reproductive success of the animals in previously culled areas. If culling is ongoing in an area, the impact of constantly changing social groups and the stresses of needing to constantly define territories may have a marked impact on recolonizing badgers. Animals may react to stressful situations by exhibiting a heightened endocrine response which causes an increase in the production of adrenal glucocorticoids (Sapolsky, 1992). If this stressor persists and adrenal glucocorticoids remain elevated, this can have negative impacts on physical parameters such as body condition and reproductive success (Creel, 2001).

Animals can experience periods of both acute and chronic stress. Acute stressors are sudden, short term events due to behavioural, physical or environmental challenges, while chronic stressors are challenges that occur frequently or last for an extended period of time (Curry, 1999; Dantzer *et al.*, 2014). While culling events can be classified as acute stressors, it is also likely that the disruption to social groups and attempts to recolonise areas after culling may prove to be a source of chronic stressors for the incoming, and any remaining, animals. Both acute and chronic stresses have been found to affect reproduction on many different species (Tillbrook *et al.*, 2000). Chronic stress has been found to have the most extensive and profound effect, by reducing reproductive output and fecundity in a variety of species (hamsters - Huck *et al.*, 1988; ungulates and monkeys - Tillbrook *et al.*, 2000; pigs - Hemsworth *et al.*, 1986; rodents - Thorpe *et al.*, 2013). Therefore, it is possible that if repeated culling, or the social disruption associated with it, constitutes a chronically stressful situation, then it may have a physiological impact on the badgers recolonising the culled areas.

Aggressive behaviours, such as bite wounding, may also be influenced by population disturbances. In Britain, bite wounding was found to decrease after culling had taken place, but to increase in frequency as the population recovered (Delahay *et al.*, 2006b). If this eventual increase in bite wounding is due to chronic stressors, it is possible that Irish badgers will exhibit similar patterns of bite wounding as seen in disturbed populations from Britain, despite the low levels of bite wounding reported in the population as a whole (Stuart, 2010).

The aim of this chapter is to develop a greater understanding of the physiological changes that may occur in response to targeted, large scale disturbance by repeated culling of specific areas within the badger population. Over a series of culling events we will examine specific setts and social groups to see whether repeated culling has had an effect on female badgers by examining:

- ☛ Physiological differences between animals in repeatedly culled and previously undisturbed areas. While Chapter 3 looked at population level differences, this chapter will focus on individual differences from each area, including body condition indexes and blastocyst numbers.
- ☛ The presence of bite wounds. If stress is affecting behaviours such bite wounding, it is possible that Irish badgers will exhibit similar patterns of bite wounding as seen in high density or disturbed populations from Britain (Delahay *et al.*, 2006b).
- ☛ Body weight and condition indices. Being able to specifically assign each animal to a particular culling event will allow for the examination of body weight and body condition scores on a more in depth level.
- ☛ Difference in reproductive potential in both areas. By investigating blastocyst numbers and litter sizes in each culling event, this study can assess if long term culling does have an effect on fecundity.

5.2 Methods

Using the post mortem data procedure described in detail in Chapter 2, a minimum of 16 and a maximum of 20 badgers were examined during each of January, February and March of 2007 and 2008. A minimum of 10 and a maximum of 42 badgers a month were examined in the remaining months of the study. To get a representative view of the Irish badger population as a whole, females were selected at random from those culled by DAFM (see Chapter 2 for county breakdown of sample size).

Post mortem examinations were carried out at the IEC by Dr. Ursula Fogarty and further processing of samples was carried out both at the IEC and in Trinity College. Parameters recorded during the examinations were as follows:

- ☛ Body weight
- ☛ Length – crown to rump
- ☛ Subcutaneous fat dept.
- ☛ Kidney weight.
- ☛ Kidney fat weight.
- ☛ The presence of bite wounds.
- ☛ Number of foetuses present (if any)
- ☛ The weight, length and sex of each foetus found.

Furthermore, the reproductive tract from the vagina to ovaries was removed and the tract was flushed to extract any blastocysts. The uterus was examined for the presence of placental scars. The detailed post mortem examination procedure and further processing carried out on samples is detailed in Chapter 2 of this thesis.

The distributions of all data were checked for normality and nonparametric tests were used with any data sets which violated the assumptions of parametric statistical testing. Two-sample comparisons were tested using t-tests or Mann-Whitney U tests. When multiple t-tests were used the Holm (1979) correction for multiple tests was applied. Multi-sample comparisons used ANOVA with Bonferonni post hoc tests, or Kruskal Wallis tests. Fisher's Exact test was also used to compare groups of categorical data. Descriptive statistics for

categorical variables are presented as percentages. The threshold for statistical significance was set at $p < 0.05$.

5.3 Results

Of the 402 females sampled, 32 were unable to be assigned to a specific culling event and so were removed from the data to be analysed. Juveniles (6) were also excluded from the study, leaving a total sample size of 364 adult females (Table 5.1). The culled badgers were assessed for the presence of bite wounding marks, however no evidence of bite wounds was found on any of the females that underwent post mortem.

Table 5.1 The population breakdown of female badgers sampled in this study.

Number of females													Total
	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	
2007	17	19	20	34	37	14	20	10	33	0	32	35	271
2008	15	17	15	27	19	-	-	-	-	-	-	-	93
Total	32	36	35	61	56	14	20	10	33	0	32	35	364
Culling Event													
1st cull	10	15	16	35	38	8	13	4	22	0	19	21	201
2nd + cull	22	21	19	26	18	6	7	6	11	0	13	14	163

5.3.1 Weight and body condition

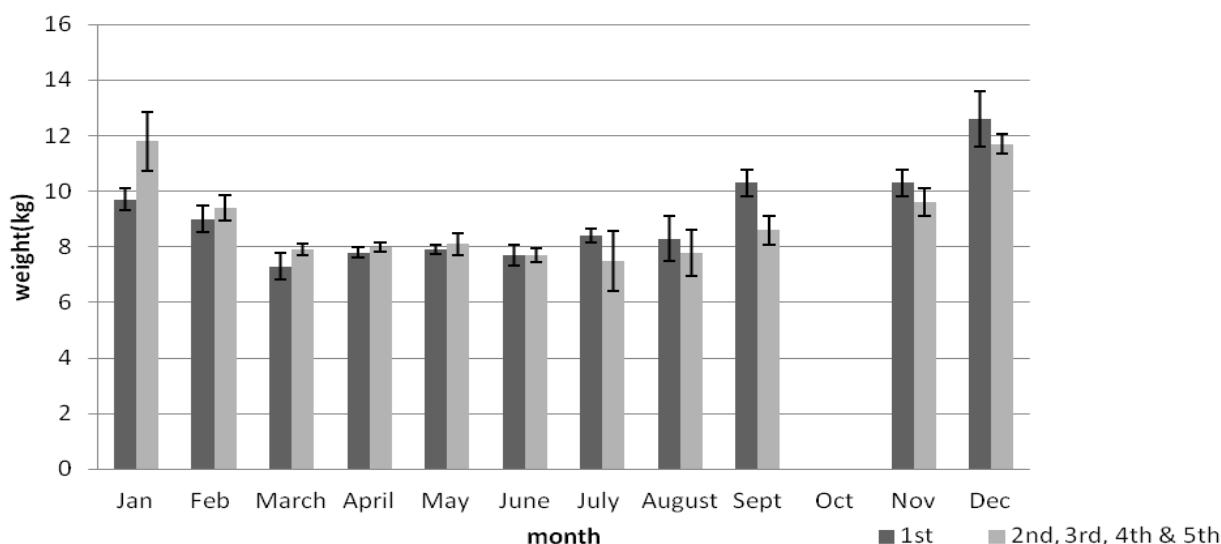
A comparison of the body weight of badgers taken from newly culled areas compared to those from areas which had undergone repeated culling show some interesting differences (Table 5.2). The monthly sample sizes vary with June, July and August being particularly small samples. Females from previously undisturbed areas (Table 5.2A) exhibited the greatest range in weights throughout the year, with individuals ranging from 4.85 kg in April to 14.30kg in December. Badgers in areas culled two or more times were heavier in January than those in previously undisturbed areas (Fig. 5.1), (t-test: $t = 37.164$; $df = 31$; $p < 0.001$). There was little difference found in body weight between the different levels of disturbance from February to August. However, the opposite trend was seen in September when

undisturbed females were significantly heavier than their disturbed counterparts (t-test: $t = 15.05$; $df = 31$; $p = 0.031$).

Table 5.2 Descriptive statistics for average monthly weight (kg) of female badgers.

Month	Females from previously undisturbed areas				Females from repeatedly disturbed areas.			
	N	Mean $\pm$ SD	Min	Max	N	Mean $\pm$ SD	Min	Max
Jan	10	9.85 $\pm$ 1.34	7.40	11.70	22	11.80 $\pm$ 1.82	10.70	13.90
Feb	15	9.03 $\pm$ 0.81	8.10	9.50	21	9.40 $\pm$ 1.55	7.50	12.30
Mar	16	7.34 $\pm$ 1.28	4.90	8.80	19	7.87 $\pm$ 0.90	6.00	9.60
Apr	35	7.87 $\pm$ 0.95	4.85	9.50	26	8.04 $\pm$ 0.97	5.90	9.80
May	38	8.19 $\pm$ 1.35	6.10	12.50	18	8.09 $\pm$ 1.42	6.40	12.50
June	8	7.73 $\pm$ 1.08	6.00	9.30	6	7.72 $\pm$ 0.57	7.20	8.70
July	13	8.35 $\pm$ 0.83	6.80	9.50	7	7.53 $\pm$ 2.00	5.50	9.50
Aug	4	7.50 $\pm$ 1.16	6.70	9.20	6	7.75 $\pm$ 1.65	6.70	10.20
Sept	22	9.30 $\pm$ 1.61	7.60	10.80	11	8.62 $\pm$ 1.17	6.70	9.80
Nov	19	10.86 $\pm$ 1.66	7.90	12.80	13	9.74 $\pm$ 1.26	7.40	11.70
Dec	21	12.60 $\pm$ 1.75	10.80	14.30	14	11.68 $\pm$ 0.76	11.00	12.90
Total	201	8.49 $\pm$ 1.61	4.85	14.30	163	8.59 $\pm$ 1.57	5.50	13.90

Overall, there was a seasonal variation seen in body weight. The body weight of female badgers changed significantly between months in both previously undisturbed areas (dark bars) and repeatedly culled areas (light bars) (Fig. 5.1) (ANOVA 1st cull: $F = 11.091$, $df = 10$, $p < 0.001$; 2nd+ cull: $F = 9.409$, $df = 10$, $p < 0.001$). In areas undergoing a first cull, females were significantly heavier in January compared to those culled in March, April, May and June but significantly lighter than females in December (Bonferonni post hoc test: January vs March-June, $p = 0.019$; January vs December, $p = 0.033$). Females culled in March, April, May and June were also significantly lighter than those culled in November and December (Bonferonni post hoc test: March vs November, December, $p < 0.001$; April vs November, December, $p < 0.001$; May vs November, December, $p < 0.001$; June vs November, December, $p < 0.001$).



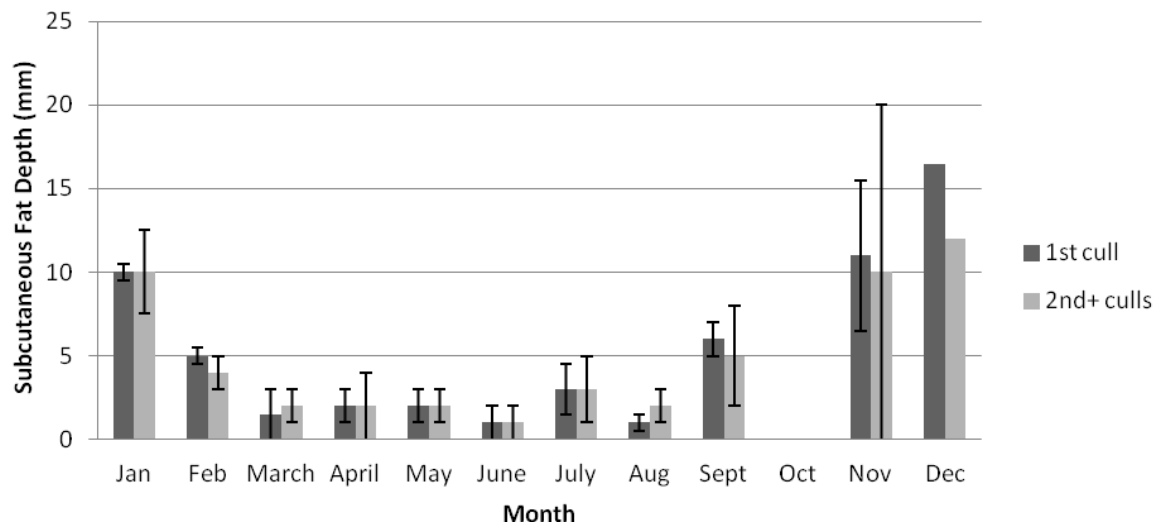
N 1st cull = 10 15 16 35 38 8 13 4 22 0 19 21
 N 2+ cull = 22 21 19 26 18 6 7 6 11 0 13 14

Figure 5.1 The average monthly weight (kg) of female badgers from previously undisturbed areas and multi-cull areas. Error bars display the standard error.

Females culled in winter months had a higher body weight compared to those found throughout the rest of the year. Female badgers in January were heavier than those in March through to November (Bonferonni post hoc test: January vs March-November, $p = 0.044$). Females in February were also heavier than those in March and April (Bonferonni post hoc test: February vs March, April, $p = 0.035$). Females culled during December were heavier than those caught in all other months with the exception of January (Bonferonni post hoc test: December vs February-November, $p = 0.0374$).

The body condition index of culled females was analysed by calculating the $\log(\text{weight}/\text{body length})$ of the animals (Tuytens *et al.*, 2002). Overall body condition of female badgers remained constant throughout different culling events (P values for simple t-tests after Holm correction for multiple tests: Cull1 vs Cull 2 = 1.000; Cull1 vs Cull 3 = 0.3834; Cull1 vs Cull 4 = 0.2726; Cull 2 vs Cull 3 = 1.000; Cull2 vs Cull 4 = 1.000; Cull 3 vs Cull 4 = 1.000).

There was no difference found in either subcutaneous fat depth (SFD) or kidney fat indices (KFI) between previously undisturbed and repeatedly culled areas (SFD - Mann-Whitney U = 3023.5; df = 1; p = 0.382; KFI - Mann-Whitney U = 3048; df = 1; p = 0.436). However, there is seasonal variation as both SFD and KFI showed a similar pattern of monthly change, which reflected the weight changes found in Fig.5.1. The highest level of both SFD (Fig. 5.2) and KFI (Fig. 5.3) were found in late autumn and winter, while late spring and summer recorded the lowest levels. There was a significant variation seen in both subcutaneous fat depth and kidney fat indices by month in both first and multiple cull areas (Kruskall Wallis, SFD : first cull, $\chi^2 = 56.028$; df = 10; p < 0.001; repeatedly culled, $\chi^2 = 35.589$; df = 10; p < 0.001; Kruskal Wallis, KFI : first cull, $\chi^2 = 51.86$; df = 10; p < 0.001; repeatedly culled, $\chi^2 = 41.326$; df = 10; p < 0.001).



1st cull N= 10 15 16 35 38 8 13 4 22 0 19 21

2nd+ cull N=22 21 19 26 18 6 7 6 11 0 13 14

Figure 5.2 The monthly median Subcutaneous Fat Dept (SFD) in female badgers. Error bars display the inter-quartile ranges.

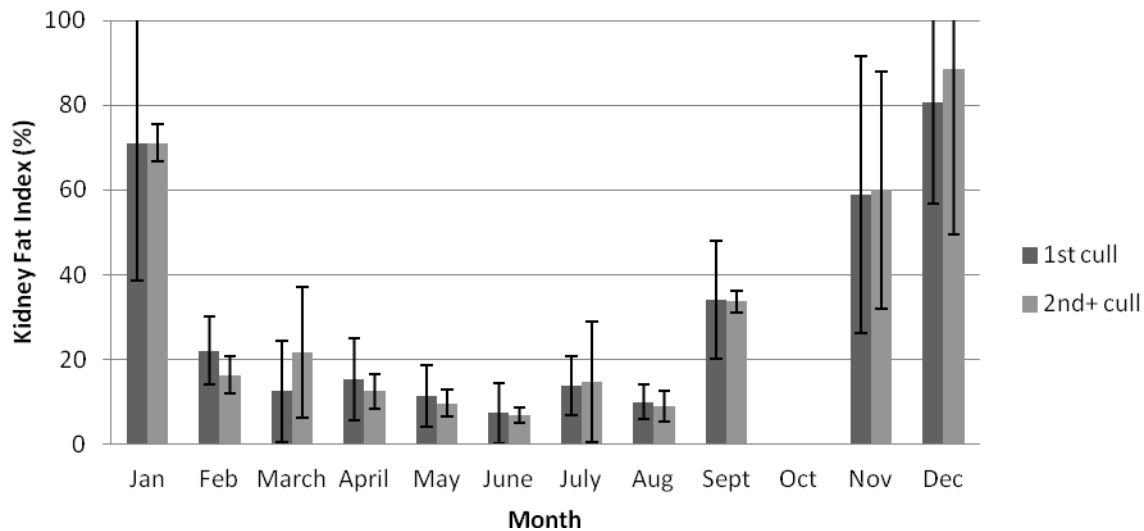
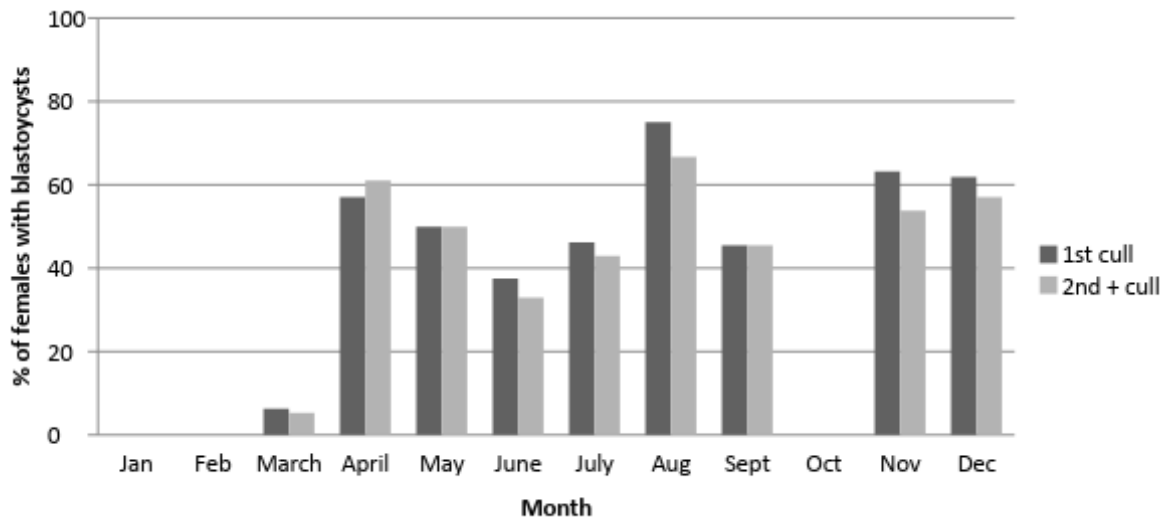


Figure 5.3 The monthly median Kidney Fat Indices (KFI) in female badgers. Error bars display the inter-quartile ranges.

5.3.2 Fecundity

Blastocysts were first found in March in just two females, one from undisturbed areas (6.25% of the badgers sampled) and one from previously culled areas (5.3% of the badgers) (Fig. 5.4). After this, there was a large increase in the proportion of females found to have blasotcysts present in the uterus. There was no significant difference between the females from repeatedly culled areas and females from previously undisturbed areas (Mann-Whitney $U = 26.5$; $df = 1$; $p = 0.215$). However, blastocyst numbers varied significantly from month to month (Kruskall Wallis: $\chi^2 = 38.477$; $df = 2$; $p = 0.0283$).



Total N 1 st cull =	16	35	38	8	13	4	22	0	19	21
Total N 2 nd + cull =	19	26	18	6	7	6	11	0	13	14

Figure 5.4 Percentage of females in 2007 – 2008 with blastocysts present in uteri.

While the proportion of females found to have blastocysts did not vary significantly with disturbance, females in repeatedly culled areas were found to have significantly lower overall numbers of blastocysts than those from previously undisturbed areas (t-test: $t = 2.223$, $df = 294$, $p = 0.027$). Females in repeatedly disturbed areas were found to have on average 1.97 blastocysts while those in areas undergoing culling for the first time had an average of 2.83 blastocysts.

During post mortem examination female badgers were assessed for the presence of placental scars. Between 61.5 and – 71.4% of females were found to have at least one placental scar, but this was not affected by culling disturbance (Fig. 5.5).

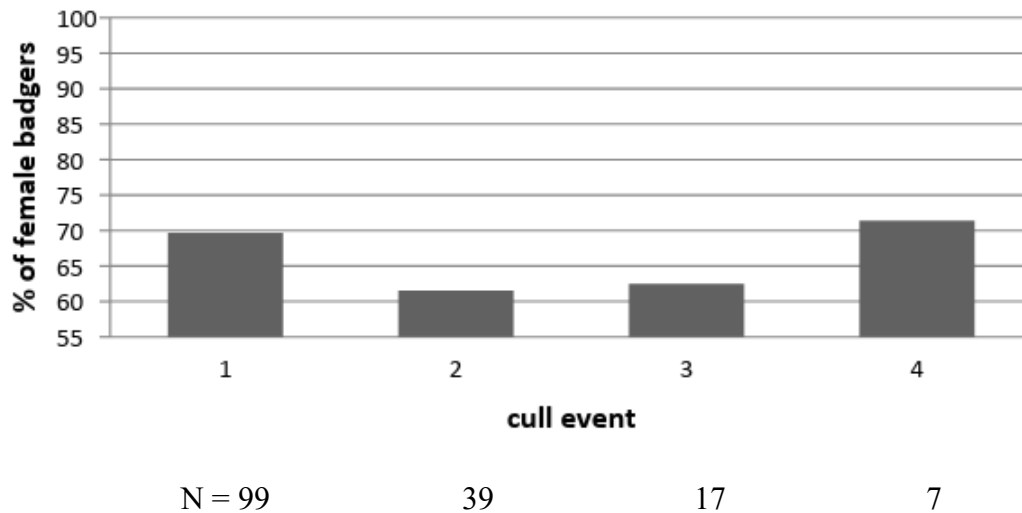


Figure 5.5 The percentage of females found with at least one placental scar over the different cull events.

While the percentage of females breeding appeared to remain the same despite repeated culling disturbance, the potential maximum litter size as assessed by placental scar presence showed some variation after different culling events (Fig. 5.6). Females from previously undisturbed areas (1st cull) were most likely to have 2 - 3 placental scars and were the only females to have evidence of a 4th implantation occurring. For these females, the average maximum litter size was 2.27 cubs. Females from an area in its second cull were also found to have 2 - 3 placental scars, although this time, females were less likely to have a single scar, and almost twice as likely to have evidence of 2 placental scars as 3. In these areas the average maximum litter size was 2.21 cubs. Females from a thrice-culled area much more rarely had evidence of 3 implantations. Instead they were more likely to have 1 - 2 placental scars and their average maximum litter size was lower, at 1.8 cubs. The biggest difference in placental scars was seen in females from setts that had undergone a 4th or more cull. Females in these areas only had evidence of 1 - 2 implantations, with one placental scar being the most common, which resulted in an average maximum litter size of 1.4 cubs. The maximum potential litter sizes from females in these areas were significantly smaller than those found in the first and second culls (ANOVA: $F = 2.779$, $df = 3$, $p = 0.045$; Bonferroni post hoc: 1st cull vs 4th + cull $p = 0.017$, 2nd cull vs 4th + cull $p = 0.029$).

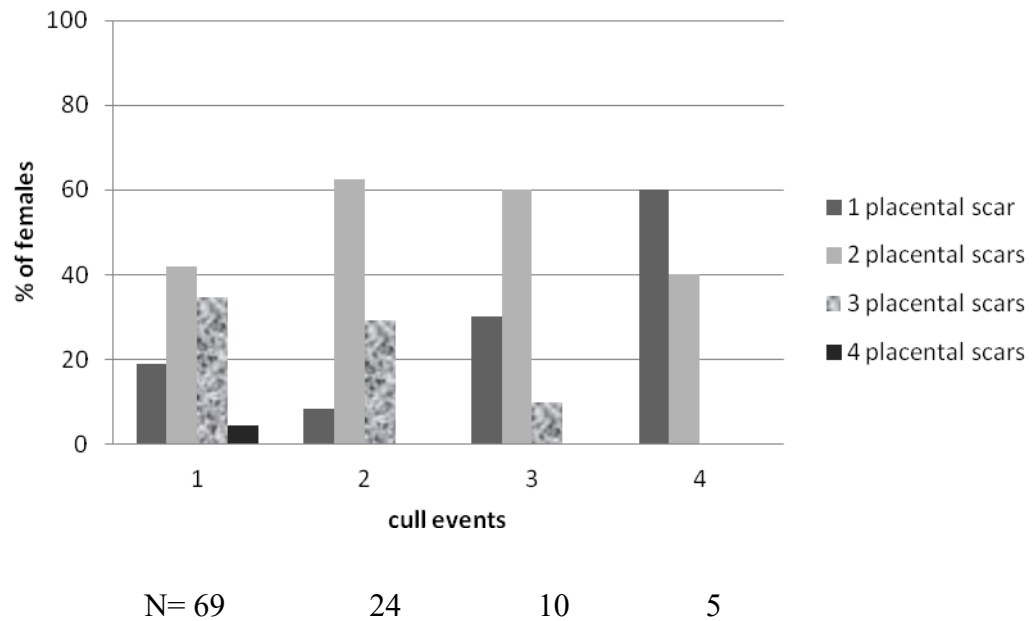


Figure 5.6 The potential litter size assessed using the presence of placental scars in female badgers over different culling events.

Of the 25 females that were found to be gravid during post mortem examination, there were 70 signs of implantation, with 35 foetuses being developed enough to sex accurately (Fig. 5.7). Areas undergoing a first cull had a sex ratio of 53.8% male vs 46.2% female foetuses, while areas repeatedly culled had a sex ratio of 44.4% male vs 55.6% female foetuses. There was no significant difference in the sex ratio of foetuses between 1st and subsequent culling event (Fishers Exact test: $p = 0.7112$).

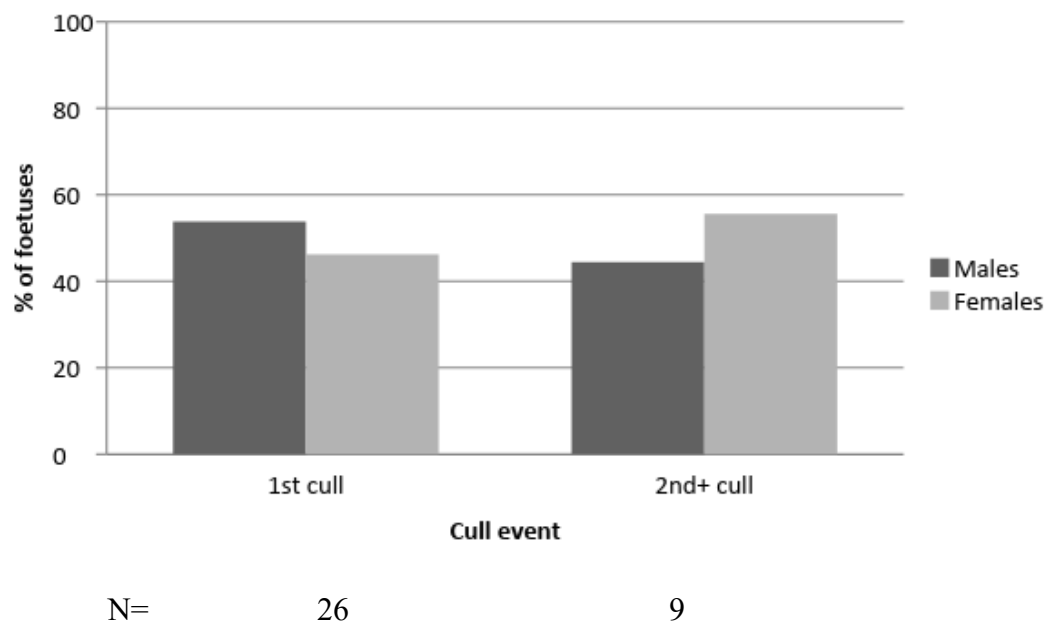


Figure 5.7 The sex ratio of fetuses from different cull events.

5.4 Discussion

This chapter gave the opportunity to explore physiological and behavioural changes happening within a repeatedly disturbed subset of the Irish badger population. Findings from this chapter will also have a wider impact, as it is possible that populations of other species which are repeatedly culled, may show similar responses to large scale/chronic disturbances. If this is the case, these effects may need to be incorporated into models for population recovery, as culling is only deemed to be a medium term solution to the bTB problem.

5.4.1 *Weight and body condition*

Females from repeatedly culled areas were significantly heavier in January than their undisturbed counterparts but this pattern reversed in September. It is possible that females in repeatedly disturbed areas were advantaged by the reduction in local animal abundance after culling (Corner *et al.*, 2008; Byrne *et al.*, 2013b) and finding better feeding opportunities and thus were on average, slightly heavier than undisturbed females in the first half of the year. Why this is only evident in the first half of the year is unclear. These findings do correlate with Whelan's Irish study (1998) that found females in previously culled areas had higher body weight compared to females from areas undergoing a first cull.

The finding in September of significantly lighter weight badgers in disturbed setts does suggest that there may be some negative implications if badgers are going into winter lethargy with a lower bodyweight than their undisturbed counterparts. As previously mentioned, female badgers with poor body condition during winter months have lower reproductive success (Woodroffe, 1995, Whelan, 1998). Therefore, the reduced body condition of females in repeatedly disturbed areas may negatively impact the following year's reproductive success, either through lack of implantation or lack of adequate body condition for successful lactation. This may be especially serious for those living in harsher climates, like badgers in Norway or Poland, who may undergo periods of inactivity for up to 6 months of the year (Kowalczyk *et al.*, 2003a). If similar responses to culling disturbance are seen in species that undergo true hibernations or extensive winter lethargy, then this may not only impact the animal's reproductive success but also their over-winter survival.

Body condition scores (SFD and KFI) had parallel patterns of wide seasonal variation, with the lowest weight and fat indices being found between March and August, gradually increasing until the peak months of December and January. As these patterns were observed in female badgers from both previously undisturbed areas and repeatedly culled areas, culling was not found to affect body condition. As body condition has been shown to decrease with increasing group size (MacDonald *et al.*, 2002) it was expected that as local badger density decreased, body condition of the repopulating animals would increase. However, female badgers had the same average annual body condition scores across all culling events. This agrees with other studies which have shown that population density has no significant effect on body condition (Cresswell *et al.*, 1992; Stuart, 2010). While seasonal mean body condition did not differ between previously undisturbed and repeatedly culled areas, badgers repopulating culled areas did not appear to gain any advantage in body condition from an extra availability of resources in culled areas which might suggest that they were already in good condition before coming into the disturbed areas.

There was no evidence of bite wounding found in any of the female badgers during post mortem examinations. This agreed strongly with data from the previous chapter, which found that bite wounding was rare and disappeared over the years of the cull. Similarly low levels of bite wounding were found by Stuart (2010), who reported only 0.5% of culled Irish badgers had evidence of bite wounding. In contrast, Murphy *et al.*, (2009) found a somewhat higher level of 4.2% of Irish badgers with evidence of bite wounds. In either case it is clear that bite wounding is not a common aggressive behaviour within the Irish badger population and did not increase during repeated culling events. This study found that badgers repopulating vacant areas did so with much lower levels of aggression than has been seen in parts of Britain, where levels of bite wounding have been found at higher frequencies in both low/medium density populations (4-10% of badgers with bite wounds) and high density populations (20-70% of badgers with bite wounds) (Cresswell *et al.*, 1992; Delahey *et al.*, 2006b). Areas undergoing repopulation in Oxfordshire also had relatively high levels of bite wounding, with 17.7% of females and 24.9% of males found to have fresh bite wounds during a period of population recovery and growth (MacDonald *et al.*, 2004).

This variation between populations at different densities in bite wounding frequency is interesting, especially when considered in conjunction with the effects of culling. As culling reduces the population density, it should also reduce bite-wounding frequency, as seen in

our results. However, it is also a disturbance to the population, which has been shown to increase bite wounding (MacDonald *et al.*, 2004). If Irish badgers are inherently less aggressive than British ones, which is possible given the low level of bite wounding found in undisturbed Irish populations (Murphy *et al.*, 2009; Stuart, 2010) then it would be unwise to assume that in England, repeated culling would have the same effect as it does here, i.e. reducing bite wounding to zero. It is possible that the influence of disturbance may outweigh the influence of density reduction, and bite wounding might even increase in response to culling. This is important because of the clear link between bite wounding and bTB transmission as reported in Chapter 4.

5.4.2 Fecundity

Blastocysts were first observed in March, albeit at very low frequencies. The proportion of females found to be carrying blastocysts remained relatively constant throughout the summer months. The percentage and frequency of blastocysts detected agrees with previous studies, which show that the majority of fertilization occurs very quickly post-partum from March onwards, with a smaller, second mating period later in the year (Ahlund, 1980; Page *et al.*, 1994; Whelan, 1998; Stuart, 2010; Corner *et al.*, 2015). Although the proportion of females found to have blastocysts in repeatedly culled areas was often smaller than that found in previously undisturbed areas, it was within the limits of natural variation. However, there was a significant reduction in the mean number of blastocysts in areas that had undergone repeat culling. This suggests that there may be some factor affecting the females in those areas, causing them to produce fewer blastocysts.

It was possible to estimate the number of blastocysts implanted during a single breeding season by counting the number of placental scars, and thus the maximum potential litter size if all blastocysts were to grow to completion. Placental scars were clearly visible during the first 9 months of the year and began to fade after mid-September. It was therefore assumed that any placental scars found were from the present year and not residual from a previous breeding season (Whelan, 1998). The presence of placental scars showed that at least 62% of females from all culls were found to have evidence of implantation. This agreed closely with previous studies on reproduction rates of badgers in Ireland, where upwards of 60% were found to have been carrying blastocysts or have signs of pregnancy (67-75% - Whelan, 1998; 60-70% - Stuart, 2010; Corner *et al.*, 2015).

When the numbers of placental scars, rather than their presence/absence was considered, a clear difference between culling events became evident. Females from undisturbed setts had the potential to have the largest litters, with an average maximum litter size of 2.27 cubs. These females were the only ones to show evidence of four placental scars, although it must be noted that the sample size for this was very small. The maximum potential litter size continued to decrease after each subsequent cull, until during the 4th or more cull average potential litter size had dropped to 1.4 cubs. This was found to be a significant reduction in maximum potential litter size. Litter size in undisturbed areas was similar to that previously found in Ireland (2.9 cubs - Whelan, 1998; 2.47 cubs - Stuart, 2010; Corner *et al.*, 2015) as well as in Britain and across Europe (Anderson and Trewhalla, 1985; Kruuk and Parish, 1987; Page *et al.*, 1994; Dugdale *et al.*, 2003). The smaller litter size of 1.4 cubs per breeding female was much lower than the expected average and similar to that found in high density populations in Britain, where it is thought that competition and breeding suppression between females may limit final litter size (Woodroffe and MacDonald, 1995b; Dugdale *et al.*, 2007). The low litter size found in disturbed areas is unlikely to be due to breeding suppression as no evidence of this behaviour has been found in any study of Irish badgers (Whelan, 1998; Corner *et al.*, 2015). Furthermore, culling has been shown to significantly reduce badger abundance (Corner *et al.*, 2008; Byrne *et al.*, 2013b) which in theory should open up breeding opportunities for individuals in those areas.

The finding that females in repeatedly culled areas had significantly fewer blastocysts and consistently smaller litter sizes is difficult to explain in the absence of breeding suppression. A previous Irish study noted that once blastocysts were maintained the majority of females went on to complete the breeding cycle (Stuart, 2010), but in that study the level of disturbance was not incorporated into the analysis. It is possible that interactions between animals in previously culled areas may increase stress hormones, leading to a reduction in fecundity in resident females.

When the sex ratio of cubs born in 2007 and 2008 was examined, no significant difference was found between years or between previously undisturbed areas and repeatedly culled areas. This is similar to a study in Wytham Woods, UK, that found no overall bias in sex ratios of cubs born in a disturbed population (Dugdale *et al.*, 2003).

5.5 Conclusion

It appears that surviving badgers are physiologically able to withstand the disturbance associated with repeated culling. Overall the body condition and body weight of females didn't change across different culling events, with similar seasonal patterns seen in SFD and KFI scores. There was no evidence of bite wounding behaviour found on any of the females examined as part of this study, suggesting that this aggressive behaviour did not increase with social group disruption.

While the proportion of females found to be carrying blastocysts in both areas were similar, blastocysts numbers were found to be significantly lower in females from repeatedly culled areas. As the majority of reproductive failures occurred after fertilization had taken place, it appears that the ability of females to find mates and become fertilised was not the cause of the low reproductive success. Instead there appeared to be a loss of foetuses in the population as a whole. As a result of these losses, females from repeatedly disturbed areas had smaller litter sizes than females from undisturbed areas.

Chapter 6: Methods to Predict Parturition Dates

Abstract

Given the insufficiencies of the current closed season in Ireland, three methods of predicting and determining parturition dates of Irish badgers were analysed in an effort to predict a more accurately timed closed season to ensure that non-lactating females are not culled before their young are still dependant on them.

Foetal measurements were a good estimation of parturition and gave a largely normal distribution of parturition dates. The peak of parturition fell between February 15th and 28th in all years. There is no consistent pattern, i.e. consistent between years, in mean female body weights which can be used to predict which sows will implant and therefore weight alone cannot be used as a method of identifying breeding status. Teat length, width, and mammary gland depth were found to be significantly greater in reproductively active females than reproductively inactive ones, with differences been seen from January through June. The difference in size of teats, seen as early as January i.e. before parturition, shows that this is an indicator of pregnancy and not just suckling young. This could potentially be a way to confirm pregnancy without the need of an ultrasound scan. As this difference in teat size is seen as early as January, this shows that this increase in teat size is not an effect of suckling young, but rather a physiological change caused by pregnancy which can be used to detect pregnancy before parturition. It appears to be a reliable way to detect pregnant females without ultrasound scan. Age of the female badger did not affect teat length or width, or the depth of mammary tissue.

Teat and mammary tissue measurements were found to be an easy and successful way of determining reproductive activity in female badgers. Teat length in particular was effective at determining not just lactation but also pregnancy as early as January and may be of use in future field trials. The findings of this study recommend that the closed season be extended

from its current 12 week period to ideally 20 weeks, running from early February until early June.

6.1 Introduction

The welfare issue of leaving dependant cubs vulnerable to starvation if lactating females are culled is a major concern when it comes to badger culling in Ireland. It is paramount that the closed season in Ireland happen at the most opportune time if the maximal number of cubs are to avoid inhumane deaths in targeted areas. Lactation has been reported to last for a minimum of 12 weeks and cubs are thought to be independent by 15 weeks, when the milk teeth have been replaced with permanent dentition (Neal and Cheesman, 1996). Therefore, it is likely that the current Irish closed season of January through March may need to be lengthened or shifted to later in the year to account for variation in parturition dates and the length of the lactation period. It is also possible that environmental factors at the time of implantation may also affect parturition dates and any such influence will need to be considered when determining a closed season. Pinpointing shifts in the parturition timeframe will ensure adequate time for cubs to wean before adult females are culled.

Badgers utilise delayed implantation as their reproductive strategy and therefore accurately predicting parturition and lactation periods can be problematic. Once fertilization takes place, the embryos go into a state of inactivity (Sandell, 1990). Reactivation of diapausing embryos occurs at a similar time in all females in a population (Sandell, 1990). In Britain, the main oestrus cycle of female badgers takes place early in the year, between February and April, with a second, smaller cycle later in the year, between September and October (Cresswell *et al.*, 1992; Page *et al.*, 1994; Stuart, 2010). Although there appears to be only two clearly defined periods of oestrus, data from south-west Britain have shown that copulations can occur any time between February and October (Neal and Harrison, 1958). Conversely in Ireland, Stuart (2010) found evidence of year-round matings in Ireland and noted that fertilization could occur throughout the year. Polygynandrous behaviour and repeated mounting have also been found to occur throughout the year (Dugdale *et al.*, 2011b). Furthermore, male testicular weight increases rapidly during the early months of the year, after which there is a gradual decline until the end of the year (Ahlund, 1980; Page *et al.*, 1994; Woodroffe *et al.*, 1997). As testicular weight can be used to indicate sexual activity

(Ahlund, 1980; Page *et al.*, 1994; Stuart, 2010), this suggests that males are capable of mating throughout much of the year.

Parturition dates can vary hugely depending on location. Both latitude and distance from the sea may also be an important factor in predicting implantation and parturition dates. As coastal areas stay cooler in summer and warmer in winter, distance from the ocean may affect the onset of spring, which may subsequently affect parturition. Ireland is considered to have a maritime temperate climate (Peel *et al.*, 2007), being at temperate latitudes, without a dry season and having warm summers. The relatively small size of Ireland and temperate climate means that animals experience less extreme temperatures across the year, which suggest that implantation and parturition dates are unlikely to be affected by extremes in temperature as they might be in other countries. In general, the more southerly and warmer the location, the earlier parturition takes place. Parturition in the Donana region of Spain has been estimated to occur during early January; in the south of France, in late January; in Britain, in early/mid-February; in Scotland, Germany and Sweden, in early March, and in Russia, in late March/early April (Sweden - Ahlund, 1980; France, Britain, Scotland, Germany and Russia - Neal and Cheesman, 1996; Spain - Revilla *et al.*, 1999). Altitude has also been found to influence parturition as female badgers in high alpine valleys in Switzerland gave birth later than those at similar latitude but lower elevation (Wandeler and Graf, 1982 cited in Woodroffe, 1995). With such a variation in parturition dates, it is clear that there are factors affecting implantation which may differ between locations.

Several factors influence when many mustelidae implant. Photoperiod was shown to affect gestation in spotted skunks (*Spilogale putorius latifrons*); Mead (1971) found that deliberately blinded females failed to implant despite equal exposure to daylight as non-blinded females. In the wild, badgers implant during the winter months. However, Canivenc and Bonnin (1979) found that captive female badgers could be compelled to implant in July if photoperiod was gradually decreased over a number of months, mimicking winter photoperiod conditions. This suggests that some aspects of implantation in mustelids are influenced by photoperiod reduction and it may be a key regulator of implantation in badgers.

Lunar cycles have also been explored to see if they influence the reproductive behaviour of badgers. Copulation events were found to peak during the lunar dark phase (Dixon *et al.*,

2006). Therefore, it has been suggested that lunar cycles can influence reproductive behaviour in badgers and could even act as a medium term regulator of such behaviour (Dixon *et al.*, 2006).

Previous studies have found that body condition influences implantation and parturition success. Female badgers that were heavier in autumn were more likely to successfully produce cubs the following spring (Whelan and Hayden, 1993; Woodroffe and MacDonald, 1995a; Whelan, 1998), while those in poorer health with less body mass were more likely to fail to produce blastocysts altogether (Cresswell *et al.*, 1992). Body condition has also been found to influence implantation and parturition dates. Using ultrasound scanning, a study in Britain found that females in poorer body condition towards the end of the year had smaller fetuses compared to females in better condition (Woodroffe, 1995). This suggests that females in poorer condition had implanted later compared to those in better condition and may imply that body condition during the latter part of the year, November-December, can regulate implantation and parturition dates on a short-term scale (Woodroffe, 1995). It follows therefore that there may be a minimum weight threshold that needs to be achieved by females to successfully implant (D. Kelly, *pers comm*). As food availability is low during winter months, when gestation occurs, females have to rely on their fat stores to provide adequate nutrients (Woodroffe, 1995; Neal and Cheeseman, 1996). Therefore, it is possible that female badgers will not implant successfully unless they have gained enough weight to maintain themselves and the developing embryos during times of insufficient food availability through the winter.

This idea of a minimum weight necessary for breeding is supported by findings in other animals. A minimum weight threshold is necessary if females in some primate species are to begin reproductive cycling. Evidence for this has been found in both Japanese macaques (*Macaca fuscata*) (Mori, 1979) and in olive baboons (*Papio anubi*) (Bercovitch, 1987). Studies have also found that in years of lower body condition index, and thus a lower body weight, African fur seals (*Arctocephalus pusillus*) showed decreased implantation success (Guinet *et al.*, 1998). The fecundity and reproductive success of female caribou (*Rangifer tarandus*) has also been found to be influenced by weight and condition. Small changes in weight distribution significantly affected parturition rates, with calving date and offspring survival being linked to summer body mass (Cameron *et al.*, 1994). It is possible that as obligate implanters, badgers may also be influenced by body weight during the late autumn

months. As the mechanisms by which diapausing blastocysts are reactivated are still unclear it is possible that a minimum weight threshold must be reached by females if successful gestation and lactation are to occur. Therefore, female body weight in December may be a means by which implantation dates can be predicted. This study will assess the weights of breeding and reproductively inactive females from November through to the following March to investigate whether there is a link between body weight and implantation dates.

Given that there are so many variables which may affect implantation and thus parturition dates, it is unsurprising that there have been numerous different methods used to try to predict the seasonality of badger reproduction. Comparing the crown-rump length of the scanned foetuses to known foetus growth rates resulted in implantation dates being successfully estimated in other studies (Huggett and Widdas, 1951; Whelan, 1998; Stuart, 2010). By measuring the average length of the foetus for each litter, it was possible to estimate the age of the foetus and subsequently, calculate both an approximate implantation and parturition date for each breeding female (Whelan, 1998; Stuart, 2010). My study used a combination of post mortem data from 1998 onwards to predict annual trends in badger pregnancy dates. In addition to this, more recent data from foetuses which I examined in 2006 - 2008 were used to predict both implantation and parturition dates for the breeding periods of those years.

Another tool for assessing fecundity and reproduction in mammals is teat length measurements. There have been numerous studies that have found a link between pregnancy and elongated teat length, including in small mammals (McCravy and Rose, 1992), badgers (Cresswell *et al.*, 1992; Dugdale *et al.*, 2011a), fishers (Frost *et al.*, 1999) and in fur seals (McKenzie *et al.*, 2007). Teat length has also been utilised to assess pregnancy in female badgers in conjunction with other methods such as ultrasound scanning (Woodroffe, 1995; Woodroffe and MacDonald, 1995b; MacDonald and Newman, 2002). These studies have found that females can be identified as having bred in the current breeding year by comparing the length of teats. Successful breeders had significantly longer teats compared to non-breeders (Frost *et al.*, 1999; Mech *et al.*, 1993; McKenzie *et al.*, 2007; Dugdale *et al.*, 2011a). This method was used here to identify reproductively active and lactating females during 2007 and 2008. In addition, teat width and the depth of mammary gland tissue were also used to determine whether they too can be used as better indicators of pregnancy and lactation in culled females.

Therefore, the aim of this chapter is to predict the annual parturition patterns of Irish badgers and to determine which, if any, method of pregnancy detection provides an accurate way to date annual parturition trends. Using a selection of data from 1998 through to 2008, we carried out the following investigations:

- Estimating cub birth dates from foetal measurements. Parturition in badgers varies by location, with more southerly populations giving birth earlier than more northerly ones (Neal and Cheesman, 1996). Timing may be affected by body condition and weight (Whelan and Hayden, 1993; Woodroffe and MacDonald, 1995a; Whelan, 1999). Previous research suggests that the cubbing season occurs in early February and march in Ireland and Britain (Stuart, 2010).
- Predicting parturition dates using female body weight. Previous studies have shown that in some species there is a minimum weight needed for reproductive cycling to occur (primates - Mori, 1979; Bercovitch, 1987, seals - Guinet *et al.*, 1998). Therefore, it might also be a factor in badger reproduction as previous studies have found that female badgers that were heavier in autumn were more likely to successfully produce cubs the following spring (Whelan and Hayden, 1993; Woodroffe and MacDonald, 1995a; Whelan, 1998), while those in poorer health with less body mass were more likely to fail to produce blastocysts altogether (Cresswell *et al.*, 1992).
- Detecting pregnancy and lactation periods using teat and mammary gland measurements. There have been several studies that have established a relationship between pregnancy and elongated teat length in mammals (McCravy and Rose, 1992; Cresswell *et al.*, 1992; Dugdale *et al.*, 2011a). Longer and wider teats are often an indication of reproductive activity and so it may be possible to predict pregnancy status without the need for post mortem examination should the changes in teat measurements be detectable before birth occurs.

6.2 Methods

Female badgers used in this study were caught by DAFM and underwent post mortem examination in the IEC, under the direction of Dr. Ursula Fogarty. A more detailed explanation of the complete process can be found in Chapter 2. Female badgers were weighed prior to post mortem examination. The uteri were extracted and flushed for blastocysts. Then they were opened, examined and if present any embryos/foetuses were removed. Teats and the underlying mammary gland were also excised and later measured for length, width and depth. These measurements were analysed to explore their use for parturition prediction.

6.2.1 Estimating birth dates using foetal measurements

During 2006 - 2008, any foetuses found during post mortem examinations were removed and examined in greater detail. The foetuses were carefully removed from the embryonic sack and any placental tissue was discarded. They were then measured from crown to rump (mm), weighed (grams) and if possible, sexed. The average weight of all foetuses from each female was calculated. Implantation dates were calculated from foetal mass with the formula used by Whelan (1998) (see page 33 of Chapter 2). It was then possible to work forward 52 days (gestation length was described as 49 - 56 days by Neal and Harrison, 1958) to estimate the birth date of the foetuses.

6.2.2 Predicting implantation and parturition period by female weight.

As body condition has been found to influence implantation in female badgers (Woodroffe, 1995), the theory that females must meet a minimum threshold weight before implantation takes place was examined in this study. Additionally, the possibility that reproductively active and reproductively inactive females could be identified by weight difference later in the season was also investigated. The weight (kg) of female badgers was examined from November 2005 - March 2006 and November 2007 - March 2008. Females were deemed to have been breeders by the presence of blastocysts, evidence of implantation, presence of foetuses or fresh placental scars. Identifying the breeding status of the female badgers involved the removal and examination of the uteri, extraction and measurement of

blastocysts/foetuses. A more detailed description of the procedures used can be found in Chapter 2 of this thesis.

6.2.3 Detecting pregnancy and lactation by teat and mammary gland measurements

During post mortem examination, teat and mammary gland samples were taken from female badgers in 2007 and 2008. In total, 185 adult and aged adults were included in this study.

6.2.4 Analysis

The distributions and variances of all data were checked for normality. Two-sample comparisons were tested using t-tests, while multi-factorial comparisons were analysed using one-way or two-way ANOVAs. Bonferroni post hoc tests were used to identify any differences found. Descriptive statistics for categorical variables are presented as percentages. The threshold for statistical significance was set at $p = 0.05$.

6.3 Results

Female badgers were generally found to be gravid between the first week of January and mid - late February in a number of years, from 1998 - 2008, although the pattern changed on a yearly basis.

6.3.1 Estimating birth dates using foetal measurements

By using the average weight of all foetuses from each female it was possible to calculate implantation date. The predicted parturition dates in 2006 showed a tightly packed and normal distribution (Fig. 6.1). The majority of foetuses were calculated to be born between February 15th and 28th, with the median parturition point being around the third week of February. Although there was some suggestion of earlier gravid females in 2007 than in other years, and a slightly earlier peak in cub numbers, the parturition fell within the same time window as in 2006.

However, the predicted parturition dates in 2008 were more varied. Parturition was more spread out over time and no single peak in parturition numbers was observed. There was evidence of parturition occurring later in 2008 with cubs being predicted to be born up until mid-March. However, the median parturition date also fell towards the end of February, from the 21st - 28th, and was similar to that seen in 2006 and 2007 despite the longer parturition time period.

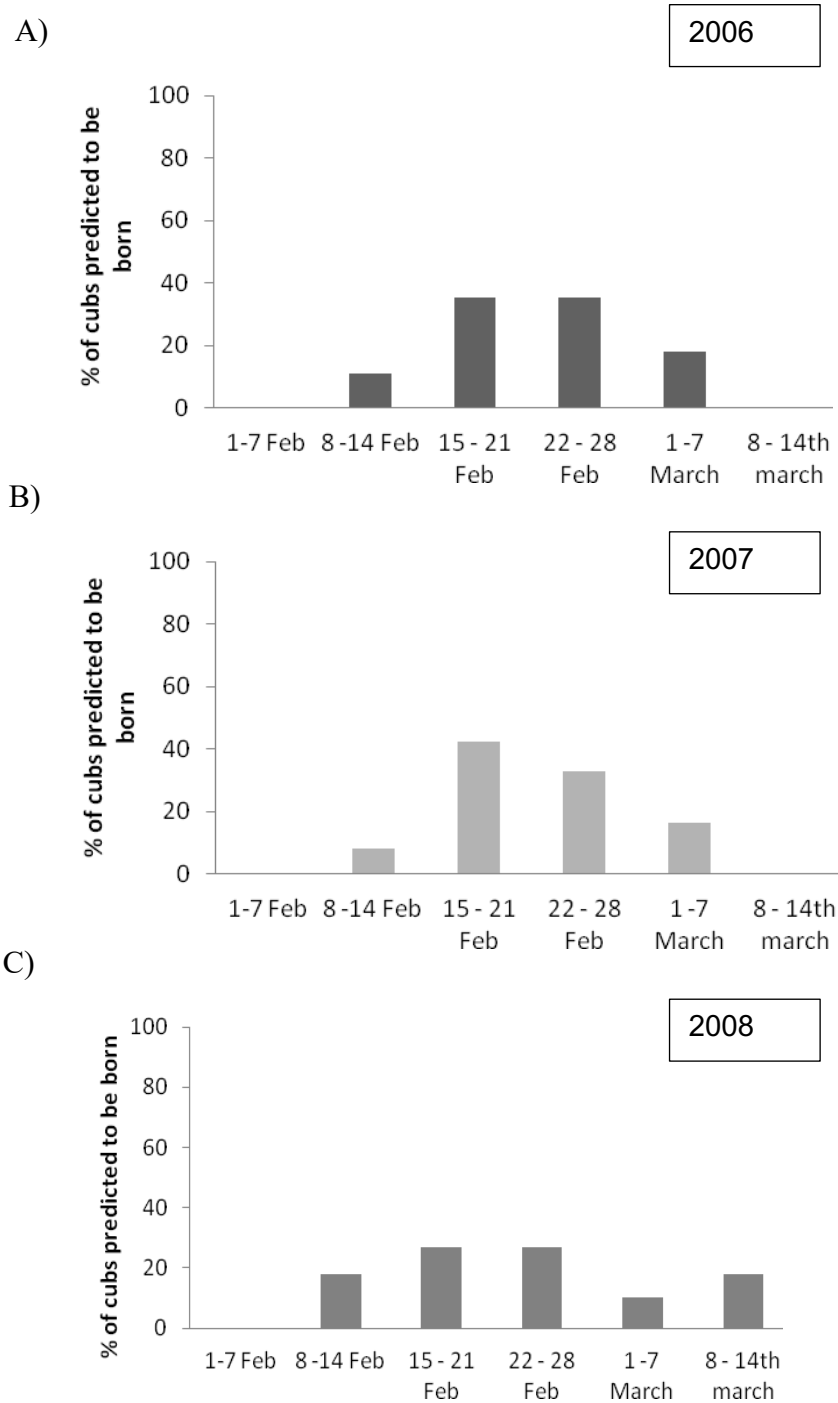
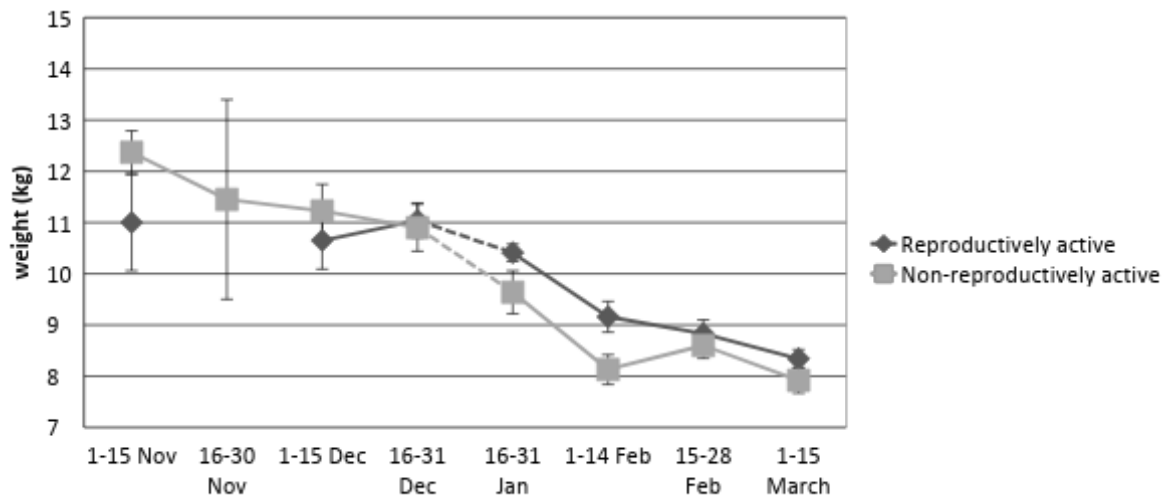


Figure 6.1 The predicted birth dates of foetuses in (A) 2006 (N=30), (B) 2007 (N=14) and (C)2008 (N=11).

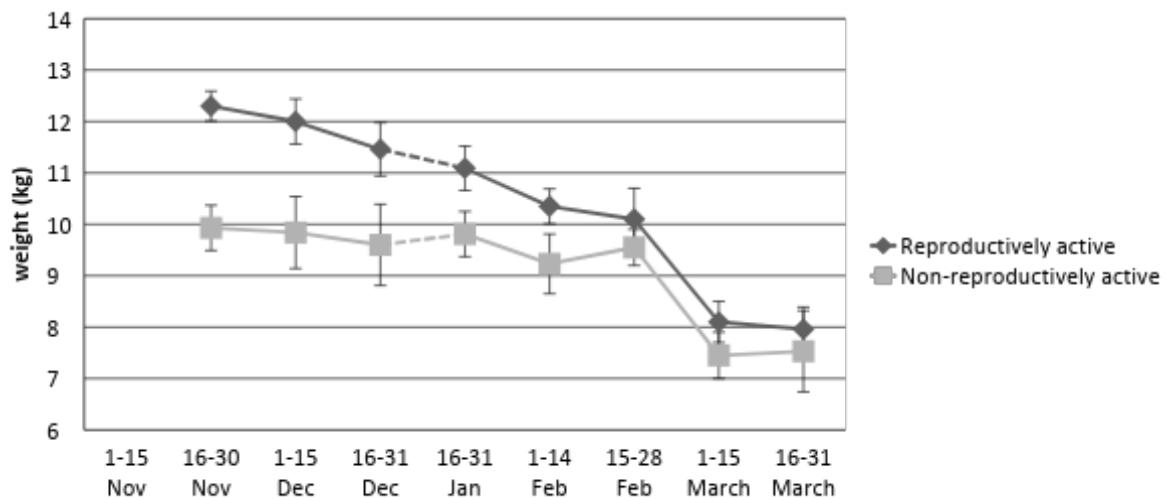
6.3.2 Predicting implantation and parturition period by weight of females in different geographical areas.

Previous studies have found that females in some species must meet a minimum threshold weight before implantation occurs. If this is true for badgers, there is a possibility that reproductively active and reproductively inactive females could be identified by weight differences leading up to and during the breeding season. In theory, if there is a minimum weight threshold which needs to be reached during the winter months before females successfully implant, identifying this threshold could make for a simple method with which to identify the females most likely to breed within a population.

Although sample size was very small, in the winter of 2005 reproductively inactive females were heavier than reproductively active females and remained so until late December when female body weight was approximately 11kg for all females (Fig. 6.2). This result is contrary to what would be expected if the theory of threshold weight was correct. Reproductively active females go on to become heavier than reproductively inactive from this date. From February 1st - 14th, reproductively active females were significantly heavier than other females (t-test: $t = 2.245$, $df = 23$, $p = 0.035$). Despite the consistently higher weights of reproductively active females from mid-February onwards, there was no overall significant difference found between the categories of females. In 2007/2008, reproductively active female badgers were significantly heavier than reproductively inactive females through most of the winter season (Fig. 6.3b) (November = t-test: $t = 3.933$, $df = 8$, $p = 0.004$; December = t-test: $t = 3.317$, $df = 19$, $p = 0.004$). Female body weight began to equalize in late January, and while females with evidence of recent pregnancy were heavier, they were not significantly so (Table 6.1). Thus, in the second year of data the weights were consistent with the theory that there might be a threshold weight for implantation but in the first year they were not.



A) Repro. active N = 3 0 5 6 14 11 7 15
 Repro. inactive N = 3 2 4 3 10 9 9 12



B) Repro. active N = 0 4 5 7 8 4 2 3 7
 Repro. inactive N = 0 6 5 4 7 3 2 2 3

Figure 6.2 The weights of reproductively active and reproductively inactive females during (A) late winter 2005/early spring 2006 and (B) late winter 2007/early spring 2008. Error bars display the standard error.

Table 6.1 Summary statistics for reproductively active and reproductively inactive female weights.

Year	Month	Sample number	Mean body weight $\pm$ SD
05/06	November	8	Reproductively active: 11.00 ± 1.67 kg Reproductively inactive: 12.00 ± 1.55 kg
	December	18	Reproductively active: 10.86 ± 1.14 kg Reproductively inactive: 11.06 ± 0.92 kg
	January	24	Reproductively active: 10.4 ± 0.73 kg Reproductively inactive: 9.64 ± 1.45 kg
	February	36	Reproductively active: 9.16 ± 1.2 kg Reproductively inactive: 8.13 ± 0.86 kg
	March	27	Reproductively active: 8.34 ± 0.73 kg Reproductively inactive: 7.91 ± 0.85 kg
07/08	November	10	Reproductively active: 12.3 ± 0.58 kg Reproductively inactive: 9.93 ± 1.08 kg
	December	21	Reproductively active: 11.68 ± 1.2 kg Reproductively inactive: 9.74 ± 1.47 kg
	January	15	Reproductively active: 11.08 ± 1.22 kg Reproductively inactive: 9.81 ± 1.16 kg
	February	11	Reproductively active: 10.26 ± 0.66 kg Reproductively inactive: 9.36 ± 0.77 kg
	March	15	Reproductively active: 8.00 ± 0.98 kg Reproductively inactive: 7.5 ± 1.01 kg

6.3.3. Detecting pregnancy and lactation by teat and mammary gland measurements

In 2007 and 2008 during post-mortem examination 185 female badgers had their teats and associated mammary glands excised during post mortem examination. Reproductively active female badgers had significantly longer teats compared to reproductively inactive females (t-test: $t = -10.077$, $df = 183$, $p < 0.001$) (Fig. 6.3). The average teat length in reproductively active females was $7.06\text{mm} \pm 3.32\text{mm}$ (S.D) while reproductively inactive female teats had an average length of $2.44\text{mm} \pm 2.64\text{mm}$ (S.D) (Table 6.2). Reproductively active females also had significantly wider teats (t-test: $t = -9.545$, $df = 183$, $p < 0.001$). The average teat width in reproductively active females was $5.22\text{mm} \pm 2.05\text{mm}$ (S.D) while

reproductively inactive female teats had an average width of $3.03\text{mm} \pm 1.06\text{mm}$ (S.D). Additionally, reproductively active females had a greater depth of mammary gland (t-test: $t = -7.829$, $df = 183$, $p < 0.001$). The average mammary gland depth in reproductively active females was $8.65\text{mm} \pm 4.95\text{mm}$ (S.D) while reproductively inactive female mammary glands had an average depth of $4.91\text{mm} \pm 1.48\text{mm}$ (S.D).

Table 6.2. The sample size, mean $\pm$ SD and range of mammary gland length, width and depth.

	N	Teat length(mm)		Teat Width (mm)		Mammary Tissue Depth (mm)	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Repro. inactive	56	2.44 ± 2.64	10.91	3.03 ± 1.06	5.04	4.91 ± 1.48	8.87
Repro. Active	129	7.06 ± 3.32	14.69	5.22 ± 2.05	10.17	8.65 ± 4.95	24.55

Reproductively active females had on average at least twice the teat length of reproductively inactive females. This may be a simple and cost-effective way to assess pregnancy during field work situations, although the success of this method may vary by month, with less differentiation in teat size seen in January.

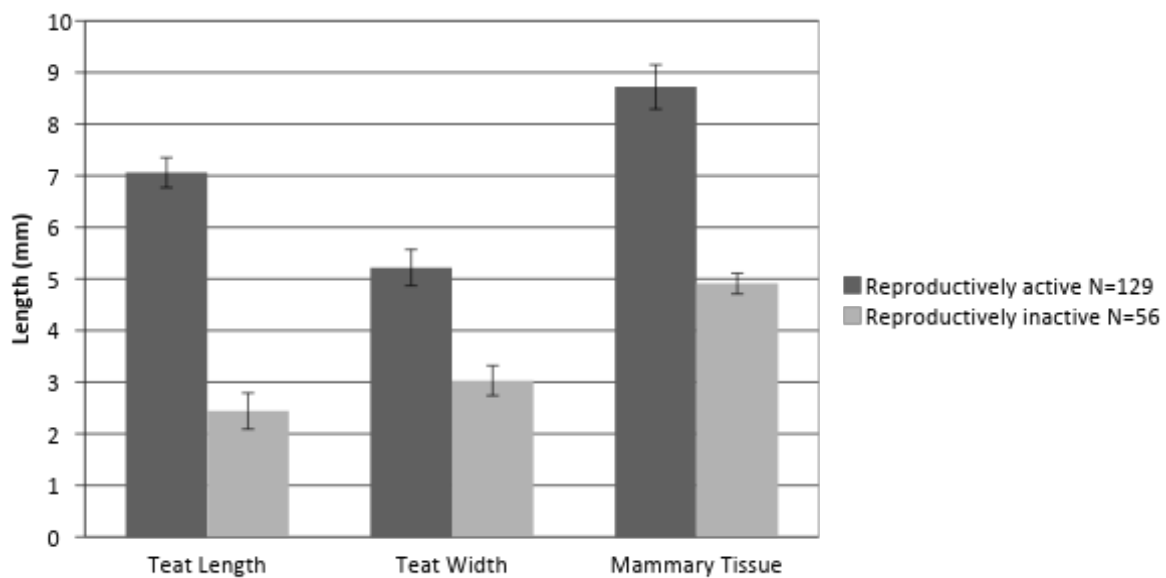
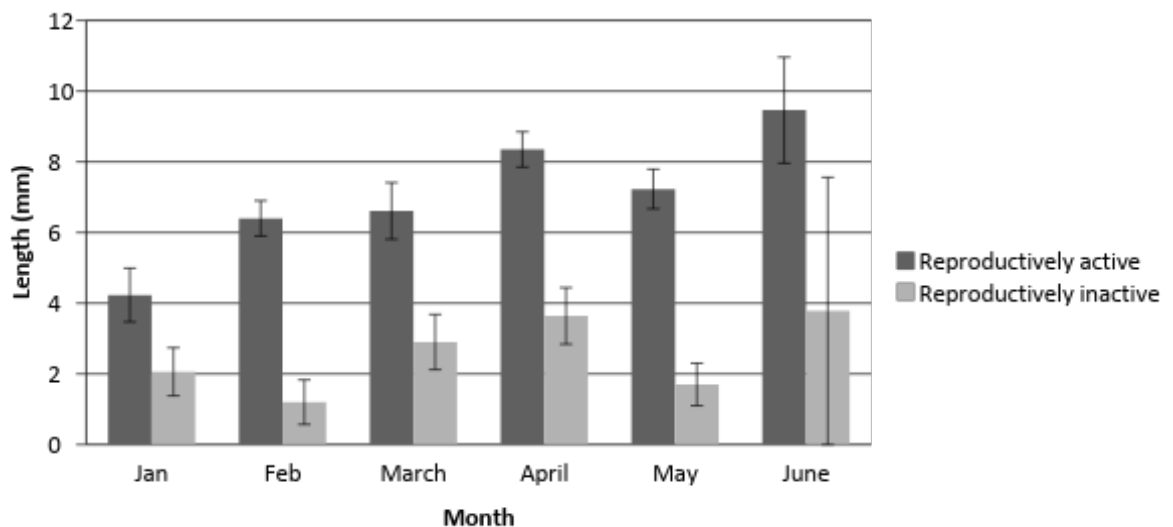


Figure 6.3 The average teat length, width and mammary tissue depth (mm) of reproductively active and reproductively inactive female badgers on 2007 and 2008. Error bars display the standard error.

Month and pregnancy status were found to significantly influence teat length, as reproductively active females had significantly longer teats in all months compared to reproductively inactive females, with the exception of June (Fig. 6.4) (two-way ANOVA: month: $F = 3.966$; $df = 5$; $p < 0.001$; pregnancy: $F = 58.202$; $df = 1$; $p < 0.001$). The effect of month as a single factor was only significant in the reproductively active females. Reproductively active females from April, May and June had significantly longer teats than those caught in January, suggesting that teat length increased during lactation (Bonferroni post hoc: January vs April, May and June $p = 0.016$). While this large increase in teat size, from 4mm to 9mm, was seen in reproductively active females, reproductively inactive females had no significant difference in teat size between the months (ANOVA: $F = 1.85$, $df = 5$, $p = 0.120$). As this difference in teat size is seen as early as January, this shows that this increase in teat size is not an effect of suckling young, but rather a physiological change caused by pregnancy which can be used to detect pregnancy before parturition. It may be a way to detect pregnant females without ultrasound scan.

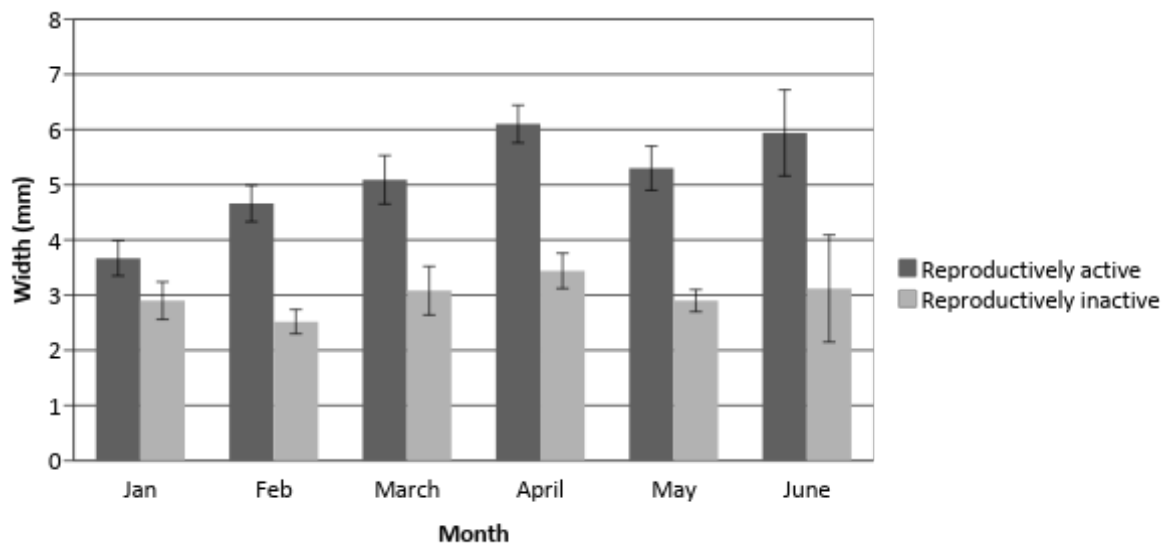


Repro. active N = 16	17	19	39	32	6
Repro. inactive N = 9	9	9	16	11	2

Figure 6.4 The average monthly teat length (mm) of reproductively active and reproductively inactive female badgers on 2007 and 2008. Error bars display the standard error.

Pregnancy status and month also significantly affected teat width in reproductively active and reproductively inactive females (Fig. 6.5), with reproductively active females having

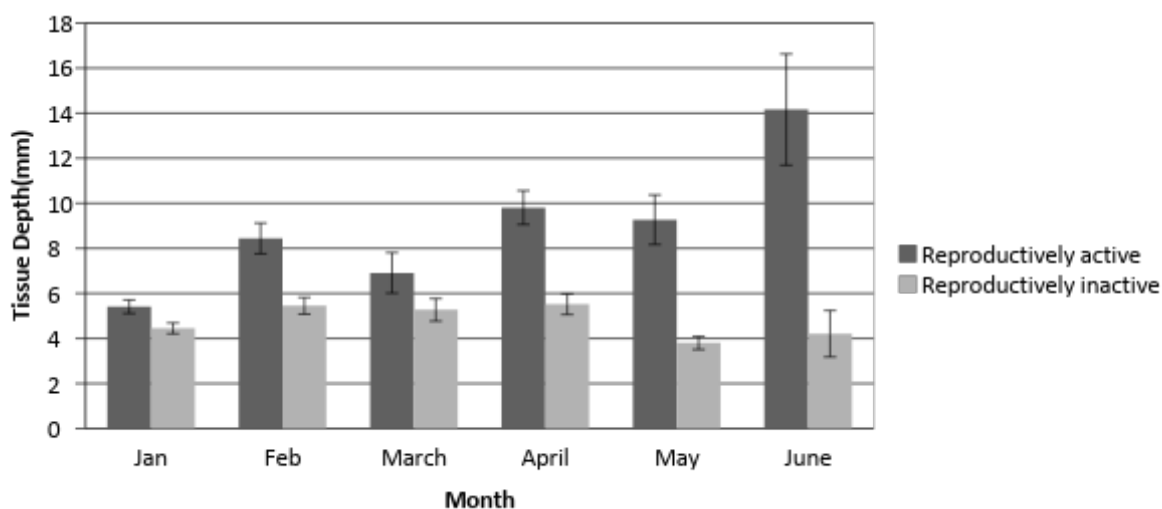
significantly wider teats in all months except January (two-way ANOVA: month: $F = 2.66$; $df = 5$; $p = 0.008$; pregnancy: $F = 39.944$; $df = 1$; $p < 0.001$). From February to May the teat width of reproductively active females grew significantly wider (Bonferroni post hoc: January vs February, March, April and May, $p = 0.038$) suggesting that teat width increased during pregnancy and lactation. The teats of reproductively inactive females remained the same from January, with very little fluctuation in width (ANOVA: $F = 0.95$, $df = 5$, $p = 0.457$).



Repro. active N = 16	17	19	39	32	6
Repro. inactive N = 9	9	9	16	11	2

Figure 6.5 The average monthly teat width (mm) of reproductively active and reproductively inactive female badgers on 2007 and 2008. Error bars display the standard error.

Monthly mammary tissue depth also varied across the year. Unsurprisingly, pregnancy status had a significant impact on mammary tissue depth, with reproductively active females having greater mammary depth than non-breeders (Fig. 6.6) (ANOVA: pregnancy: $F = 25.519$; $df = 1$; $p < 0.001$). Reproductively inactive females had only slight variation in mammary tissue depth across the six months. However, reproductively active females showed a marked increase in mammary tissue depth in June compared to the original levels assessed in January (t-test: $t = -2.625$, $df = 33$, $p < 0.027$), suggesting that mammary gland depths increase throughout pregnancy, parturition and lactation.



Repro. active N = 16	17	19	39	32	6
Repro. inactive N = 9	9	9	16	11	2

Figure 6.6 The average monthly mammary tissue depth (mm) of reproductively active and reproductively inactive female badgers on 2007 and 2008. Error bars display the standard error.

When reproductively active females were separated into different age categories, there was no significant difference found (Fig. 6.7). Yearlings had the most variation across all three categories, though this might have been caused by the much smaller sample size ($N = 13$) of this age group. When all three age groups were compared, there was no significant difference found in teat length, width or depth of mammary tissue (Teat Length = ANOVA: $F = 1.476$, $df = 2$, $p = 0.235$; Teat Width = ANOVA: $F = 0.411$, $df = 2$, $p = 0.664$; Mammary Tissue = ANOVA: $F = 1.058$, $df = 2$, $p = 0.353$). This suggests that age had no effect on teat length, width or mammary tissue. This implies that the age of the individual badger should not bias the results when these measurements are used to determine pregnancy or lactation.

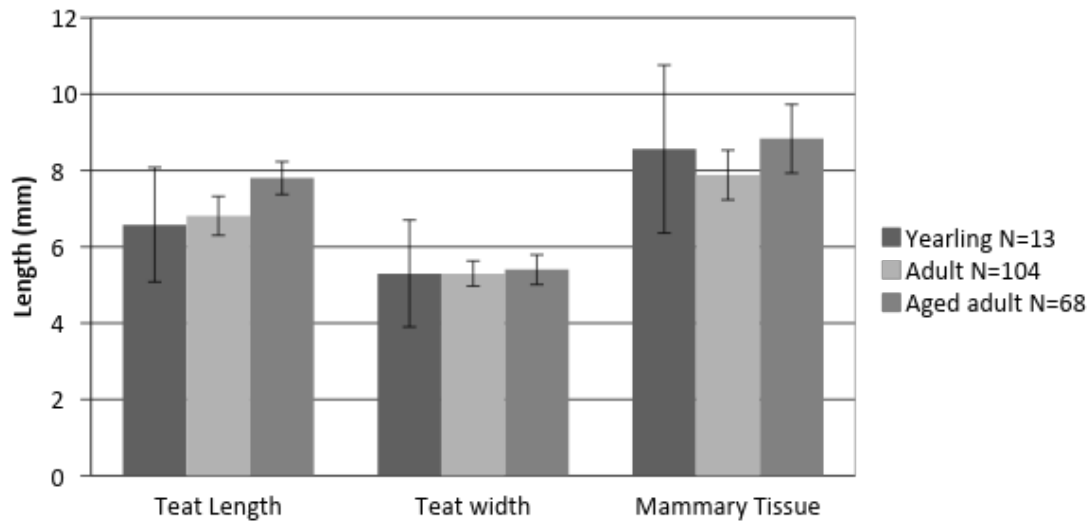


Figure 6.7 The average teat length (mm), width (mm) and mammary tissue depth in reproductively active yearling, adult and aged adult female badgers. Error bars display the standard error.

6.4 Discussion

Parturition dates of badgers are difficult to determine accurately as parturition takes place underground and, as in any species population, there are factors such as poor body condition, which can cause substantial individual variation in the timing of implantation (Roper, 2010). This study has investigated several ways in which pregnancy and implantation may be calculated or detected.

6.4.1 Estimating birth dates using foetal measurements

Foetal measurements have been used in several ways to predict parturition dates. In Britain, ultrasound scanning is a method used to measure the crown to rump length of the foetus (Woodroffe, 1995). This system has advantages as the females are anaesthetized during the procedure with no loss of life. In Ireland, foetal measurements taken during post mortem examination are the most common methods used to estimate parturition dates of the population (Whelan, 1998). This method predicted that parturition would take place over a four week period, February 8th - March 7th in 2006 and 2007, and over a 5 week period, February 8 to March 14th in 2008. This corresponds closely with findings in other Irish studies which have used similar methods (Whelan and Hayden, 1993; Whelan, 1998). Using foetal length as a predictor, the timeframe within which parturition occurs is approximately five weeks long from the second week of February onwards. It is also possible to determine earlier in the season when parturition is due to occur by using foetal length of culled females as an indicator for the rest of the population.

6.4.2 Predicting implantation and parturition period by female weight

Whelan (1998), working in Ireland, found that the mean body weight of female badgers varied significantly during September to December, with females with blastocysts being significantly heavier than those without. This correlated with studies in Britain which found that heavier females with better body condition were more likely successfully to produce blastocysts and cubs (Cresswell *et al.*, 1992; Woodroffe and MacDonald, 1995a; Delahay *et al.*, 2006a). However, the results of this study have shown that that body weight is not a reliable indicator of which females are going to become pregnant. While reproductively

active female badgers were significantly heavier throughout the winter and spring months in 2007/2008, it was the reproductively inactive females which were heavier in 2005/2006. These findings suggest that for this species, winter weights of females is not a good indicator of breeding status, unlike in other mammalian species (Mori, 1979; Bercovitch, 1987; Cameron *et al.*, 1994; Guinet *et al.*, 1998). Therefore, winter mean body weight is not an accurate means to ascertain when females implant based on the findings of this study. However, as mentioned previously, this study had limited access to data, resulting in small sample size and only data from two years. This could account for temporal variation within the sample size. no definite conclusions can be made regarding the predicting of implantation and parturition period by maternal body weight.

6.4.3 Detecting pregnancy and lactation by teat and mammary gland measurements

Teat length has been used in several studies in Britain to determine the breeding status of female badgers (Woodroffe and MacDonald, 1995a; Woodroffe and MacDonald, 1995b; MacDonald and Newman, 2002; Dugdale *et al.*, 2011a). Predominantly, these measures were used in conjunction with ultrasound scanning to provide an accurate assessment of litter size and possible parturition dates. MacDonald and Newman (2002) found that lactating females had a teat length and width in excess of 10mm for both measurements and retained a length of >5mm until August, while Dugdale *et al.*, (2011a) identified reproductively active females as having teats longer than 6.5mm and wider than 5.1mm, with the teats of non-breeders measuring approximately half this.

This study also found that teat length, width and mammary tissue depth were significantly greater in reproductively active females in Irish badgers. On average, reproductively active females had teat lengths of 7mm, teats wider than 5mm and mammary tissue depth of more than 8mm compared to teat length and width of less than 3mm and mammary tissue less than 6mm in reproductively inactive females. These measurements also varied from month to month. Reproductively active females culled in April, May and June had significantly longer and wider teats than females caught in January, suggesting that teat length increased during lactation. This is similar to studies in Britain which found that month had a significant effect on teat length (Dugdale *et al.*, 2011a). However, the English study only assessed data from May-July, while this current study has shown that differences in teat length and width

in reproductively active and reproductively inactive females can be detected as early as January i.e. before parturition. Additionally, reproductively active females showed a marked increase in mammary tissue depth compared to the original levels assessed in January. Therefore, using the monthly measurements identified in this study it would be possible to determine which female badgers were pregnant/gravid, as well as which have successfully bred and lactated in any given year. Although this study tested this hypothesis only on dead animals if these changes could also be noted in live animals it may be possible to determine gravid females in the field without need of ultrasound equipment. This may be a simple and useful tool for determining breeding status in the field and warrants further investigation.

When the three age categories of females were compared, no difference was found in either teat length, width or in mammary tissue depth. This suggests that these reproductive measurements are not influenced by age of the individual and therefore age should not impact on the assessment of the breeding status of an individual badger. Previous studies found that badgers assigned successful breeding status the previous year, had significantly smaller teats the following year if not breeding (Dugdale *et al.*, 2011a). It should also be noted that mammary tissue depth continued to be significantly greater in reproductively active females through to June, which could suggest that lactation was still ongoing in later months, a finding also reported from Britain (Neal and Cheeseman, 1996). However, as it is not known how quickly teats and mammary glands shrink after a breeding period, the significantly larger teats and greater mammary gland depth in June may not mean late lactation but may simply be engorged teats and glands after breeding.

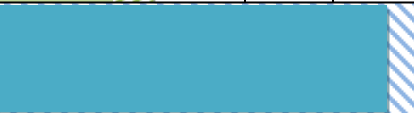
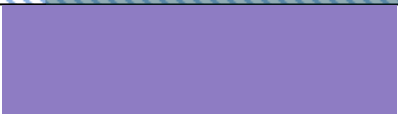
Although there is a clear correlation of teat size with pregnancy, caution must be used in the interpretation of teat size as it is possible that these physical changes may still be evident for some time after a pregnancy is lost. This would give a false positive indication of gestation or lactation success. In Britain, females that were deemed by ultrasound scanning to be pregnant but did not successfully lactate later in the season had slightly extended teats but no mammary tissue activation (Woodroffe and MacDonald, 1995b). Furthermore, lactation rather than gestation has been found to have a significant negative effect on female body condition (Woodroffe and MacDonald, 1995b). Therefore, it is possible that some females who did implant may not have enough fat reserves to successfully complete a pregnancy or lactate long enough to fully wean cubs, even though changes in teat length, width and mammary gland activation may suggest they did so. It should also be noted that while teats

may not reduce in length or width fully after a pregnancy as in many mammal species, this study found no age effect on teat length, width or depth of mammary tissue. This implies that the age of the individual badger and/or previous breeding events should not bias the results when these measurements are used to determining pregnancy or lactation.

Parturition prediction methods examined in this study and predictions from previous research could be used to select a suitable annual closed season. This study predicted that parturition would begin in Ireland during the second week of February and end in mid-March. This information added to previous research by Neal and Cheeseman (1996) that suggests 15 weeks for badger cubs to gain independence can be used to estimate the length of the annual closed season. If this timeframe is applied to the parturition dates calculated from foetal measurements in this study, this predicts that lactation occurs in Ireland until mid-June. Thus, it would be detrimental for cub survival to cull females during this time, i.e mid-February to mid-June.

The results of this study strongly recommend a change to the current Irish closed season, which at present takes place between January and March. While Britain has a 3 month closed season of February 1st to April 30th (Woodroffe *et al.*, 2005a; Bourne, 2007), this study suggests an even longer closed season of February through mid-June if cub starvation is to be reduced to a minimum (Table 6.3). Re-commencement of badger culling before this date may result in the welfare of suckling cubs being compromised, as some may still be dependent on their mothers for survival.

Table 6.3 The timing of parturition, lactation and proposed closed season assessed by this study, compared to British data. (Solid colours Irish data, hatched lines British data)

	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Parturition Period												
Lactation Period												
Proposed closed Season												

6.5 Conclusion

In this chapter, three methods of predicting and determining parturition dates of Irish badgers were analysed: foetal measurement, female badger weight and teat and mammary tissue measurement. The foetal measurement method appears to be more precise and can be used to predict parturition earlier in the season, which may benefit the implementation of a fluctuating annual closed season.

Predicting implantation and subsequent parturition dates using mean female body weight was unsuccessful, whereas teat and mammary tissue measurements were an easy and successful way of determining pregnancy and lactation in female badgers. The significant differences seen in teat length and width and mammary tissue depth all suggest that pregnancy and the subsequent lactation period can be easily identified through simple measurement of the teats. Teat length in particular may be a simple and effective means to determine pregnancy if it can be shown to work equally well in field-based situation.

Finally, it is suggested that the closed season be extended from its current 12 week period to ideally 20 weeks, to allow for the successful weaning of badger cubs in targeted areas. This would result in the current Irish closed season of January - March changing to a later and longer closed season of February until June.

7.1 Project overview

The bTB eradication program and the subsequent badger culling provided an opportunity to conduct a novel study on changing population dynamics of badgers in Ireland. It allowed for the comprehensive assessment of the effects of culling from population level to individuals, especially in areas undergoing repeated and long-term culling. The information found here will be important for future work, especially with the potential vaccine program as accurate epidemiological modelling depends on the population size, movements, intra-specific transmission and regrowth parameters of vectors such as the badger, to be incorporated. Finally, this study investigated how to predict parturition dates accurately and addresses the ethical concerns regarding the culling of reproductively active and lactating females. It seeks to highlight current insufficiencies in the closed season.

7.2 What has changed?

Based on what was previously known about the effects of culling on badger population from studies carried out in Britain (Cresswell *et al.*, 1992; Delahay *et al.*, 2006b; Tuijttens *et al.*, 2000b; Sadler and Montgomery, 2004), this study expected to find that long term culling had significantly changed many aspects of badger dynamics in Ireland also. In Britain, badger populations that have been greatly depleted by large scale culling were found to have reduced fecundity rates and took up to 10 years to recover to their pre-removal densities (Tuijttens *et al.*, 2000b; Sadler and Montgomery, 2004). As young females are normally the primary colonisers of culled areas, Tuijttens *et al.*, (2000b) concluded that the reduction in fecundity could be attributed to lack of mating opportunities due to the sex bias of recolonising females or possibly due to those females being sexually immature. In Britain, bite wounding can range from over 40% in high density populations (Cresswell *et al.*, 1992) to 7.5% in lower density, disturbed populations (Delahay *et al.*, 2006b) with males more likely to have moderate or severe wounds compared to females and cubs (Britain - Cresswell

et al., 1992; Tuytens *et al.*, 2000b; Delahay *et al.*, 2006b). In contrast, in disturbed populations females were more likely to have bite wounds than males, which may be due to disputes occurring between recolonising females (Delahay *et al.*, 2006b)

In short, based on previous research, this study expected to find similar reductions in the proportion of females breeding and possible changes in body weight/condition, an increase in bite wounding behaviour and primarily young females being the main recolonisers of repeatedly culled areas. None of this happened. Bite wounding behaviour, instead of increasing, decreased to the point of no occurrences which was unexpected given so much repeated disturbance to social groups in targeted areas (Chapters 3 and 5). The age profile of the recolonising female badgers was also surprising, as it appears that a varied range of ages recolonised vacant areas in Ireland (Chapter 4). Therefore, it is not just young, sexually immature females who come into vacant areas, as seen in Britain (Tuytens *et al.*, 2000b). There was also no change seen in body weight or body condition scores that could be attributed to culling (Chapter 3 and 5). And perhaps most surprisingly, and importantly, the proportion of females breeding within repeatedly culled areas did not change in response to long term culling (Chapter 3). Therefore, it would seem that in Ireland, badger populations appear to be able to absorb many of the effects of long term culling that have been reported in Britain. This means that any assumptions about what may or may not be happening in repeatedly culled areas may need to be reassessed.

7.3 Is there an argument to amend national policy?

Since badger culling began, there have been ethical concerns regarding the culling of reproductively active and lactating females. In Ireland, a voluntary closed season of January - March has been initiated in order to avoid dependant cubs starving. Under present guidelines, this closed season only occurs in previously uncultured areas. Previous research has shown a significant reduction in fecundity in culled areas (Tuytens *et al.*, 2000b; Sadler and Montgomery, 2004), and this maybe the rationale on which DAFM chose not to implement a closed season in culled areas, believing that few, if any females would successfully breed in recently culled areas. However, this study highlights several flaws with the current closed season. It found that female badgers in both previously undisturbed areas and repeatedly culled areas can breed successfully and in similar numbers. Consequently, the lack of a closed season in repeatedly culled areas will be detrimentally affecting the

survival of dependant cubs. These findings lead me to recommend that the closed season be extended to all areas to avoid the culling of lactating females and the subsequent starvation of dependant cubs. Furthermore, the timing of the current closed season is not ideal. It begins too early in January, is too short as it ceases in March, when the majority of cubs are still dependent on their mothers. Parturition in Ireland begins in February and as cubs suckle for 15 weeks (Neal and Cheeseman, 1996), this means that lactation may occur up to early June (Chapter 6). Therefore, I would also recommend that the timing of the current closed season be amended.

7.4 Implications of the fecundity findings

Females from previously undisturbed areas had the largest litters, but litter size decreased after sustained culling (Chapter 5). While litter size in undisturbed areas was similar to that previously found in Ireland, Britain and Europe (Anderson and Trehalla, 1985; Page *et al.*, 1994; Whelan, 1998; Dugdale *et al.*, 2003; Stuart, 2010; Corner *et al.*, 2015), litter sizes in repeatedly culled areas were significantly lower, with single cubs or twins being the most common litter sizes. There was also a significant reduction in the mean number of blastocysts found in females from repeatedly culled areas (Chapter 5).

The evidence from this study suggests the loss of some foetuses throughout the culled badger population, which may be attributed to culling, given that other parameters that affect implantation and reproductive success e.g. body condition, did not change. This low rate of fecundity and low reproductive output means that recovery of the badger population will be much slower than previously predicted, and this must be taken into account in the planning of future culling events. These findings also have ramifications for the Irish badger vaccine trials which will need to take into account the slow recovery to enhance vaccination efficiency and modelling of population spread. If culling were to be carried out without adjustment for reduced fecundity, then there is a danger to the sustainability of the local population.

7.5 Is stress a factor?

Embryonic resorption has been observed in undisturbed but very high-density badger populations (Neal and Harrison, 1958; Woodroffe and MacDonald, 1995b). Ultrasound

examinations showed that up to 38% of females had resorbed some or all of their foetuses during the course of gestation (Woodroffe and MacDonald, 1995b). Embryonic resorption might partially explain the reduction in the number of females found to be carrying foetuses in the present study, compared to those with evidence of placental scars (Chapter 3 and 5). Studies on species such as dogs and cats have shown that maternal environmental stress can adversely change the uterine environment which resulted in the resorption or abortion of the foetus at various stages of the pregnancy (Verstegen *et al.*, 2008). While embryonic resorption may also be due to insufficient maternal body condition to allow for the continued development of the growing foetus, as in dogs (Vannucchi *et al.*, 2007), as body condition did not significantly change over multiple culls (Chapter 5) it was unlikely that poor body condition contributed to high reproductive failure rates. If chronic stress is a similar issue for badgers, it may also be the reason why females from repeatedly culled areas had significantly fewer blastocysts and smaller litter sizes, when other factors such as body condition and weight remained relatively unaffected.

If stress is affecting badgers, it may be due to factors such re-forming of social groups. Survivors of culling operations, likely mainly young/small individuals, still present in these areas may be faced with an influx of new animals into their territories. Similarly, new animals to the area will need to seek and define their own new territories. It could be that these animals need to increase scent marking behaviours which may put more physical stress on them. Therefore, establishing a new social structure after culling has taken place may increase stressful interactions, leading to the higher loss of embryos after implantation.

7.6 Identifying pregnant and lactating females during fieldwork.

Teat length has been used in several studies in England to determine the breeding status of female badgers (Woodroffe and MacDonald, 1995a; Woodroffe and MacDonald, 1995b; MacDonald and Newman, 2002; Dugdale *et al.*, 2011a). Dugdale *et al.*, (2011a) identified reproductively active females as having teats longer than 6.5mm and wider than 5.1mm, with the teats of reproductively inactive females measuring approximately half this. However, Dugdale's study only assessed data from May – July. The present Irish study has shown that differences in teat length and width in reproductively active and reproductively inactive females can be detected much earlier, soon after implantation has taken place. As these differences can be detected as early as January, it is possible to determine pregnant

females in the field without need of ultrasound equipment. This may be a simple and useful tool in determining breeding status in the field. In 2013, DAFM suspended culling in 6 areas to allow for a 4 year mark-recapture and vaccination field study (Byrne *et al.*, 2013a). The resulting study found that vaccination of the Irish badger population is possible so long as it is done in conjunction with the current culling program (Anzar *et al.*, 2018). Therefore, badger culling seems likely to continue in Ireland in its present form, and at its current intensity, for the foreseeable future. In addition to monitoring bTB levels within these areas, DAFM have also indicated that they wish to investigate any changes in reproductive success while culling has ceased. To do this they will use the methods described in this study to assess the reproductive status of females within these areas (M. Good, DAFM, *pers.comms*).

7.7 Considerations for the vaccine trial program

This study has also highlighted some areas for consideration which will be relevant to the impending vaccination program. Understanding the dynamics of the recovering population will be needed if vaccination is to be as effective as possible. The results of this study will need to be taken into consideration when assessing disease transmission models. A reduced population means less badger to badger transmission of bTB (Ní Bhuachalla *et al.*, 2015). With lowered fecundity in an already sparse population, it may be that culling does not need to be carried out as regularly (Chapter 6). Additionally, given the low reproductive success of Irish badgers in repeatedly culled areas, this factor should be considered in future population models and epidemiological projections, resulting in a more accurate assessment of population recovery times. This new information along with the information about the potential age range of the recolonising animals (Chapter 4) will need to be accounted for if programs such as the badger bTB vaccine delivery plan are to be implemented, as the dynamics and recovery could vary from what may have been previously expected based on what was found in British studies.

When bite wounding frequency was assessed in this study, the data suggest that it exists at very low levels in undisturbed populations. Furthermore, as culling continued these low levels decreased further and eventually disappeared (Chapter 3 and 5). As bite wounding has been shown to be a small, but significant route of bTB transmission from badger to badger, this suggests that the spread of bTB in repeatedly culled areas may be reduced more than previously thought as this route of transmission disappears with repeated culling. This

will need to be included in any further models when planning vaccination projects so as not to overestimate the success of vaccination in reducing bTB in badgers in test areas.

Furthermore, if badgers are experiencing more stress than previously thought (Chapter 5), this may have a serious impact on bTB within the population. Previous research has suggested that higher stress leads to higher incidence of disease (Glaser and Kiecolt-Glaser, 2005). Stressors can increase the risk of developing infectious disease, prolong infectious illness episodes and interfere with wound healing processes (Glaser and Kiecolt-Glaser, 2005). This may be problematic for bTB control as badgers in repeatedly culled areas which are undergoing chronic stress may be more susceptible to new bTB infection or to the activation of latent infections and the spread of the disease. Again, this is something that has not been included in current models and needs to be considered during future culling and/or vaccination programs.

A final point of interest highlighted by this study is the variability of social group size after culling (Chapter 4). My evidence suggests that the recovery of social groups and local populations is much more variable than previously thought and may suggest a high degree of movement within and through repeatedly culled areas. This may impact the outcome of the vaccine trials, as vaccinated animals may not remain in study areas and unvaccinated individuals may also take up residence. This means that any future results will need to be carefully analysed, taking into consideration the increased instability of the repeatedly culled social groups. Additionally, as the only evidence for social group size stabilisation occurred more than 31 months post culling, it could be suggested that vaccine trials should not take place until such a time period has passed to allow the social groups to recover, territories to be established and a more stable population to develop. This could allow for a clearer understanding of the results of future vaccination programs.

7.8 Wider implications?

As mentioned at the beginning of this thesis, there is a lack of research into the physiological, behavioural and morphological changes which may occur as a result of culling. Many studies have focused on a reduction in density and abundance of the targeted species, with little thought as to the other impacts that such population disturbances may have on both populations and individuals. In Ireland the badger cull aims to significantly reduce badger

density in local, targeted areas, from ≥ 2 badgers per km² to less than 0.5 badgers per km² (Ní Bhuachalla *et al.*, 2015). As recent studies have found that this reduced density is a vital requirement of the ongoing vaccine program (Anzar *et al.*, 2018), the continued reduction in the Irish badger population density looks set to continue for some years to come. Especially given that any reduction in bTB levels in badgers will take some time to have a noticeable effect on the surrounding cattle herds. Therefore, it is more important than ever that the effects of such long term culling and its impact on the remaining population are fully understood and incorporated into future recovery models.

This body of work adds to the current literature by describing the effects that can result from extensive, long term culling and may be of relevance to other species undergoing culling. The significant reduction in blastocysts and litter size is of particular interest, as it suggests that something negative is happening to animals in culled areas. This will need to be included in future repopulation models of other species. This will be most necessary if targeted populations are a protected species, where the aim of culling is a temporary reduction in overall density and not a complete local extermination of the species. Failure to account for the reduced reproductive success may result in unwanted and detrimental effects on the target species, to the point where it may not be possible for the remaining population to recover. This may be especially important where culling may be due to unlicensed hunting and the animal in question is an endangered species.

7.9 Limitations and future work

While this study has added to the knowledge of the Irish badger population, there is still much to learn about the dynamics of the badger population as a whole. It was unfortunate that the full dataset for all years of culling was not made available. Furthermore, the study was hampered by the lack of information sharing between agencies and departments. If more access had been granted to the existing information, then perhaps more precise conclusions could have been drawn from some of these data.

Why there is a fall in blastocyst numbers and litter size in repeatedly culled areas still needs to be investigated. Determining whether badgers in repeatedly culled areas are chronically stressed could be accomplished by testing stress hormone levels from blood samples, either from the animal themselves or from parasites such as ticks. As the culling program is set to

continue for the foreseeable future, it would provide a source of culled badgers and their associated ticks, from which blood hormone levels could be tested for stress indicators in a relatively easy study. As the process of catching the animal stresses them, the captured badgers will in all likelihood have higher levels of stress hormones, however, the ticks will have the pre-catch blood in them, making them an ideal source of pre capture stress blood for which to test. Furthermore, little is known about the movement and ranging behaviour of these animals. It is important to understand this aspect of badger life and to determine whether ranging is affected by bTB infection or by culling. Knowing how badgers move within and outside their territories would also aid in our understanding of which badgers recolonise culled-out areas. Finally, it would be advantageous to have a better understanding of the genetic variability and relatedness of the Irish badger population, as it might help to explain the lack of aggression seen in this study and also account for the apparent lack of territoriality reported in other studies(Cresswell *et al.*, 1992; Delahay *et al.*, 2006b).

7.10 Conclusions and recommendations

While this study highlights some novel insights into how badgers in Ireland respond to long term culling, the patchy and sometimes sparse nature of the available data proved problematic at times.

Recommendation: A comprehensive database that can be easily made available would ensure future studies can be carried out in complete detail and add to the knowledge from this and previous studies. This would be most advantageous to future researchers.

Badgers can absorb a lot of population and social group disturbance, as seen in this study with little or no effect on badger weight or body condition. Social group size was also unaffected by culling. Aggressive behaviours such as bite wounding were initially low throughout the population and disappeared completely during repeated culling. However, this study did find a significant link between the presence of bite wounds and bTB infection, with bitten animals significantly more likely to test positive for bTB.

Recommendation: As culling appears to reduce the frequency of bite wounding, continued culling could be a useful method to reduce this bTB transmission pathway.

This study found no difference in the age profile of female badgers in previously undisturbed and repeatedly culled areas. This may suggest that a retrospective sample of the surrounding population, containing a range of ages, recolonises the culled areas. Repeated culling had little effect on the reproductive success. There was no significant difference in the proportion of reproductively active females in repeatedly culled or previously undisturbed areas.

Recommendation: Given the mixed age profile found in repeatedly culled areas which allows for the potential of breeding females in these areas, it is recommended that policymakers consider changing the voluntary closed season to a mandatory one and extended it to include all previously culled areas during the breeding season.

The mean number of blastocysts and the maximum potential litter sizes in these areas were significantly smaller than in previously undisturbed areas. It is possible that the stress associated with repeated culling is causing females to lose or reabsorb foetuses after implantation.

Recommendation: Further investigation is needed into the cause of the decline in reproductive success in repeatedly culled areas. If stress is adversely affecting the animals in these areas, then this may also adversely impact the disease levels in these areas as high stress levels have been linked with increased risk of disease.

Parturition dates showed evidence of annual fluctuation. Different methods to predict parturition dates were assessed for accuracy in predicting annual parturition. This study recommends that either foetal measurements taken during post mortem examination, or teat measurements be used to predict the beginning of parturition.

Recommendation: The use of teat measurements could be used as a quick and simple field method to assess levels of both pregnancy and lactation in the badger population, without the need for post mortem examinations.

This study found that badger cubbing begins in early February each year and last for 4 – 5 weeks. Given that cubs are dependent on their mothers for a minimum of 12 weeks, the current closed season ends at a time when the cubs are still vulnerable to starvation should their mothers be culled.

Recommendation: This study recommends that the length of the closed season should be extended, and the timing changed to more accurately reflect the dates between which cubs

are dependent on their mothers. Ideally, it would run from February through to the beginning of June and take place in all culled areas, not only in previously undisturbed areas.

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