Monitoring European pine martens (*Martes martes*) in Scottish forested landscapes

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Declaration

I hereby declare that this thesis has been composed by myself and that it embodies the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

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Laura Michelle Kubasiewicz

Abstract

Monitoring the distribution, abundance and demography of species is vital to ensure that conservation efforts are appropriate and effective. Monitoring enables evaluation of responses to natural or human disturbance, highlights the need for management interventions and enables these interventions to be assessed and refined. The methods used largely depend on the specific aim of monitoring and behaviour of the target species, as well as the time and spatial scale that monitoring is required to cover. The European pine marten (*Martes martes*) is one of few remaining mammalian predators native to the UK. Since persecution in the early 19th century resulted in their near extinction, pine martens have recovered part of their former range in Scotland. Their recent recovery and an overlap in territory with vulnerable prey species such as the capercaillie (*Tetrao urogallus*) make the collection of accurate baseline data and subsequent monitoring of population trends vital for conservation efforts.

Faecal counts have traditionally been used to provide a relative measure of population density for pine marten. In most cases, absolute measures of population density require individual identification. Non-invasive genotyping can provide this information but the quality of DNA from these samples is often poor. Here, the process is refined for pine marten faeces (scats) and hair samples. DNA degradation increased significantly for scats exposed to rainfall, with the rate of DNA amplification success reduced by 38% over a 16 day period. Success rates for hair samples were higher when more hair follicles were included in the analysis. Population densities were estimated for three forests in Scotland using a robust combination of non-invasive genotyping of hair samples and spatially explicit capture recapture modelling. Population density estimates of 0.07 (95% CI 0.03 - 0.16) to 0.38 km^{-2} (95% CI 0.11 - 1.07) were obtained which are within the range of previous estimates for forests elsewhere in Scotland. The first attempt to quantify the relationship between traditional scat counts and home range size was then conducted; a significant negative correlation exists but only when population density is relatively low. Previous studies suggest that pine martens in Western Europe are less forest dependent than those in Eastern Europe. Results from this thesis support this, with populations at the highest density found at sites with intermediate forest cover. This tolerance of lower forest cover is also apparent in the diet. Despite a preference for *Myodes* voles in populations in Eastern Europe, those in Western Europe show a strong preference for *Microtus* voles as well as a higher level of frugivory. We assess the autumn diet of four populations in Scotland assess the effect of forest cover and sex on the diet. There was no evidence of differential consumption of *Microtus* voles or birds between the sexes. Our analysis shows that frugivory is influenced by forest fragmentation, with a 5-fold increase in the occurrence of fruit (from 2% to 10%) as forest cover increased from 4% to 47%.

Diversionary feeding has been suggested as a management technique to reduce the depredation of capercaillie by pine martens. This thesis presents the first attempt to quantify the success and cost-efficacy of diversionary feeding for a range of problems (crop damage, threatened safety, livestock depredation) across 30 experimental trials. The strategy proved more effective when targeted towards food-limited populations, and when aiming to alleviate habitat damage or risks to human safety rather than depredation. A novel decision-making framework was developed to aid managers with the initial planning of the strategy and its subsequent implementation within an adaptive format. Further to this, the feasibility of using diversionary feeding with a view to reduce the depredation of capercaillie by pine martens was assessed. Questionnaire responses were collected from people who have provided food for pine martens throughout Scotland. A positive reaction to food was observed, with 58% of respondents reporting that initial visits occurred within one week of placement and 46% reporting that subsequent visits were received daily. These results suggest that diversionary feeding may be a viable option for pine marten management, although testing of its impact on capercaillie productivity would be required.

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Chapter 1

General introduction

1.1 Ecological monitoring

Effective ecological conservation relies on accurate knowledge of the distribution, density, demography and behaviour of a population (Gibbs, Snell & Causton 1999; Collen *et al.* 2013). Surveys of these parameters provide a snapshot of the status of a population but, more importantly, they provide a baseline from which to measure changes through time (Lindenmayer & Likens 2010). Monitoring can be defined as the repeated empirical measurement or estimation of parameters through time (Gitzen *et al.* 2012) and forms the basis of ecological research and its application for management (Spellerberg 2005). The key to an effective monitoring regime is to ensure that the aim is well defined and that appropriate techniques are employed to meet this aim.

Monitoring can allow the detection of ecological responses to natural or anthropogenic disturbance (Remis & Kpanou 2011), provides data to enable ecological theory to be tested and aids the development of ecological models to further our understanding of the natural world (Lindenmayer & Likens 2010). In terms of management, sound baseline data coupled with continued monitoring may provide evidence of the outcome or efficacy of management as part of an iterative approach (Nichols & Williams 2006; Head *et al.* 2013). Managers, however, are more often rewarded for implementing the action itself, rather than for its subsequent effectiveness (Gitzen *et al.* 2012) and monitoring programmes may not receive the funding or attention required to effectively inform management decisions.

The decision of whether to employ monitoring consists of three main drivers (Lindenmayer & Likens 2010). Firstly, curiosity-driven or ‘passive’ monitoring may be carried out purely for the interest of the observer. This type of monitoring is valuable for environmental education and awareness raising (Cosquer, Raymond & Prevot-Julliard 2012; Toomey & Domroese 2013), but may lack the detail or control needed for scientific application. Mandated monitoring, where data are gathered in response to a legislative requirement (Liu *et al.* 2014; Sanchirico *et al.* 2014), follows strict protocols and provides data of a more detailed nature; the focus tends to be on the identification of trends, rather than the underlying mechanisms causing these trends. Finally, 'question-driven' monitoring is based on hypotheses and employs a more rigorous study design. This will often include monitoring of the effects of a management intervention or other disruption to the subject or ecosystem. In this case, an understanding of the how a system will react leads to the capacity to predict the effects of future interventions or natural events, placing a high value on this type of monitoring for ecologists and decision-makers. There is inevitably some overlap between these distinctions; passive monitoring may, in some cases, feed into mandated monitoring as long as the results can be verified; long-term trends may also be used post-hoc to test hypotheses if the collection methods adhere to the study design.

The aim of monitoring, as well as the available resources and the nature of the target species will largely dictate the methods, spatial scale and timescales over which monitoring is carried out (Newman, Buesching & Macdonald 2003; Boitani & Powell 2012). The monitoring of parameters such as demographic rates (birth, survival and fecundity), individual behaviour, or population density tend to require a higher investment of time and resources than monitoring trends in distribution. These investments may be further amplified where the species are elusive, rare, or occupy a large area (Zylstra, Steidl & Swann 2010; Hui *et al.* 2011).

For easily observed animals, surveys can be carried out using direct observation. The spatial scale and cost of these programmes tends to dictate whether monitoring of more detailed aspects of demography and population size are possible in addition to measuring basic trends in presence and distribution (Dickinson, Zuckerberg & Bonter 2010). Citizen science, where observational data collected by volunteers contributes to a scientific study, has, however, proven extremely effective for territory mapping (Morrison *et al.* 2014), as well as monitoring the fecundity and survival of bird species (Morrison *et al.* 2014; Sullivan *et al.* 2014) on a national scale at relatively low cost. Despite the ease of collection, concerns remain regarding the reliability of data collected via the citizen science approach as observations can be difficult to verify (Snall *et al.* 2011).

Where species are difficult to observe (Pereira *et al.* 2010), indirect methods of detection may be more cost effective. Animal track observations (Conover & Linder 2009; Loughry *et al.* 2012); acoustic sampling, where calls and sounds of the species of interest are recorded (wolf howling; Stenglein *et al.* 2010; acoustic recording of bats; Lintott *et al.* 2013); or counts of non-invasive samples such as faeces (Jarman & Caparano 1997; Gormley *et al.* 2011) negate the need to observe or disturb the animals. These methods can provide information on presence or distribution, as well as relative trends in population density through time when repeated surveys are carried out in the same area (Summers & Denny 2010; Guethlin *et al.* 2012). Camera traps have also been widely used to monitor the species diversity, distribution and behaviour of terrestrial and arboreal mammals (Oliveira-Santos, Tortato & Graipel 2008; Ahumada *et al.* 2011; Di Cerbo & Biancardi 2013); to a lesser extent, this method has been used to monitor the presence and feeding behaviour of bird species (Campos, Steiner & Zillikens 2012; Samejima *et al.* 2012) and breeding behaviour in fishes (Butler & Rowland 2009). Population density estimates can be obtained when individuals are identifiable from photographs (Durban *et al.* 2010; Rich *et al.* 2014) or, when animal behaviour conforms to certain model requirements, without the need for individual identification (Rowcliffe *et al.* 2008); aside from this notable exception, individual identification of elusive or rare species usually requires the individuals to be physically captured. This can be extremely time consuming and expensive, particularly for protected species, as licences may need to be obtained for trapping and handling. Further to this, estimates of population abundance and density obtained this way are usually based on extrapolation from a relatively small sample size.

1.2 Non-invasive genetic sampling

Over the last two decades, advances in molecular techniques have enabled the identification of individuals from DNA extracted from non-invasive samples (i.e. those collected without direct contact with the animal, such as faeces or hair). DNA has been isolated from a number of non-invasive sources (Waits & Paetkau 2005), including urine found on snow patches (Sastre *et al.* 2009), saliva found around bite wounds on prey (Sundqvist, Ellegren & Vilà 2008) and feathers (Vazquez *et al.* 2012; Bosnjak *et al.* 2013) as well as faeces (Kohn & Wayne 1997; Roques *et al.* 2014) and hair (Mullins *et al.* 2009; Berry *et al.* 2013; Steyer *et al.* 2013). For individual identification, microsatellites (short tandem repeats of a sequence of DNA bases) are the most popular tool (Taberlet & Luikart 1999; Selkoe & Toonen 2006), with species specific markers being relatively quick to develop at a reasonable cost. Several other genetic techniques, including Single Nuclear Polymorphisms (SNPs) and, more recently, Next Generation Sequencing (NGS) allow the estimation of a number of ecological parameters, such as migration rates, genetic diversity, relatedness of individuals, estimation of effective population sizes, and identification of cryptic species (Selkoe & Toonen 2006; Goossens & Salgado-Lynn 2013). Much of this information would not be gained by observational sampling alone and can be used to enhance conservation management plans (Goossens & Salgado-Lynn 2013).

Genetic methods are not without drawbacks. Non-invasive hair samples are usually collected using snagging devices, which are subject to bias due to differences in individual behaviour (Ebert *et al.* 2009). Sample processing can be costly and DNA extracted from non-invasive samples, particularly faeces, is often of poor quality and prone to errors in the genotyping process (Lucchini *et al.* 2002). These errors can be exacerbated at each stage of the process; environmental variables such as temperature (Lucchini *et al.* 2002; Nsubuga *et al.* 2004) and rainfall (Murphy *et al.* 2007; Brinkman *et al.* 2010) as well as the diet of the defecator affect sample quality prior to collection (Murphy, Waits & Kendall 2003; Vynne *et al.* 2012). The method used to store samples (Frantzen *et al.* 1998; Piggott & Taylor 2003; Panasci *et al.* 2011) and the method of DNA extraction may further decrease sample quality during sample processing (Piggott & Taylor 2003; Vynne *et al.* 2012). If studies do not take these factors into account and plan accordingly, error rates could increase to an unacceptably high level.

1.3 Conflict management

When contact between people and wildlife results in problems such as damage to crops or timber (Sullivan & Sullivan 2008; Barrio, Bueno & Tortosa 2010), risks to safely (Kaplan *et al.* 2011; Rogers 2011), or increased depredation of livestock (Smith *et al.* 2000), management may be required. Where different stakeholders disagree on how to alleviate these problems, conflict can arise (Young *et al.* 2010; Redpath *et al.* 2013). Environmental variation, lack of knowledge of population status and trends, as well as poor understanding of the underlying processes influencing population dynamics all contribute to the uncertainty associated with the likely effects of management. Monitoring the effects of management is therefore paramount, not only to ensure that the management objectives are being met, but also to provide feedback to stakeholders and ensure clarity and trust in the management process (Volkmann *et al.* 2014). Management can be increasingly difficult when the target species is protected or vulnerable, as managers aim to reduce the impact of the target species without adversely affecting it in the process. For example, depredation by hen harriers (*Circus cyaneus*) can, in some circumstances, limit red grouse (*Lagopus lagopus scotica*) populations but some stakeholders argue that interventions to reduce the effects of predation on grouse density must consider the conservation of the raptor (Redpath 2001). Non-lethal methods can be employed to manage such species and, although some methods may have a negative effect on the target species in the short term, they present a compromise for stakeholders on opposing sides of the argument.

Visual, physical and sonic deterrents and chemical repellents have been targeted towards a number of mammalian predators to reduce livestock depredation with varying levels of success. Smith (2000) reviews these methods, suggesting that most are only effective for a limited time or on a small scale. Aversive conditioning, where bait is treated with an unpleasant compound to deter the target species from a feeding behaviour, has proven effective at reducing both nuisance feeding by bears in proximity to human settlements and the destruction of ground-nests by predators, but has been abandoned by many livestock owners as ineffective at protecting livestock (Conover & Kessler 1994; Mason & Bodenchuk 2002). This highlights the importance of using the appropriate management for a specific type of problem. For the protection of endangered ground-nesting birds, direct protection of the nest may not be the most appropriate aim of management. For example, the nests of the capercaillie (*Tetrao urogallus*) are extremely difficult to locate and hens are sensitive to disturbance (Summers, Willi & Selvidge 2009). In such cases, management aimed at changing the behaviour of the predator would be preferable and diversionary feeding is one such option (Gasaway *et al.* 1992; Reynolds & Tapper 1996; Conover 2002; Rea 2003; Thirgood & Redpath 2008; Massei *et al.* 2011). In its broadest sense, diversionary feeding is the use of food to divert the activity or behaviour of a target species away from an action which causes conflict, without the intention to improve or increase the density of the target population (Conover 2002). Despite widespread use, the technique is considered expensive (Witmer, Nolte & Stewart 2000; Conover 2002; Rea 2003) and in order to make evidence based management decisions, its efficacy needs to be quantified and reviewed.

1.4 European pine marten

1.4.1 Status and distribution

The European pine marten (*Martes martes*) is a forest dwelling mammalian predator, approximately the size of a domestic cat, characterised by its dark brown fur and a white throat bib (Velander 1983). It is one of eight members of the genus *Martes* within the family *Mustelidae* (Balestrieri *et al.* 2010), and the only member of the genus native to the UK (Birks 2002; Caryl 2008). The species native range covers most of Europe, from Portugal and Ireland in the west, Iran and parts of Siberia in the east and Fennoscandia in the north (Proulx *et al.* 2004). Although the pine marten is listed as 'Least Concern' in the IUCN red list of endangered species (Kranz 2008), its conservation status varies considerably between countries. Legal hunting occurs in some countries within its range (Proulx *et al.* 2004) but exploitation is regulated by listing under Appendix III of the Bern convention and Annex V of the Habitats Directive (Kranz 2008).

Habitat associations and diet differ considerably within the pine martens range; in Eastern Europe they are strongly associated with late-successional coniferous forests containing a high level of forest cover (Storch 1990; Brainerd *et al.* 1995). This type of habitat is thought to provide protection from predators (Buskirk & Powell 1994) and contains arboreal cavities which provide insulated denning sites and reduce thermoregulation requirements over winter (Birks, Messenger & Halliwell 2005). In contrast, populations in Western Europe are less reliant on forested landscapes (Pereboom 2008; Mortelliti *et al.* 2010; Caryl, Quine & Park 2012a), with Scottish populations showing selection for open areas such as tussock grassland within their home ranges, as well as higher populations densities at intermediate levels of forest cover (Caryl *et al.* 2012a). This may be the result of a behavioural adaptation to the much lower levels of forest cover in Western Europe (Caryl *et al.* 2012a), and this adaptation is also reflected in the diet.

1.4.2 Dietary profile and niche partitioning

Pine martens are omnivorous and are considered to be dietary generalists (see reviews: Clevenger 1994; Martin 1994; De Marinis & Masseti 1995; Zalewski 2004), focussing on different food types such as mammals, birds, invertebrates and fruits, depending on their availability (Clevenger 1993; Helldin 2000; Caryl *et al.* 2012b). Despite a generalist diet overall, pine martens show a preference for rodents, especially microtines (Caryl *et al.* 2012b). Whilst pine martens in north eastern Europe show a marked preference for voles of the genus *Myodes*, those in western Europe show a preference for voles of the genus *Microtus* (Putman 2000; Caryl *et al.* 2012b), a species found in open tussock grasslands. This preference is particularly apparent in Morangie, Scotland where, despite a ratio of *Microtus* voles to *Myodes* voles of one to five within the pine martens home range, dietary analyses show that 10 *Microtus* voles are eaten for every *Myodes* vole (Caryl *et al.* 2012b).

Pine martens are sexually size dimorphic, with females weighing approximately 1,950g and males approximately 1,350g (Caryl 2008). Males maintain home ranges up to 8 times larger than females (Caryl *et al.* 2012a) and display intra-sexual territoriality (Balharry 1993), with low levels of overlap between the home ranges of animals of the same sex (e.g. 4-6%; Zalewski & Jedrzejewski 2006). Females tend to select their range based on the availability of resources required for raising young (Powell 1994), whilst males select their home ranges based on the presence of females (Caryl 2008). Females may show a higher level of risk aversion than males by avoiding open areas, as seen by fewer female road casualties (Coope 2012, pers. comm; Caryl *et al.* 2012a) This, along with differences in habitat selection may mean that dietary profiles differ between the sexes, although very limited research exists (Balharry 1993).

1.4.3 History of pine martens in the UK

In the UK, widespread habitat loss and human persecution during the late 19th century led to a severe decline in pine marten abundance (Lockie 1964; Langley & Yalden 1977). By the start of the 20th century, only a few populations remained in North West Scotland (Ritchie 1921; Langley & Yalden 1977). The two subsequent World Wars substantially reduced trapping pressure as focus was turned away from the management of game species (Lockie 1964; Caryl 2008); this, along with the establishment of the Forestry Commission in 1919 (Heitzman 2003) and the resulting afforestation enabled populations to start recovering (Lockie 1964; Caryl 2008). Pine martens were listed in Schedule 5 of the Wildlife and Countryside Act 1981 (Balharry 1993) in 1988, leading to a substantial reclamation of their former range in Scotland (Croose, Birks & Schofield 2013; Croose *et al.* 2014). This rapid recovery has led to concern by some stakeholders regarding the effect of increasing depredation on vulnerable prey species. As a protected native predator, considerable scope exists for conflict concerning the conservation of pine martens alongside their prey, particularly where their prey may include other protected and commercially valuable species such as capercaillie and red grouse. Reliable methods of measuring and monitoring population density, as well as in depth knowledge of the behaviour, diet and the likely response to management actions, are essential to ensure effective management.

1.5 Thesis outline

1.5.1 Chapter 2 – Fur and faeces: an experimental assessment of non-invasive DNA sampling for the European pine marten

The feasibility of using non-invasive genetic sampling, with hair and scat samples, is tested as a survey method for studies of European pine martens in Scotland. Firstly, the effect of spatial sampling regime (distance from forest tracks) on overall visitation rates to hair tubes, then on the sex ratio of visitors using hair tubes is tested. Second, genotyping success for both scat and hair samples is assessed. For hair, the effect of the number of hair follicles used per reaction on DNA amplification is measured. For scats, the effect of time since defecation, with scats kept under contrasting environmental conditions, on genotyping error rates and on overall genotyping success is quantified.

1.5.2 Chapter 3 – Use of non-invasive genetic sampling and spatial mark-recapture to monitor European pine martens in forested landscapes

Estimates of population density for pine martens are currently reliant of extrapolation from home range sizes obtained through radio tracking (Balharry 1993; Bright & Smithson 1997; Halliwell 1997; Caryl *et al.* 2012a). To provide a more direct method of estimating population density, individual genotypes are derived from hair samples using a set of eight microsatellite markers, then incorporated into Spatially Explicit Capture Recapture (SECR) models to obtain population density estimates for three forests in Scotland. These data are added to previous density estimates to enable assessment of the effects of forest fragmentation on marten density. This is the first step to identifying a relationship between scat counts and population density for pine martens in Scotland.

1.5.3 Chapter 4 – Dietary composition between the sexes for European pine martens in fragmented landscapes

The diet of Scottish pine martens is analysed to ascertain the dietary differences between males and females, as well as between forests with different levels of forest cover. Firstly, it is hypothesised that the preference for *Microtus* voles seen in the Scottish population may be due to higher consumption of this species by males, and that this focus on hunting for *Microtus* voles will cause males to have a narrower dietary niche breadth than females. Secondly, it is predicted that the consumption of *Microtus* voles will be higher in forests with higher levels of forest cover. Finally, to further the understanding of population life history and behaviour, the differences in bird depredation between the sexes and between forests with increasing forest cover is assessed.

1.5.4 Chapter 5 – Diversionary feeding: An effective management strategy for conservation conflict?

This chapter provides a review of the efficacy of diversionary feeding and identification of the common causes of both success and failure. These factors are incorporated into a decision making framework which proposes an evidence-based process for future planning and implementation of this strategy. Although some success has been found, the results of diversionary feeding are mixed at best, with a lack of follow-through from the initial uptake of diversionary food to achievement of management objectives. Experiments often fail to report the effects of feeding in sufficient detail to allow an evidence-based evaluation by stakeholders considering using the strategy. Clear reporting of findings, as well as costs, would enable the return-on-investment to be calculated for different levels of management effort, and future decisions to be driven by cost-effectiveness and efficiency of the action.

1.5.5 Chapter 6 – The response of pine martens to diversionary food sources: testing the feasibility of diversionary feeding

Although there is call to manage pine marten populations in some areas, the species are native to the UK and protected under the Wildlife and Countryside Act, 1981 (Balharry *et al.* 1996). Non-intrusive methods must be explored, of which diversionary feeding is one such option. Through the analysis of questionnaire data, this chapter reports on the effect of food placement methods and the types of food used on the time taken for pine martens to respond to diversionary food, as well as the time scale that these visits occur over, to allow an assessment of the feasibility of the strategy for pine martens.

Chapter 2

Fur and faeces: an experimental assessment of non-invasive DNA sampling for the European pine marten

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2.1 Abstract

Non-invasive genetic sampling using materials such as faeces, hair or feathers can be used to monitor wildlife populations, although the quality of the DNA obtained is often poor. Improving the efficiency of sampling and minimising the factors that reduce DNA quality are therefore critical for designing an effective survey. After a severe decline, European pine martens have reclaimed much of their former range in Scotland. This rapid range expansion makes accurate monitoring vital, but this is hampered by the species' elusive behaviour. We tested two sampling methods; hair collected from hair tubes and faeces (scat) collected along tracks, to identify the effects of key variables on DNA quality and sampling efficiency. For hair, we tested the influence of hair tube location (distance from forest tracks) on collection rate and sex ratio of animals successfully sampled. For scat, we assessed the effect of time since defecation (1 to 16 days), with scats kept under contrasting environmental conditions (exposed to rainfall or sheltered), on genotyping success and genotype error rates. We found no bias in the overall collection rate or sex ratio of animals detected by hair samples with differing proximity to forest tracks. DNA amplification failure for scats exposed to rainfall increased from 28% to 65% over the 16 day experimental period. During periods of low rainfall, the length of collection sessions may therefore be extended to increase sample number without risk of DNA degradation. Lack of bias in hair collection rates with proximity to forest tracks provides justification for tube placement close to tracks, thus reducing survey effort. These findings provide guidance for the development of efficient and cost effective non-invasive sampling of Scottish pine martens.

2.2 Introduction

Accurate baseline data on species' presence, abundance and demographic rates is a key component of effective wildlife management (Gibbs *et al.* 1999), particularly for species of conservation concern, and in order to monitor a species, there must be a reliable method of detection. Traditional methods of detection often involve capturing animals, which can be difficult when species are elusive or protected and stressful for animals vulnerable to disturbance. Non-invasive genetic sampling has been suggested as an alternative survey tool, with genetic samples extracted from hair, faeces or feathers potentially negating the need to physically capture or even observe the animal (Taberlet *et al.* 1996; Taberlet & Luikart 1999). Non-invasive DNA methods have been used to map the distribution of species such as the Andean cat in Peru (*Orealilurus jacobita*; Cossios *et al.* 2007) and the jaguar in Belize (*Panthera onca*; Weckel, Giuliano & Silver 2006); to estimate population densities of the coyote (*Canis latrans*; Kohn *et al.* 1999) and the ship rat (*Rattus rattus*; Wilson *et al.* 2007); and to compare survival estimates between the sexes in Wolverine populations (*Gulo gulo*; Brøseth *et al.* 2010).

Genetic methods, however, are not without drawbacks. Sample processing is costly and, in the case of wide-ranging or low density populations, collecting sufficient samples can also be time consuming and expensive. These issues may be exacerbated when using samples of poor quality DNA such as faeces (Lucchini *et al.* 2002), which contain compounds that inhibit the DNA amplification process. Quality is measured by the rate at which DNA amplification, through polymerase chain reaction (PCR), yields a detectable quantity of DNA and the rate of occurrence of amplification errors. Two types of error are prominent: allelic dropout, where one allele from a heterozygous individual fails to amplify; and false alleles, where an allele differing from the consensus, or agreed, genotype is produced (Broquet, Menard & Petit 2007). Data with an acceptable level of precision may only be achieved through increased collection of samples and repeated amplifications, as well as through the use of more expensive DNA extraction techniques (Taberlet *et al.* 1996). Improving the efficiency of sampling and minimising the factors that reduce DNA quality are therefore critical when designing a cost effective surveying strategy.

Despite previous findings that suggest a decrease in faecal DNA quality over time (Murphy *et al.* 2007; Lampa *et al.* 2008; Brinkman *et al.* 2010; Panasci *et al.* 2011; Reddy *et al.* 2012), as well as with increased temperature and rainfall (Lucchini *et al.* 2002; Nsubuga *et al.* 2004; Murphy *et al.* 2007; Brinkman *et al.* 2010), there is considerable variation in the effect of these factors between taxa. For example, rainfall significantly degrades DNA in Sitka black-tailed deer pellets (*Odocoileus hemionus sitkensis*), but does not affect DNA sample quality from mountain gorilla faeces (*beringei beringei*; Nsubuga *et al.* 2004). Over one month, amplification success for faecal samples decreased by 65% for the brush-tailed rock-wallaby (*Petrogale penicillata*; Piggott & Taylor 2003), but only 5% for coyote (*Canis latrans*; Panasci et al. 2011). In reality, the combination of the environmental conditions in which samples are found, as well as inter-species differences, most likely cause a considerable amount of variation in DNA degradation rates, but very few studies have assessed this under controlled conditions.

Pine marten populations in Scotland have shown a recent range expansion after near-extinction in the early 20th century (Lockie 1964; Croose *et al.* 2013). As a predatory species, their status is of interest to stakeholders concerned with the conservation of potential prey species, as well as that of the pine martens themselves. Their elusive behaviour makes non-invasive sampling such as DNA extraction from hair or faeces potentially useful. Genetic analyses of scats has been successfully used for mapping the distribution and diet of martens (Caryl *et al.* 2012b; Croose *et al.* 2013). Hair sampling has previously been used to estimate pine marten populations in Ireland (Lynch, Brown & Rochford 2006; Roche 2008; Mullins *et al.*

2009), but has thus far been unsuccessful in Scotland. Mixed success has also been found in Ireland, with hair traps placed in lowland forests collecting more samples than those in upland forests, despite similar population densities in both habitats (Lynch *et al.* 2006). Individual identification has also been derived from hair samples in Ireland and used for population density estimation (O'Mahony, Turner & O'Reilly 2012), suggesting that an investment in the refinement of the technique in Scotland would be of value.

Most methods of population abundance and density estimation rely on an equal probability of capture for each individual. Sampling regimes must, therefore, provide an unbiased sample of the population. For studies using non-invasive hair sampling, time constraints usually make it unfeasible to relocate hair traps between sampling sessions, which may introduce a temporal bias and violate assumptions of sampling independence (Boulanger *et al.* 2006). Spatial biases can also occur; hair tubes are most accessible if placed close to forest tracks; pine marten scats are also collected from forest tracks due to the relative ease of collection compared to searching the dense forest floor. If some individuals use forest tracks less frequently than others, the samples collected may only represent a sub-set of the population. Female pine martens, for instance, are thought to be more risk averse than males due to the reporting of a higher proportion of male road casualties (Rob Coope, pers. comm.); females also maintain smaller home ranges than males, which are therefore less likely to contain forest tracks.

In this paper we assess the sample quality and sampling regime of hair and scat collection for DNA studies of pine martens in Scotland. Firstly, we test the effect of temporal and spatial sampling regime on hair tube visitation rates; we test the effect of time between sampling session to give an indication of sample independence; and distance from forest tracks to show whether there is a pattern in usage, both overall and between the sexes. Secondly, we experimentally assess the genotyping process for both scat and hair and identify the key factors that adversely affect DNA quality. For hair, we test how PCR success is affected by the number of hair follicles included in the reaction. For scats, we assess the effect of time since defecation (up to 16 days), with scats kept under different (exposed and sheltered) environmental conditions, on genotyping success and error rates.

2.3 Methods

2.3.1 Study areas

Four sites in the Cairngorms National Park or Moray region of Scotland known to have pine martens present were surveyed. Abernethy Forest National Nature Reserve (57°15'N, 3°40'W; hereafter Abernethy) is a Royal Society for the Protection of Birds (RSPB) reserve in the northern Cairngorms covering 36km^2 of both ancient native pinewood (approx. 24km^2) and Scots pine (*Pinus sylvestris)* plantation (Summers, Dugan & Proctor 2010). Mar Lodge Estate (57°00'N, 3°37'W; hereafter Mar), owned by the National Trust for Scotland, comprises Caledonian pinewood concentrated mainly along Glen Lui and Glen Quioch, north west of Braemar (Davies & Legg 2008). Inshriach Forest (57°06'N, 3°56'W, hereafter Inshriach) is a Forestry Commission owned site in the Northern Cairngorms consisting mainly of managed Scots pine plantation with some remnants of Caledonian pinewood (Twiddle & Quine 2011). Darnaway Forest (57°33'N, 3°45'W; hereafter Darnaway), which is managed by Moray Development Company Ltd, consists of commercial Scots pine, Sitka spruce (*Picea sitchensis*) and Douglas fir (*Pseudotsuga sp*.) plantation, with some areas of deciduous woodland.

2.3.2 Sample collection

Hair was sampled during Sept-Nov 2011 (Abernethy, Mar) and 2012 (Darnaway, Inshriach) using hair tubes fitted with sticky pads (Mullins *et al.* 2009). The number of sampling sessions held was four (Abernethy, Darnaway) or five (Inshriach, Mar; Appendix 2A). Each session lasted from four to six days and session length was kept constant within a site. Hair samples from each tube were collected in individual polythene bags and labelled with a unique identifier. All samples were frozen at -20 ˚C with 8 hrs and transferred to -80 ˚C within three weeks to await DNA analysis.

Hair tube placement within each study site was planned using 1:25,000 Ordinance Survey maps. One (Abernethy, Mar) or two (Inshriach, Darnaway) hair tubes were placed in each 1 km^2 grid cell within the study area (Fig 2.1a). For ease of access, only cells containing forest tracks were used. In the field, fine scale placement was chosen based on the presence of woodland. Cells that did not contain trees were excluded. Hair tubes were placed at distances of between 0 m and 200 m from the nearest forest track (in increments of 50 m) with approximately the same number of tubes at each distance within sites. A combination of Hawbakers marten lure (F&T Fur Harvester's Trading Post, 10681 Bushey Road, Alpena, MI 49707), peanut butter and bread were used as attractants as these have previously proven effective (Chandrasekhar 2005; Roche 2008; Burki *et al.* 2009). Details of hair tube construction can be found in Appendix 2B).

Scats were collected from Abernethy during May 2011 (Fig 2.1b). Scats were cleared 24 hrs prior to the first survey, then two surveys were conducted on consecutive days so that scats were ≤24 hrs old. Twenty two scats were collected in individual pots and labelled with a unique identifier, then frozen at -20 ˚C before transfer to a -80 ˚C freezer in the laboratory at Stirling University. In order to test the effect of exposure and time on DNA quality, scats were thawed and a small section taken for DNA extraction (day zero samples). The remainder of the scat was split into two equal sections and allocated to one of two treatment groups. Samples in treatment one (exposed) were placed directly on a woodland floor to replicate the conditions in which they were found. Samples in treatment two (sheltered) were placed in the same location, but raised off the floor and covered by a waterproof canopy. To test the effect of time since deposition (time), a small section of each scat was taken from both treatments at intervals of 2, 5, 9, 12 and 16 days.

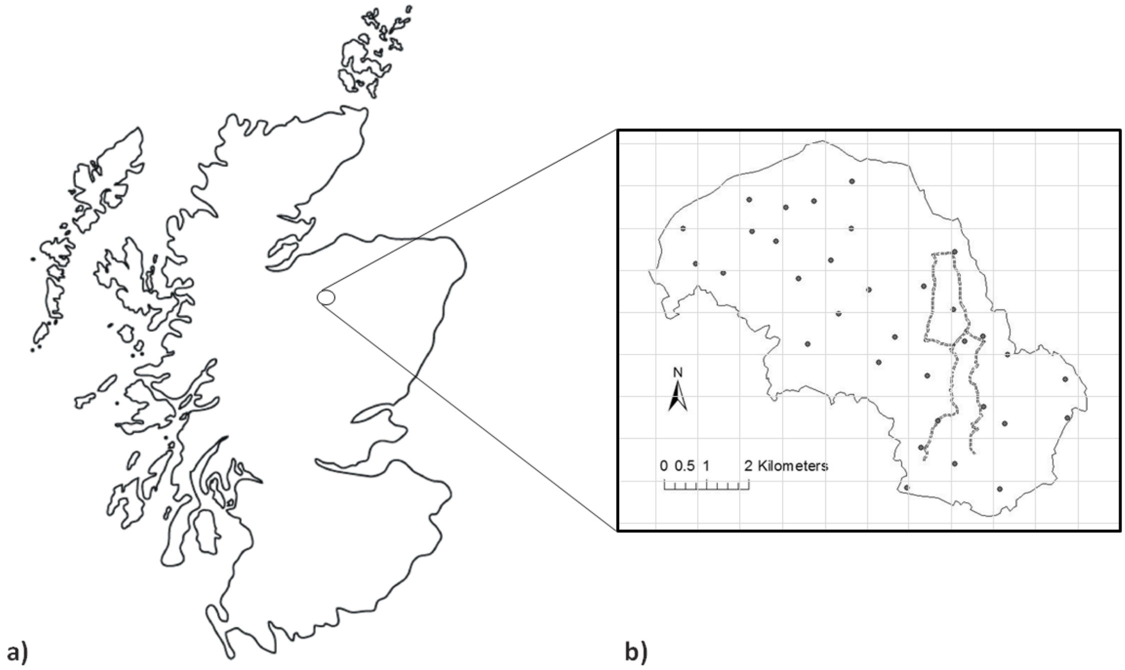

Figure 2.1 Hairtube placement (a) and scat transects (b) in Abernethy, with a grid of 1km^{-2}. Hair tubes were also placed in Inshrach, Mar and Darnaway (not presented). The grey dots are hairtubes, placed at approximate density of 1km^{-1}. Dashed lines are transects used for scat collection and are placed along vehicle tracks. Transects were surveyed by walking up one side of the track and down the other, hence checking each track twice per survey.

2.3.3 Genetic analysis

Hair samples were removed from sticky pads with xylene. Extractions were performed using an adapted chelex-100 method (Walsh, Metzger & Higuchi 1991); a 1 cm root-section of hair was placed in 200 µl chelex (5%) 7 µl dithiothreitol (DDT) and 1 µl proteinase K and agitated at 56 °C for approx. 5 hrs, centrifuged for 3 minutes and the supernatant incubated at 95 °C for 10 minutes. DNA was stored at -20 °C until required. The number of hair follicles in each extraction was recorded. Sex typing was performed using a 5′ nuclease TaqMan assay developed by Mullins (2009) and Real-time PCR using 5 µl Precision Master Mix (Primer Designs), 0.2 mM of either MMX or MMY forward and reverse primers and probes (see Appendix 2C) and 3 µl DNA template in a total volume of 10 µl. Amplification of ZFX (MMX) only signifies female DNA, whereas amplification of both ZFX and ZFY (MMY) signifies male

DNA (Mullins *et al.* 2009). The PCR conditions were 2 min at 50 °C, 10 min at 95 °C, then 50 cycles of 15 s at 95 °C and 1 min at 60 °C. Two replicate amplifications were performed for each primer/probe. For real-time product detection, Ct value (i.e. the number of PCR cycles needed to obtain the required quantity of DNA) was recorded at a ΔRn threshold of 0.2.

For scat samples, genomic DNA was immediately extracted from day zero samples using the QIAamp DNA stool mini kit (Qiagen, #51504) with a negative control. To avoid contamination, extractions were performed in an area of the laboratory reserved for DNA extraction. To test DNA amplification failure and error rates, two microsatellite loci were amplified (Mar08, Mar43; Natali *et al.* 2010) in one multiplex reaction of 10 µl containing 0.4 µM forward and reverse primers, 5 µl Qiagen Type-it PCR mastermix, 1 µl Q solution and 2 µl DNA template. After initial denaturation at 95 °C for 5 mins, 40 cycles of 95 °C for 30 s, 63 °C for 90 s and 72 °C for 30 s were used followed by a final extension step of 60 °C for 30 mins. Fragment analysis was performed at DNA Sequencing and Services (University of Dundee, Scotland, DD1 5EH) with negative and positive controls. Samples were scored using GeneMarker (Version 2.4.0) and verified by eye. Consensus genotypes were obtained for day zero samples following the comparative multi-tubes approach (Frantz *et al.* 2003); each sample was initially amplified twice, then further replications were performed until a consensus was reached. Samples without a consensus after seven amplifications were discarded. Samples from each treatment and time period were extracted and amplified twice then compared to the consensus to quantify error rates, with a negative and positive control in each plate.

2.3.4 Statistical analysis

Darnaway was excluded from all hair analyses due to lack of samples. Visitation rate to hair tubes was analysed using a Generalised Linear Mixed effects Model (GLMM) with a binomial error distribution. The response variable was recorded as ‘visit’ or ‘no visit’ for each hair tube, replicated per session. Distance and session were included as fixed covariates, forest as a fixed factor and two way interaction terms between distance and forests, and distance and session were included. Hair tube ID was included as a random factor. To test the effect of these variables on the sex-ratio of visitors, the same analysis was used, but with the proportion of males as the response variable restricting analyses to samples with a positive sex ID only.

To determine whether PCR success for pine marten sex-typing is affected by the number of hair follicles used in the extraction process, we calculated the mean Ct value per sample over rtPCR replicates. As the ZFX region is present in male and female pine martens and a Ct value is only obtained for positive samples, we included positive amplifications using the MMX locus only. A Generalised Linear Model (GLM) with poisson error distribution was used with Ct value as the response variable and number of hairs as the explanatory variable.

Genotyping errors per amplification were categorised as allelic drop out (p), false alleles (f) and failure as described in Murphy *et al.* (2007), relative to the consensus genotype for each sample. Overall error rates were calculated using equations from Broquet (2004):

$$p = \bar{p}_w = \frac{\sum_{j=1}^{L} D_j}{\sum_{j=1}^{L} A_{het_j}} \qquad f = \bar{f}_w = \frac{\sum_{j=1}^{L} F_j}{\sum_{j=1}^{L} A_j}$$

Where p and f are the probability of allelic drop out and false alleles, respectively, at locus j; L refers to each scat within the treatment block; A_j and A_{het_j} are the number of positive amplifications, and the number of positive heterozygous amplifications, respectively, for the scat at locus j; D_j and F_j are the number of amplifications at locus j containing an allelic drop out and a false allele respectively (Broquet et al. 2004).

Error rates (allelic drop out, false alleles and failure) were analysed using three GLMMs with a binomial (logit) distribution using proportional data from two repeated amplifications per sample for each combination of treatment, time and locus. As treatment commenced on day two, samples from day zero were not subject to the treatment conditions and were not included in the models. Only successful samples (i.e. those that produced DNA) were included in the models for false alleles and allelic drop out. Treatment (exposed, sheltered) and locus were included as fixed factors, time (days) as a fixed covariate and an interaction between time and treatment included in all models. To account for pseudo-replication of scat samples, scat and 'scat-half' were included as random effects, with scat-half nested within scat. Scat-half refers to the division of each scat between the two treatments.

For all analyses, we present estimates of the full model to avoid bias associated with stepwise deletion of non-significant terms (Whittingham *et al*. 2006). We present likelihood ratio test results for the deletion of each interaction term from the full model, or each main effect from a model with main effects only (Faraway 2005; Zuur *et al.* 2009). Prediction uncertainty of the full models is calculated using N = 1,000 random draws from the estimated parameter distributions and presented as the 95 % quantiles of the resulting distributions (Gelman & HIll 2007; Zuur *et al.* 2009). Analyses were performed in R version 3.1.0 (R core team 2014).

2.4 Results

Overall, hair samples were obtained on 20 % of occasions (115 samples, 572 trap nights; Appendix 2A). Of the 115 samples, 69 (60%) provided a positive sex-type, with 23 males and 46 females.

2.4.1 Hair tube placement

Neither the distance of the tube from the nearest track nor the identity of the forest significantly improved model fit for hair tube visitation rate or the sex ratio of visitors (Table 2.1). Sampling session significantly affected overall visitation rate, with a higher predicted visitation rate as sessions progressed from one (0.07; 0.01 - 0.10) to four (0.18; 0.13 – 0.25; Fig 2.2), but did not significantly affect the sex-ratio of visitors.

Table 2.1 Coefficient estimates for the GLMM for visitation rate of hair tubes. Results are shown for visitation rate of all pine marten (overall) and proportions of visits to hair tubes attributed to male pine marten (proportion males). Estimates are for the full model. Log-likelihood χ^2 statistic and associated p-values are for the deletion of each term from the full model (for interaction terms); or the model with main effects only (for main effect terms). Darnaway was excluded from the analysis due to lack of data.

	Visitation rate			**Visitation rate (proportion males)**		
Predictor	**Estimate ± SE**	χ^2_{df}	**P**	**Estimate ± SE**	χ^2_{df}	**P**
Intercept	-2.863 ± 0.695			-0.726 ± 1.28		
Distance	-0.005 ± 0.003	0.01_1	0.906	-0.001 ± 0.005	0.15_1	0.696
Session	0.544 ± 0.191	15.12_1	**<0.001**	0.149 ± 0.379	0.70_1	0.404
Forest (Inshraich)	-0.509 ± 0.866	3.54_2	0.170	-0.004 ± 1.439	1.26_2	0.532
Forest (Mar)	-0.645 ± 0.923			1.084 ± 1.559		
Distance*Forest (Inshriach)	0.007 ± 0.004	3.68_2	0.159	0.001 ± 0.006	0.35_2	0.838
Distance*Forest (Mar)	0.008 ± 0.005			-0.001 ± 0.007		
Session*Forest (Inshriach)	-0.229 ± 0.221	5.19_2	0.075	-0.012 ± 0.412	0.42_2	0.812
Session*Forest (Mar)	-0.479 ± 0.234			-0.174 ± 0.433		

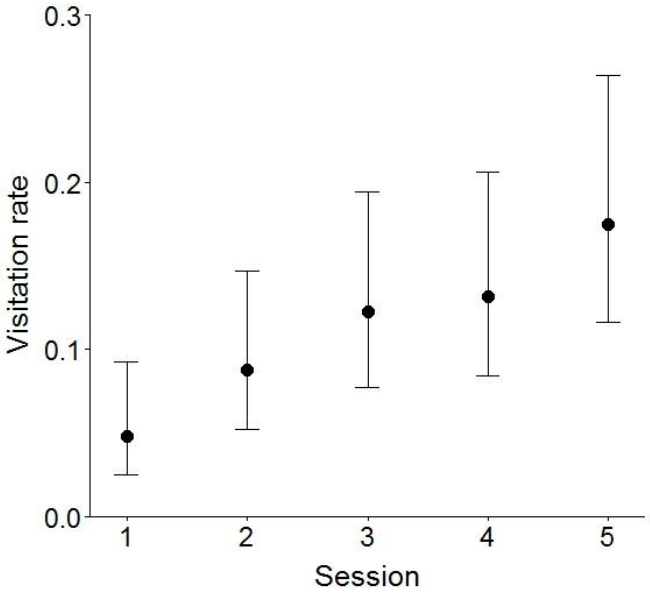

Figure 2.2. Visitation rate (proportion of hair tubes visited per session) to hair tubes by pine marten in Scotland. Data points represent predicted visitation rate from the GLMM (Table 2.1) and error bars represent the 95% confidence intervals for the model from repeated model simluations using random draws from the estimated parameter distributions. The ‘forest’ parameter was set for Inshriach. The ‘distance’ parameter was set to its median value of 100.

2.4.2 *Hair sex-typing success*

The number of PCR cycles needed to obtain the required quantity of DNA, as measured by Ct value, depended significantly on the number of hair follicles used for DNA extraction (χ^2= -2.08, df = 61, p =0.036). As the number of hair follicles increased from one to >13, the Ct value decreased by 13% (Fig 2.3).

2.4.3 *Scat genotyping success*

For the experimental study, a consensus genotype was established for 28 of 44 sample loci (22 samples, two loci). DNA amplification was successful in 63 % (421/666) of attempts over all loci, treatments and time periods. The average temperature for the duration of the study was 15 °C (7.7 – 23.7 °C), with 21.6 mm rainfall overall.

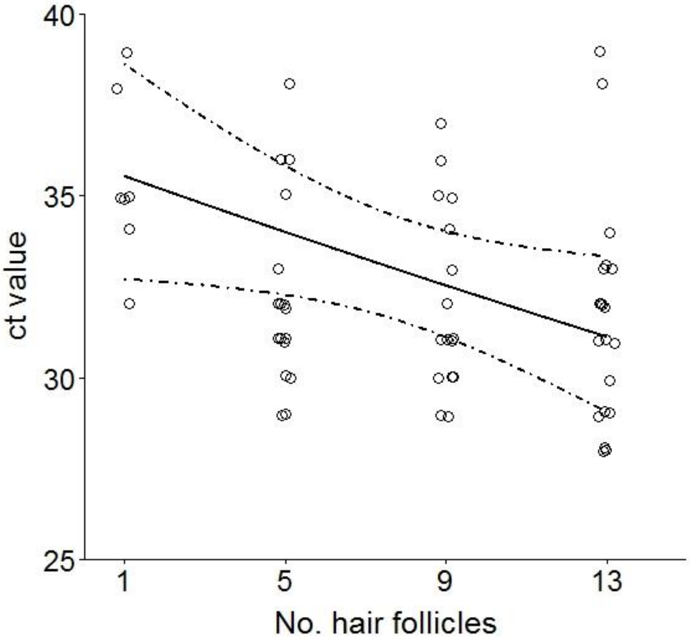

Figure 2.3. Ct value obtained from rtPCR of the ZFX region of each pine marten hair sample plotted against the amount of hair used in the extraction process. Data points are for observed data, solid lines represent predicted Ct value from the GLM using and dashed lines represent the 95% confidence intervals for the model prediction from repeated model simluations using random draws from the estimated parameter distributions.

2.4.4 PCR failure

Time, treatment and locus all significantly improved model fit for failure rate (Table 2.2). Failure rate increased from 0.28 (0.18 - 0.43) at day two to 0.65 (0.48 - 0.79) at day 16 for exposed samples, but did not change significantly for sheltered samples: 0.22 (0.13 - 0.35) at day two to 0.29 (0.15 - 0.42) at day 16 (Fig 2.4). Locus also improved model fit with the average failure rate over all treatments and time periods being higher for locus m08 than locus m43, with proportions of 0.58 (0.51 - 0.61) and 0.44 (0.37 - 0.51) respectively.

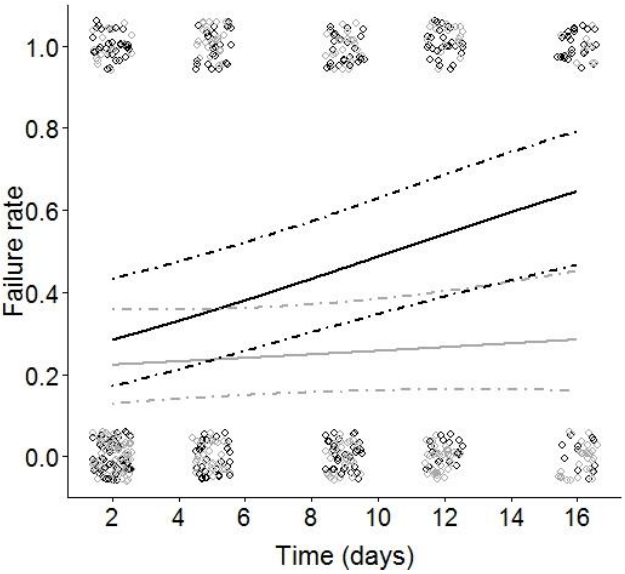

Figure2.4. Failure rate of PCR amplifications with increasing sample age, for samples exposed to rainfall (black line) and those under shelter (grey line). Data points are for observed data, solid lines represent predicted failure rates from the GLMM (Table 2.2) and dashed lines represent the 95% confidence intervals for the model prediction calculated from repeated model simulations using random draws from the estimated parameter distributions. The ‘locus’ parameter was set to locus m43.

2.4.5 *Allelic drop out and false alleles*

For successful amplifications, overall rates of allelic drop out and false alleles were 0.25 and 0.33 respectively. Neither treatment, time, nor genetic locus significantly improved model fit for allelic drop out (Table 2.2). The rate of false alelles increased with time for exposed samples only, from 0.19 (0.10 – 0.38) to 0.52 (0.28 – 0.78; Table 2.2; Fig 2.5). Samples amplified using locus m08 contained false alleles in 0.47 (0.37 – 0.58) of cases, compared to 0.30 (0.21 – 0.41) of cases for samples amplified with locus m43.

Table 2.2. Coefficient estimates for the GLMM's for failure, allelic dropout and false alleles associated with PCR amplification results. Estimates are for the full model. Estimates are for the full model. Log-likelihood χ^2 statistic and associated p-values are for the deletion of each term from the full model (for interaction terms); or the model with main effects only (for main effect terms).

	Failure			**Allelic drop out**			**False alleles**		
Predictor	**Estimate ± SE**	χ^2_{df}	**P**	**Estimate ± SE**	χ^2_{df}	**P**	**Estimate ± SE**	χ^2_{df}	**P**
Intercept	0.699 ± 0.358			1.659 ± 0.478			0.382 ± 0.435		
Treatment (exposed)	-0.150 ± 0.394	8.85_1	0.003	0.115 ± 0.590	0.05_1	0.817	0.366 ± 0.590	1.31_1	0.251
Time	-0.023 ± 0.028	10.80_1	0.001	-0.040 ± 0.042	1.95_1	0.162	0.004 ± 0.036	2.08_1	0.149
Locus (43)	0.594 ± 0.197	9.07_1	0.003	-0.534 ± 0.339	2.36_1	0.125	0.930 ± 0.279	10.80_1	0.001
Treatment* Time	-0.086 ± 0.040	4.55_1	0.033	-0.027 ± 0.071	0.14_1	0.705	-0.118 ± 0.058	4.02_1	0.045

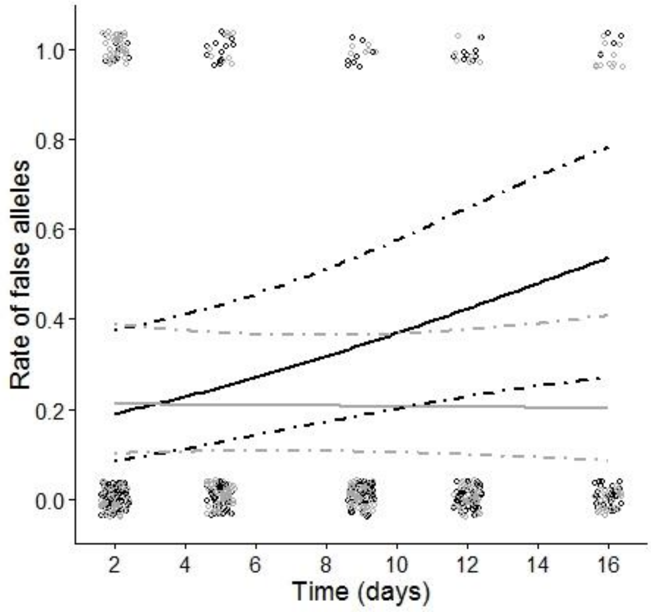

Figure 2.5. Rate of occurrence of false alleles with increasing sample age, for exposed (black line) and sheltered (grey line) samples. Data points are for observed data, solid lines represent predicted failure rates from the GLMM (Table 2.2) and dashed lines represent the 95% confidence intervals for the model from repeated model simluations using random draws from the estimated parameter distributions. The 'locus' parameter was set to locus m43.

2.5 Discussion

We assessed the potential of using hair and scat samples in DNA studies of pine marten populations in Scotland, including experimental tests of temporal and spatial hair tube use and assessments of the impacts of time and rainfall on scat DNA quality. Hair tube visitation rates increased over time with, on average, 2.6x as many samples collected in the fourth session compared to the first. This supports previous findings in Ireland, where sampling success increased with time when hair tubes were checked every four to six weeks for six months (O'Mahony *et al.* 2012). The shorter time period of the current study clarifies that this is not due to increased population density or behavioural changes through time, but suggests the influence of two factors; an increase in the likelihood of different animals locating hair tubes over time; and the habituation of individual pine martens to particular hair tubes. The latter of these factors is less apparent as, of 15 identified individuals that made multiple visits during the study period, only one individual used one hair tube exclusively (chapter 3).

The proximity of hair tubes to forest tracks did not affect the overall visitation rate, or the sex-ratio of visitors, suggesting that tracks are not avoided by either sex and that surveying along tracks does not bias the sample collected by sampling more males than females. There is no evidence to suggest that placing hair tubes on the edge of forest tracks favours certain individuals; of the 15 pine martens that visited more than one hair tube, 14 (93%) visited tubes at multiple distances (chapter 3). Placing hair tubes directly next to forest tracks reduces sampling effort, potentially allowing more samples to be collected per session or more sessions to be conducted.

The amount of amplifiable DNA obtained from hair samples is significantly increased by including more hair follicles in each reaction. Previous studies suggest that one hair is sufficient for accurate genotyping (Higuchi *et al.* 1988), however our analysis suggests that including more follicles (up to 13) reduces PCR failure rates. Where funding or time prevent processing of all samples, researchers should favour samples with the most follicles to increase PCR success.

Both the age of the scat and the level of exposure affected DNA amplification, reinforcing previous findings of the importance of these factors. An increase in PCR failure occurred with time up to 16 days after deposition, but only for scats that were exposed to rainfall. Although this effect was also seen for false alleles, allelic drop out did not increase significantly with time or treatment. As only a small number of repeat amplifications were performed, the increased failure rate over time could have masked any decrease in quality i.e fewer successful amplifications were available for errors to occur in. Nevertheless, our results highlight the interacting effects of time and rainfall on pine marten scats and we would encourage other studies to assess the drivers of DNA degradation in faecal samples from other mammals.

There was a significant difference in DNA quality and amplification success between the two loci tested. During initial planning of a project, we would recommend testing a range of potential microsatellite loci for relative success and error rates so that the most effective can be chosen. This should be considered as essential as optimising sample collection and storage conditions in developing an efficient and cost effective process.

For scat collection, researchers must strike a balance between leaving sufficient time for samples to accumulate and collecting samples before DNA degrades, particularly during periods of rainfall. If longer sampling sessions are required where populations are thought to be at low density, genotyping success may be improved by sampling during drier months. For hair, samples are usually collected from stationary sources such as hair tubes. As such, the time between sampling sessions must also take into account sample independence. For pine martens in Scotland, our data suggest that sessions of longer than four days are required to achieve this. Sample quality, however, can be maximised by using as many hair follicles as possible per sample in the DNA extraction process. Sampling efficiency can also be improved by placing hair tubes on the edge of forest tracks to improve access by surveyors. Given the high rate of error associated with non-invasive genetic sampling, refinement of the process and consideration of environmental conditions associated with each species is paramount to making the process efficient and cost effective. This study provides guidance for improvements to non-invasive surveys of pine martens in Scotland, and also highlights key areas for assessment prior to surveys of other mammalian species.

2.6 Acknowledgements

Thank you to David Bavin, Lizzie Croose, Tara Curry, Melissa Simmons and Kayleigh McCrory for help with fieldwork; Lucy Woodall, Stuart A'hara, Bridget Lau and Catherine O'Reilly for advice on genetic analysis; Jeroen Minderman for advice on statistical analysis.

Appendix 2A Details of hair collection sessions and data collected

Number of hair samples collected per collection session and overall, from Abernethy, Mar and Inshriach during Sept-Oct 2011 and 2012. The total number of hair tubes installed per forest, total number of hair tube checks (trap nights) and density of hair tubes within each forest is also shown.

		Abernethy	**Mar**	**Inshriach**	**Darnaway**	**Total (all forests)**
	No. hair tubes	33	26	62	47	121
	Trap nights	132	130	310	141	572
	Hair tube density (Nkm^{-2})	0.8	1.1	1.9	2	1.5
No. samples	**Session 1**	2	7	2	0	11
	Session 2	5	6	9	0	20
	Session 3	11	4	13	0	28
	Session 4	13	4	13	0	30
	Session 5	n/a	10	16	n/a	26
	Total hair samples	31	31	53	0	115

Appendix 2B Details of hair tube construction

To construct the sticky hair patches, a 1cm^2 patch of mouse-trap glue (‘The Big Cheese STV182 Mouse Glue Trap’) was attached to a small square of corrugated plastic with super glue. Two holes were drilled through the plastic and the ends of a piece of wire pushed through each hole. The wire was then pushed through the holes in the bottom section of the hair tube and twisted until secure. Two sticky patches were placed in each tube, one on each side.

Appendix 2C MMX and MMY probe sequence information

MMX and MMY probe sequences are reversed from the text provided in (Mullins *et al.*, 2009) and are as follows: MMX, 5′-VIC-CCTGGTCTGAAAACT-MGB-3′ and MMY 5′-6FAM-TGTGTCTCTCTCTGTCAAMGB-3′.

Chapter 3

Use of non-invasive genetic sampling and spatial mark-recapture to monitor European pine martens in forested landscapes

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3.1 Abstract

Accurate estimates of population density are important for many areas of natural resource management but can be difficult to obtain especially where species are elusive, rare, and/or occur at low densities. Non-invasive genotyping based on hyper-variable microsatellite markers from samples such as hair, faeces or feathers has provided a promising solution and allowed individual identification from genotypes to inform population assessment and models. Here we apply such methods to estimate the population density of the elusive European pine marten in Scotland (UK). After a severe population bottleneck, this species is beginning to reoccupy its former range in Scotland but estimating population density is problematic. Relative trends in pine marten abundance have been measured by observing changes in the number of scats in an area through time but these have not been calibrated to provide an absolute measure of population density. Here, we use individual genotypes derived from hair samples to calculate population density estimates for three forests in Scotland, using spatially explicit capture recapture (SECR) models. Using our own density estimates and data from previous studies we examine the effects of forest fragmentation on population size. We then attempt to calibrate a traditional method of measuring population trends to enable population density estimation without the need for genetic analysis. Estimates ranged from 0.07 km^2 (95% CI 0.03 - 0.16) to 0.38 km^2 (95% CI 0.11 - 1.07) with an unequal sex ratio at one forest (Abernethy) of eight females to two males. We found support for the previous finding that pine marten density in the UK increases with forest fragmentation up to a threshold level, beyond which it decreases, and suggest that forest cover of 20 – 35% enables maximum pine marten density. We provide the first calibration of scat counts for population density estimation of pine martens, which suggests that density may be inferred from scat counts only where population density is relatively low.

3.2 Introduction

Robust measures of population density are a key component in wildlife conservation. The effects of human activities on vulnerable species can be assessed (Remis & Kpanou 2011), sustainable quotas for game species can be set (Brøseth, Nilsen & Pedersen 2012), and insight can be gained into the life history of species of interest (Souza *et al.* 2014). For rare or threatened species, knowledge of population status enables informed management decisions to be made, and adaptive conservation relies on the ability to monitor the effects of management (Nichols & Williams 2006; Head *et al.* 2013). As most traditional methods of population monitoring rely on the ability to detect individuals, it can be extremely difficult when focal species are elusive (Marucco *et al.* 2009), have large home ranges (Brøseth *et al.* 2010), or are present at low density. Under such circumstances the establishment of accurate measures of population density may prove difficult, as these usually involve the capture and recapture of marked individuals (Luikart *et al.* 2010).

To overcome these issues, non-invasive sampling is being increasingly adopted because samples such as hair, faeces, egg shells or feathers (Morin & Woodruff 1996) can be collected without the need to capture or disturb individuals of vulnerable species, or observe elusive ones. Faecal surveys, in particular, have been used assess mammal populations. On a national scale, these surveys can establish the presence of a species and its range, with subsequent surveys providing data on range expansion or contraction (Croose *et al.* 2013; Croose *et al.*

2014). On a local scale, changes in the abundance of scats over time can be used to infer a relative change in density within an area (Harrison, Barr & Dragoo 2002; Summers & Denny 2010). This method of counting scats does not, however, provide an outright measure of population density. Despite some success in relating faecal abundance to absolute population abundance for sika deer (*Cervus nippon*; Marques *et al.* 2001), red fox (*Vulpes vulpes*; Webbon, Baker & Harris 2004) and sambar (*Cervus unicolor*; Brodie 2006), other studies have been less successful (White tailed deer; *Odocoileus virginianus*; Fuller 1991; ungulate sp.; Ariefiandy *et al.* 2013). All studies highlight the uncertain relationship between faecal abundance and population density, with variation in defecation rate depending on diet (Panasci *et al.* 2011); degradation rate depending on weather (Murphy *et al.* 2007; Brinkman *et al.* 2010); and errors in detection due to identification error (Davison *et al.* 2002; Birks *et al.* 2004).

Over the last 20 years, advances in molecular techniques have enabled individuals to be identified from non-invasive samples by producing DNA fingerprints based on multi-locus genotypes from a set of highly variable microsatellite loci (Morin & Woodruff 1996; Taberlet & Luikart 1999). Samples identified to individual level can then be used within a traditional capture-mark recapture (CMR) framework (Nichols 1992) to obtain accurate estimates of population abundance. Traditionally, population density is usually inferred by dividing the abundance estimate by an arbitrarily selected ‘effective trapping area’ containing the traps and a buffer, usually set at a distance equal to the radius of one home range (Dice 1938). Recent advances in CMR models integrate a spatial element into the modelling process to provide a reliable estimate of density. Empirical trapping data is used to estimate the extent of a detection function, which is then used to estimate the probability of capture at increasing distance from traps at the edge of the trapping area (Efford & Fewster 2013). Non-invasive genetic sampling combined with a SECR framework has proven extremely effective for population estimation of elusive species such as the European wildcat (*Felis silvestris*; Kéry *et* *al.* 2011) Asian tapir (*Tapirus indicus*; Rayan 2013) and jaguar (*Panthera onca*; Tobler *et al.* 2013). These techniques provide a promising basis for studying European pine marten populations in the UK, as accurate measure of population density are currently lacking.

After heavy persecution in the 19th and early 20th century, pine marten populations have now recolonized much of their historic range in Scotland (Lockie 1964; Croose *et al.* 2013). As a protected native predator, there is strong stakeholder interest in their conservation as well as some concern about their impact on prey species, such as through nest depredation of vulnerable capercaillie populations (Summers *et al.* 2009). To inform conservation management, it is desirable to gather as much information as possible on the species, including spatio-temporal trends in population density and how the availability of habitat may influence future range expansion in order to guide species conservation policy and practice.

Conservation management based on inference from research conducted in other parts of the European pine martens range is, however, likely to be misguided, as there appear to be strong differences in life history traits between the populations in different parts of their range. In contrast to the specialism for closed canopy forests by Eastern European populations, those in Western Europe, including Scotland, show more generalist behaviour and favour scrub and tussock grassland as well as mature forest within their home range (Pereboom 2008; Mergey, Helder & Roeder 2011; Caryl *et al.* 2012a). These differences are associated with in smaller home ranges, suggesting higher population densities, found with moderate levels of forest fragmentation (Caryl *et al.* 2012a). This raises the question of how to incorporate the conservation of this species with other management goals for the forested landscape, particularly in light of the dynamic nature of land use in Scotland and ongoing debate regarding the extent and placement of woodland (Sing, Towers & Ellis 2013).

Here, we use a combination of non-invasive genotyping of both scat and hair samples and spatially explicit capture recapture models to provide estimates of pine marten population density in Scotland. We use these data to test an existing suggested curvilinear relationship between population density and forest fragmentation, such that marten densities reach a peak at moderate levels of forest fragmentation. Finally, we examine whether our method of population estimation can be used to calibrate the traditional method of counting scats.

3.3 Methods

3.3.1 Study sites

Four sites were surveyed for scat and hair samples from mid-September to mid-November 2011 and 2012 (Fig 3.1). Abernethy (57°15'N, 3°40'W) is a Royal Society for the Protection of Birds (RSPB) reserve consisting of a Scots pine (*Pinus sylvestris)* plantation and Caledonian pinewood. Mar (57°00'N, 3°37'W) is a Caledonian pinewood in the southern Cairngorms, owned by the National Trust for Scotland. Inshriach (57°06'N, 3°56'W) is a Forestry Commission owned site near Aviemore consisting of a managed Scots pine plantation with some remnants of Caledonian pinewood (Twiddle & Quine 2011). Darnaway (57°33'N, 3°45'W) contains a plantation Scots pine, and exotic species such as Sitka spruce (*Picea sitchensis*) and Douglas fir (*Pseudotsuga menziesii*), with some deciduous woodland and is managed by Moray Development Company Ltd.

Data from four previous studies were used for the analysis of the effect of forest fragmentation, providing home range sizes for six sites within Scotland. Home ranges were estimated using a 100% minimum convex polygon from radio tracking data collected from June 2006 to July 2007 in Morangie Forest (Caryl *et al.* 2012a), August 1993 to April 1995 in Novar Forest (Halliwell 1997), during 1988 to 1989 in Kinlochewe, 1989 to 1991 in Strathglass (Balharry 1993) and July 1995 to December 1996 in Glen Trool and Minnoch (Bright & Smithson 1997). Estimates of percentage forest cover and forest edge density within a 9.77 km radius of the forest centre (300 km^2) were taken from Caryl et al. (2012) for all of the above forests.

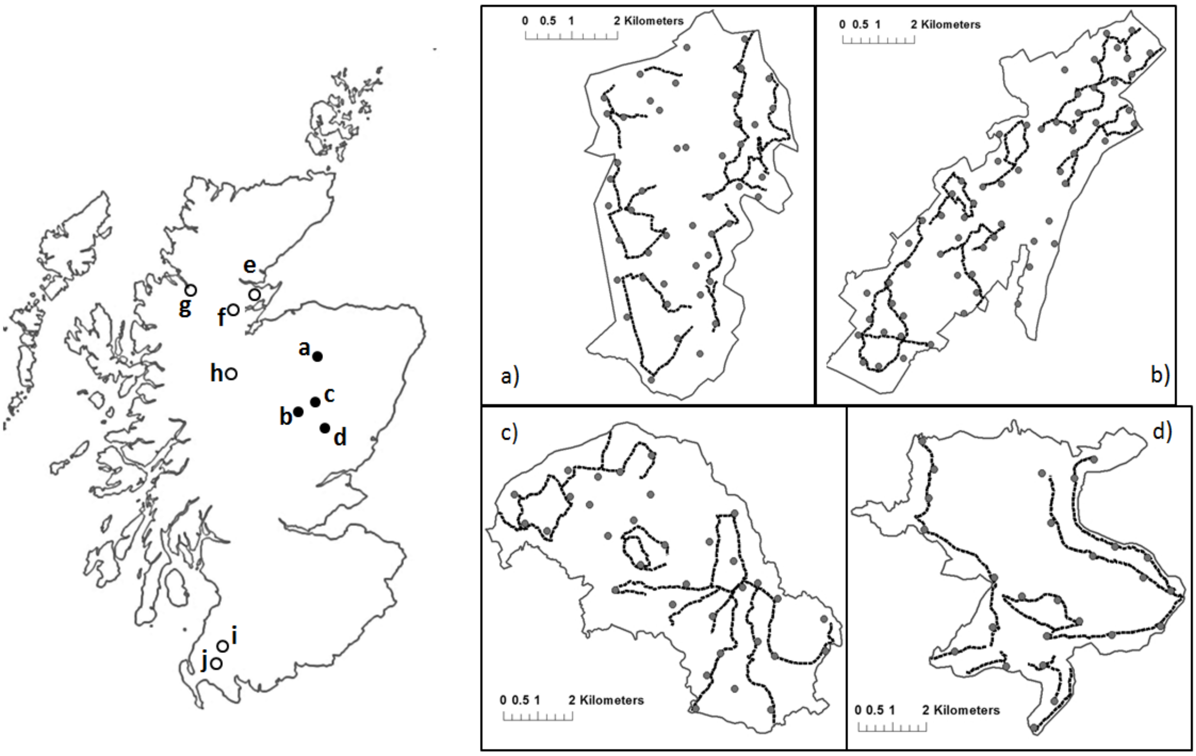


Figure 3.1. Location of the four sites (a - d) used for the collection of hair and scat samples for population density estimates using genetic SECR modelling: a) Darnaway Forest b) Inshriach Forest c) Abernethy NNR and d) Mar Lodge Estate. Thin grey lines are site boundaries. Thick black lines are scat transects and grey dots are hair tubes. The remaining sites were used to assess the effect of habitat fragmentation on pine marten home range size: e – Morangie (Caryl *et al.* 2012), f - Novar (Halliwell 1997), g – Kinlochewe and h - Strathglas (Balharry 1993), i – Minnoch and j – Glen Trool (Bright & Smithson 1997).

3.3.2 Sample collection

Scat collection was undertaken on transects distributed along vehicle tracks within each forest (Fig 3.1). Transects were cleared of all scats five days prior to sample collection. Three sessions were held for collection of scat samples from each site, with each session taking five days (Mar; Inshriach) or six days (Darnaway; Abernethy), depending on the length of the transects (Table 3.1). Sessions were carried out on successive days to complete the session for one forest before moving on to the next. All scats were photographed and the collection location mapped (using a handheld GPS). Using a fresh toothpick for every scat, a portion of each scat was placed into an individually labelled tube for subsequent DNA analysis. The remainder of each scat was then collected in a pot and labelled for diet analysis (data not presented). To avoid cross contamination, care was taken to ensure that the scats were only touched with toothpicks and did not come into contact with the outside of the tubes.

Table 3.1. Details of the study sites used for pine marten scat and hair collection during Sept-Nov 2011 and 2012.

	Abernethy	**Mar**	**Inshriach**	**Darnaway**
Total forested area (km^2)	39	24	35	24
Total transect length (km)	106	74	96	80
No. scat collection sessions	3	3	3	3
No. hair collection sessions	4	5	5	4
No. scats collected	239	84	167	92

Plucked hair samples were collected using hair tubes fitted with 1 cm^2 sticky pads (Mullins *et al.* 2009) during Sept-Nov 2011 (Abernethy; Mar) and 2012 (Darnaway; Inshriach). Prior to surveying, hair tube placement locations were chosen using 1:25,000 Ordnance Survey maps, with one (Abernethy; Mar) or two (Inshriach; Darnaway) hair tubes placed in each 1 km^2 grid cell within each study area. For ease of access, hair tubes were placed in grid squares containing forest tracks only. In the field, fine scale placement was chosen based on the presence of woodland. Cells were excluded if no trees were present. A combination of Hawbakers marten lure (F&T Fur Harvester's Trading Post, 10681 Bushey Road, Alpena, MI 49707), peanut butter and bread were used as attractants as these have previously proven effective (Chandrasekhar 2005; Roche 2008; Burki *et al.* 2009).

Hair samples from each tube were collected in separate polythene bags and labelled with a unique identifier. All samples were frozen at -20 ˚C within 8 hrs and transferred to -80 ˚C within three weeks to await DNA analysis. Scat and hair collection was carried out simultaneously, with an extra one (Abernethy; Darnaway) or two (Mar; Inshriach) hair collection sessions held immediately after scat sampling had been completed for each successive forest. No hair samples were obtained for Darnaway, so this site was excluded from further analysis.

3.3.3 Genetic analysis

To avoid contamination, extractions were performed in an area of the laboratory reserved for DNA extraction. DNA extractions from hair samples were carried out using an adapted chelex-100 protocol (Walsh *et al.* 1991) with a negative control; a 1 cm root-section of hair was agitated in 200µl chelex (5%), 7 µl dithiothreitol (DDT) and 1 µl proteinase at 56 °C for approximately 5 hrs, centrifuged for 3 minutes and incubated at 95 °C for 10 minutes. DNA was stored at -20 °C. For scat samples, the QIAamp DNA stool mini kit (Qiagen, #51504) was used with a negative control. Both scat and hair samples were amplified using eight species-specific microsatellite markers (Mar08, Mar21, Mar36, Mar43, Mar53, Mar56, Mar58, Mar64; Natali *et al.* 2010) 10 µl reaction containing 0.4 µM forward and reverse primers, 5 µl Qiagen Type-it PCR mastermix, 1 µl Q solution and 2 µl of DNA template. After initial denaturation at 95 °C for 5 mins, 40 cycles of 95 °C for 30 s, 63 °C for 90 s and 72 °C for 30 s were used followed by a final extension step of 60 °C for 30 minutes. Fragment analysis was performed at DNA Sequencing and Services (University of Dundee, Scotland, DD1 5EH) with a negative and positive control. Samples were scored using GeneMarker (Version 2.4.0) and verified by eye.

Sex typing was performed using a 5′ nuclease TaqMan assay developed by Mullins (2009) and Real-time PCR using 5 µl Precision Master Mix (Primer Designs), 0.2 mM of either MMX or MMY forward and reverse primers and probes (see Appendix 2C) and 3 µl DNA template in a total volume of 10 µl. Amplification of ZFX (MMX) only signifies female DNA, whereas amplification of both ZFX and ZFY (MMY) signifies male DNA (Mullins *et al.* 2009). The PCR conditions were 2 minutes at 50 °C, 10 minutes at 95 °C, then 50 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Two replicate amplifications were performed for each primer/probe combination. For real-time product detection, Ct value (i.e. the number of PCR cycles needed to obtain the required quantity of DNA) was recorded at a ΔRn threshold of 0.2. Amplifications were deemed successful when the Ct value was below 40. A sex type was accepted when both replicate amplifications were successful.

Genotyping errors such as allelic drop out, where an allele from the consensus genotype fails to amplify; and false alleles, where an allele not present in the consensus genotype is amplified, can cause an overestimation of the number of individuals in a population, as samples from the same individual appear to differ at the loci containing an error. Conversely, low genetic diversity can cause the opposite bias, as more than one individual may have the same genotype at the selected loci. To reduce the possibility of these erroneous or concealed genotypes, consensus genotypes were obtained following the comparative multi-tubes approach, for which at least two positive PCR reactions are required to confirm a heterozygous genotype and three for a homozygous genotype (Frantz *et al.* 2003). A sample genotype was only accepted once a consensus was reached for all markers. A positive sex-type was not required for acceptance of an individual genotype, but samples with sex-types that did not match the consensus were discarded. One DNA extraction was carried out per sample, and error rates were quantified across all amplification attempts from this template DNA for each locus (Broquet & Petit 2004). To provide a level of confidence in the individual genotypes obtained, the probability of sampling more than one individual with the same genotype, or genotype probability (P_{gen}), was calculated for each unique genotype (Parks & Werth 1993). Genetic diversity was assessed using the mean number of alleles, observed heterozygosity (Ho) and expected heterozygosity (He) per locus, calculated in GIMLET version1.3.3 (Valiere 2002).

3.3.4 Population assessment

For population density estimation, a Spatially Explicit Capture Recapture (SECR) model was applied to hair genotypes (Borchers & Efford 2008) using the 'secr' package (v2.8.1; Efford 2014) in R version 3.1.0 (R core team 2014), with selection of full likelihood estimation for a Poisson distribution of home range centres; half normal spatial capture probability distribution; and detection via 'proximity detectors'. This type of detector can capture more than one animal during a sampling occasion but does not restrict their movement (e.g. camera traps; Borchers 2012). Hair tubes could potentially obtain a sample from more than one individual during a sampling occasion, allowing classification as a proximity detector; lack of amplifications containing more than two alleles, however, shows that this did not occur during our survey. A buffer was extended beyond the location of traps for each forest. To ensure that this buffer encompassed all habitat with a positive detection probability (i.e. area with a positive probability of occupation by a member of the population) models were tested with increasing buffer widths until a stable density estimate was reached. This provided buffer widths of 2 km for Abernethy and Inshriach and 3 km for Mar. To determine whether the sex ratio of animals within a forest differs from 1:1, a hybrid mixture model was fitted with fixed class membership (male or female) for individuals of known sex and class-specific detection parameters (g0 and σ). Deviation from the 1:1 sex-ratio was tested using a likelihood ratio test between the full model and a model with a fixed 1:1 mixing proportion.

Abundance estimates were calculated using the region.N function in the 'secr' package (v2.8.1; Efford 2014). Previous analyses on the effect of forest fragmentation used home range size (based on 100% minimum convex polygons) as an index of abundance rather than population density (Balharry 1993; Bright & Smithson 1997; Halliwell 1997; Caryl *et al.* 2012a). To enable comparison, our data were converted to 95% home range sizes for males and females using

$\pi*(2.24*\sigma)^2$ (Efford, unpublished data), where σ is the spatial scaling parameter of the detection function generated from the SECR models for each forest.

3.3.5 Fragmentation analysis

To allow comparison with previous data, the percentage forest cover and forest edge density (m/ha) were calculated as indices of forest fragmentation within a 9.77 km radius from the centre of each forest (300 km^2; Caryl *et al.* 2012a), using data from the Land Cover Map (LCM) 2007 land-use layer (Morton *et al*. 2011), and added to previous data (Balharry 1993; Bright & Smithson 1997; Halliwell 1997; Caryl *et al.* 2012a). The size of study sites was selected by Caryl *et al*. (2012) as the minimum convex polygon containing all home ranges of radio-tracked pine martens, plus a buffer of the average length of one female home range (1.8km) to ensure that the habitat composition represented that of the greater landscape. Separate generalised linear models with a log link function and Gaussian distribution were used to assess the effect of forest cover on home range size for male and female pine marten. A quadratic term for forest cover or edge density was included in the starting model and its significance tested using a likelihood ratio test between the starting model and a model with the linear term only. Correlation between the fragmentation indices was tested using cor.test in R version 3.1.0 (R core team 2014).

3.3.6 Scat count calibration

To test an association between the number of scats found per km of transect and population density, a generalised linear model with poisson distribution was performed between scat count values and average home range size for data from the four current sites and from previous studies (Bright & Smithson 1997; Halliwell 1997; Caryl *et al.* 2012b). For each study, time periods of between four days to one month were left between the clearance of transects and subsequent collection of scats. Home range estimates were made simultaneously to scat collections for each study. Pine marten scats have been observed with minimal deterioration after one month (L. Kubasiewicz, pers. obs.). To standardise conditions between studies, the number of scats per km was scaled to five days, assuming equal deposition of scats per day, and only collections carried out in autumn were included. Analyses were performed in R version 3.1.0 (R core team 2014).

3.4 Results

3.4.1 Genetic analysis

In total, 579 scats were collected from all forests over the three sampling occasions. A total of 1,920 amplification attempts were made across 76 samples at eight loci, with 29.6% success. A consensus genotype at all eight loci was gained for 6 samples (7.9%). Due to low levels of initial success with scats, individual genotyping was carried out for hair samples only.

Over all sampling occasions, a total of 115 hair samples were collected, with 31 samples from Abernethy, 53 from Inshriach and 31 from Mar (Appendix 3A). One locus (mar58) was monomorphic across all samples and was therefore excluded from further analyses. A total of 2079 amplifications attempts were made (115 samples, 7 loci) with a consensus genotype reached for 73% of loci. Overall, error rates were 6% allelic drop out and 7% false alleles. Per locus error rates were also slightly higher for false alleles, with a range of 2-14% compared to 0-11% for allelic drop out (Table 3.2). Sex-types were obtained for seven individuals from Abernethy (63%), five from Mar (83%) and 11 from Inshriach (100%).

An average of 3.3 alleles per locus was found across all samples. Overall, observed heterozygosity (Ho) was higher than expected heterozygosity (He), with values of 0.5 for Ho and 0.42 for He. Significant deviation from Hardy-Weinberg equilibrium was observed at two loci; mar53 ($\chi^2 = 23.9$, df = 3, $p < 0.001$) and mar64 ($\chi^2 = 62.8$, df = 6, $p < 0.001$). A consensus genotype at all seven loci was gained for 82 samples (71%). Genotype probabilities (P_{gen}) for individuals ranged from $1.9\text{x}10^{-5}$ (Individual 'AL', Abernethy) to $4.1\text{x}10^{-2}$ (Individual 'ME', Mar,

Appendix 3B); genotype probabilities for forests were 5.0×10^{-4} for Abernethy, 1.1×10^{-3} for Inshriach and 2.0×10^{-2} for Mar. Within Abernethy, four pairs of individuals differed at only one locus, whilst Mar and Inshriach contained three pairs each (Table 3.3).

Table 3.2. Rates of allelic drop out (ADO) and false alleles (FA); expected (exp) and observed (obs) heterozygosity; and number of unique alleles found for each marker for all hair samples collected from Abernethy, Mar and Inshriach combined.

Marker	ADO (%)	FA (%)	H (exp)	H (obs)	No. alleles
m08	11	2	0.63	0.6	5
m43	0	8	0.02	0.02	2
m56	8	15	0.51	0.8	5
m21	2	7	0.5	0.57	2
m53	6	4	0.6	0.72	3
m64	4	4	0.66	0.91	4
m36	0	4	0.01	0.01	2
Mean (all markers)	6	7	0.42	0.5	3.3

Table 3.3. The number of alleles that each pair of individuals differs by within a) Abernethy b) Mar and c) Inshriach. Individuals are represented by unique letter combinations in the left and top columns. Pairs that differ at one marker only are shown in bold, with the marker in parentheses. P_{gen} is the probability of more than one individual in the population having an identical genotype to each individual sampled.

a)

	AA	AB	AC	AD	AE	AF	AG	AH	AI	AK	P(gen)
AA											3.95x10^{-4}
AB	**1 (64)**										1.32x10^{-3}
AC	5	5									3.00x10^{-5}
AD	3	3	3								4.72x10^{-4}
AE	2	**1 (53)**	5	3							1.32x10^{-3}
AF	2	**1 (08)**	5	4	2						6.58x10^{-4}
AG	6	6	3	4	4	6					2.75x10^{-4}
AH	4	3	3	5	4	3	5				2.04x10^{-4}
AI	4	3	4	2	3	4	4	3			7.59x10^{-4}
AK	4	3	4	4	3	4	5	3	**1 (56)**		5.80x10^{-5}
AL	3	3	5	4	4	4	6	5	5	5	1.90x10^{-5}

b)

	MA	MB	MC	MD	ME	P(gen)
MA						2.04x10^{-2}
MB	**1 (64)**					2.72x10^{-2}
MC	4	3				4.08x10^{-2}
MD	4	3	3			9.08x10^{-3}
ME	3	2	**1 (56)**	3		2.04x10^{-2}
MF	4	3	2	4	**1 (43)**	2.48x10^{-3}

c)

	IA	IF	**IG**	IH	IJ	IL	IK	IE	ID	IB	P(gen)
IA											2.17x10^{-4}
IF	3										4.87x10^{-4}
IG	4	3									9.27x10^{-4}
IH	**1 (08)**	3	2								1.74x10^{-3}
IJ	2	4	3	**1 (56)**							9.13x10^{-4}
IL	4	3	4	3	5						5.70x10^{-4}
IK	3	3	**1 (08)**	3	4	3					1.52x10^{-3}
IE	2	3	3	3	4	3	2				1.74x10^{-3}
ID	4	2	3	3	4	4	2	2			1.06x10^{-3}
IB	3	3	3	3	4	3	3	3	3		2.78x10^{-3}
IC	3	3	2	3	4	3	2	2	3	2	2.35x10^{-4}

3.4.2 Density estimates

Of the 83 samples that provided a consensus genotype, 28 individual pine martens were identified, with a total of 55 recaptures. This equates to 11 unique genotypes for Abernethy, six for Mar and 11 for Inshriach. The maximum number of re-captures of an individual was 11, whilst 13 pine martens were only captured once (Table 3.4).

The proportion of males in the population did not differ significantly from 0.5 in Mar (proportion males = 0.43; $\chi^2 = 7.11$, df = 3, p = 0.068) or Inshriach (proportion males = 0.45; $\chi^2 = 0.07$, df = 1, p = 0.792), but was only 0.15 in Abernethy ($\chi^2 = 4.596$, df = 1, p = 0.032). Subsequent population density estimates were 0.38 km^{-2} (95% CI 0.11 – 1.07) for Abernethy, 0.07 km^{-2} (0.03 – 0.16) for Mar and 0.24 km^{-2} (0.13 – 0.45) for Inshriach. Population estimates were 17 (95%CI 12-47) for Abernethy, 6 (95%CI 6-11) for Mar and 11 (95%CI 11 – 24) for Inshriach (Table 3.5). Lower confidence intervals are constrained by the minimum population number of each forest.

Table 3.4. Visits to hair tubes in a) Abernethy b) Mar and c) Inshriach by individually identified pine marten, where unique letters represent each individual. All hair tubes were checked once per session. Each row represents a session.

a)

	Hair tube																
	A01	**A02**	**A03**	**A04**	**A05**	**A06**	**A07**	**A08**	**A09**	**A12**	**A13**	**A14**	**A15**	**A16**	**A17**	**A27**	**A33**
1	--	--	--	--	--	--	--	AB	--	--	--	--	--	--	--	--	--
2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	AD
3	--	AB	AB	--	--	--	AC	AB	AC	AF	--	AI	--	--	AL	AE	AD
4	AA	AB	AB	AD	AH	AG	--	--	--	--	AI	AK	--	AI	AI	--	--

b)

	Hair tube										
	M42	**M43**	**M44**	**M45**	**M46**	**M47**	**M48**	**M49**	**M50**	**M51**	**M59**
1	--	--	--	--	--	MD	MD	--	MB	MB	--
2	--	ME	MC	MA	MC	--	MB	MB	--	--	--
3	--	--	--	MA	--	MD	--	MD	--	--	--
4	--	--	--	--	MF	MD	--	--	MB	--	--
5	MB	--	ME	MA	--	MB	MD	MB	MB	MB	MC

c)

	Hair tube																		
	N01	**N11**	**N13**	**N21**	**N23**	**N24**	**N33**	**N39**	**N42**	**N43**	**N44**	**N46**	**N47**	**N48**	**N49**	**N50**	**N52**	**N53**	**N54**
1	--	--	--	--	--	--	--	--	--	--	--	--	--	IJ	--	IJ	--	--	--
2	--	--	--	IL	IL	IL	--	--	--	IJ	--	--	IJ	IJ	--	--	IH	--	--
3	--	--	--	--	IL	IL	IL	--	IK	--	IJ	--	--	--	IC	IG	IA	--	--
4	--	IF	--	IL	--	--	--	IB	--	--	IJ	ID	IC	--	--	IH	--	ID	ID
5	IE	IF	IF	IL	IL	IL	IL	--	IJ	--	--	--	--	--	--	IH	IH	--	--

Table 3.5. Population density and abundance estimates for pine marten in Abernethy, Mar and Inshriach. Sex-specific detection parameter estimates (g0, σ, pmix), where pmix represents the proportion of the population belonging to each sex, σ is the spatial scaling parameter and g0 is the magnitude of the detection function. Minimum population numbers (Min. pop.) represent the number of unique genotypes detected in each forest, and so the minimum population present. The population estimate (pop. Est.), standard error and confidence intervals are the model results from the region.N function in the "secr" package in R. Home range estimates were calculate using the formula $\pi*(2.24*\sigma)^2$ (Efford; unpublished data). Estimates are provided using the mean value of sigma within each forest between males and females, as well as for males and females separately.

	Parameter	**Estimate**	**SE**	**LCL**	**UCL**	**Min. pop.**	**Pop. Est.**	**SE**	**LCL**	**UCL**	**Home range (km^2)**
Abernethy	Density	0.384	0.217	0.113	1.076	11	17	6.9	12	47	7.0
Female	g0	0.156	0.125	0.028	0.543						3.63
	σ	480	185	232	995						
	pmix	0.849	0.106	0.523	0.967						
Male	g0	0.317	0.129	0.126	0.600						11.6
	σ	856	153	605	1212						
	pmix	0.151	0.107	0.033	0.477						
Mar	Density	0.065	0.029	0.028	0.148	6	6	0.8	6	11	18.8
Female	g0	0.368	0.129	0.164	0.633						21.9
	σ	1179	230	808	1721						
	pmix	0.535	0.236	0.153	0.881						
Male	g0	0.316	0.153	0.104	0.649						15.9
	σ	1006	203	679	1489						
	pmix	0.464	0.236	0.119	0.847						
Inshriach	Density	0.241	0.079	0.129	0.449	11	11	1.9	11	24	11.7
Female	g0	0.34	0.096	0.178	0.551						5.23
	σ	576	81	436	759						
	pmix	0.499	0.159	0.222	0.776						
Male	g0	0.413	0.023	0.013	0.120						19.9
	σ	1125	349	621	204						
	pmix	0.501	0.159	0.224	0.778						

3.4.3 Fragmentation analysis

Forest fragmentation ranged from 4.9% forest cover and 145.7 mha^{-1} forest edge (Mar) to 32.3% forest cover, 86.6 mha^{-1} forest edge (Abernethy), with Inshriach exhibiting intermediate values of 19.7% forest cover and 106.6 mha^{-1} forest edge. A strong negative correlation was found between edge density and forest cover, including data from previous analyses (Pearson correlation r = -0.856, df = 7, P = 0.003). Results are therefore presented for the analysis between home range size and percentage forest cover only. A highly significant non-linear relationship was found between percentage forest cover and female home range size (Fig 3.2; Table 3.6), with a decrease in home range size from 16 km^2 (95% CI: 4 – 55) to 4 km^2 (95% CI: 2 – 8) as forest cover increases from 4% to 20%, followed by an increase in home range size to 7 km^2 (2 – 22) as forest cover increases to 48%. The relationship between male home ranges and forest cover was non-significant (χ^2= 1.60, df = 1, p = 0.08).

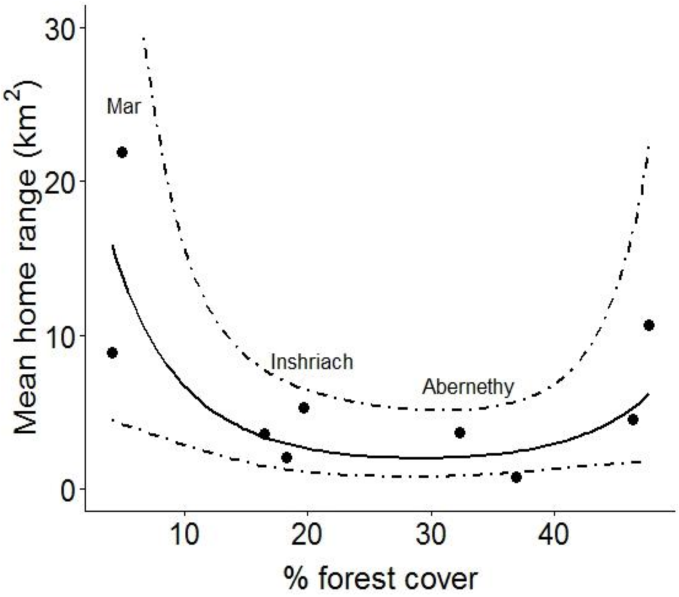


Figure 3.2. Average home range size of female pine marten with increasing levels of forest fragmentation (% forest cover). Data points are for observed data, solid lines represent predicted home range size from the GLMM and dashed lines represent the 95% confidence intervals for the model prediction calculated from 1,000 repeated model simulations using random draws from the estimated parameter distributions. Data from the current study were added to previous datasets from Balharry 1993; Halliwell 1997; Bright and Smithson 1997 and Caryl et al. 2008.

Table 3.6. Coefficient estimates for the GLM for the effect of forest cover on pine marten home range size. Estimates are for the full model. Log-likelihood χ^2 statistic and associated p-values are for the deletion of Cover^2 from the full model; or Cover from the model with main effects only.

***Microtus* voles**	**Estimate ± SE**	χ^2	**df**	**P**
Intercept	3.505 ± 0.739			
Cover	-0.194 ± 0.069	7.027	1	0.206
Cover^2	0.003 ± 0.001	3.313	1	0.009

3.4.4 Scat count calibration

A significant negative non-linear relationship was found between scat count and home range size (χ^2= 10.96, df = 1, p = 0.005; Fig 3.3). As scat count increases from 0.1 to 0.5 km^{-1}, there is a substantial decline in home range from 21 km^2 (14 - 32 km^2) to 4 km^2 (2 - 6 km^2), followed by a much more gradual decline to 3 km^2 (1 - 7 km^2) as scat count reached 1.5 km^{-1}.

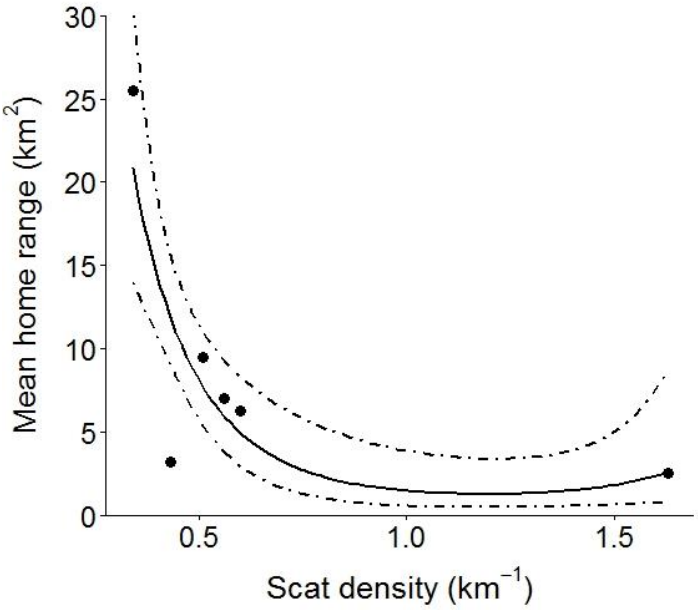


Figure 3.3. Average pine marten home range size with increasing scat count (scats km^{-1}). Data points are observed data, solid line is the predicted home range size from the GLM and dashed lines are the 95% confidence intervals for the model prediction calculated from 1,000 repeated model simulations using random draws from the estimated parameter distributions. Data were taken from the current study; Halliwell 1997; Bright and Smithson 1997 and Caryl et al 2008. Where home ranges were provided per sex, a mean was calculated. Scat counts were standardised to give values for day 5 between clearance and collection.

3.5 Discussion

We were able to provide the first estimates of population density for pine martens in Scotland using on a combination of non-invasive genotyping and SECR modelling. There was considerable variation in population density among forests with differing levels of forest fragmentation, providing a valuable baseline to support more sophisticated monitoring and refined management decisions.

The martens from the Scottish populations sampled here have relatively low allelic diversity (mean alleles per locus = 3.3) and heterozygosity ($H_o = 0.5$, $H_e = 0.42$) compared to the Italian population used to develop the microsatellites, which had a mean number of alleles per locus of 7.25 and mean H_o and H_e values of 0.713 and 0.77 respectively. The relatively recent population bottleneck experienced by Scottish pine martens may have had lasting impacts on the genetic diversity of the species, although a larger scale study incorporating more of their range in Scotland would be needed to confirm this. Compared to hair samples, scats are relatively easy to collect in large numbers, making this a preferable method of data collection for large scale studies. Scat samples are, however, particularly difficult to genotype due to the high levels of genotyping error associated with the poor quality DNA (Lucchini *et al.* 2002). Evaluation of alternative methods such as the use of SNPs (single nucleotide polymorphisms), which are more successful for degraded samples (Fabbri *et al.* 2012), may be of benefit in developing this approach for pine martens.

Despite relatively low genetic diversity, the probability of matching genotypes for individuals was <0.05 for Mar and <0.01 for both Abernethy and Inshriach, providing support that all repeat samples with the same genotype are from the same individual (Parks & Werth 1993). Genotyping errors are, however, higher than for previous studies using hair (Mullins *et al.* 2009; Baldwin *et al.* 2010; Fickel *et al.* 2012; Uno *et al.* 2012), meaning the number of individuals and resulting population densities may have been overestimated within the three forests. Further to this, the home ranges derived from previous data are based on mean 100% minimum convex polygons, whereas those from current data are 95% estimates. Currently, there is no tested formula to convert these estimates to 100% home ranges (Murray Efford, pers. comm.) Our home range sizes may therefore be slightly underestimated relative to those from previous studies included in the dataset. Forest cover in Mar is one of the lowest of all sampled forest sites. A lower population density (and larger home range size) here would serve to strengthen the finding that pine marten density is lowest at the extreme ends of the scale of forest fragmentation. Lower population density at Abernethy and Inshriach may reduce confidence in the relationship, but considering the strength of the existing relationship, is unlikely to alter the overall trend.

The current results provide significant support for previous findings that pine martens in Scotland are less forest dependent than the forest-specialist populations in the Eastern parts of their range (Pereboom 2008; Mortelliti *et al.* 2010; Caryl *et al.* 2012a). We provide a clearer picture of the level of forest cover that supports the highest population densities of pine martens in Scotland. The highest population densities (and smallest home ranges sizes) are found with forest cover of between 20% and 35% due to a balance between the availability of forest resources such as den sites (Birks *et al.* 2005) and the foraging opportunities provided by non-forested habitats (Caryl *et al.* 2012a). Further to this, we suggest that this trend is attributed to female pine martens, whilst males are less affected by the level of forest cover. Female pine martens are known to select their territory based on the availability of suitable resources for rearing young (Powell 1994), whilst males locate their territory with respect to the location of females (Caryl 2008). It follows that forest cover would have a more substantial effect on female population density, particularly the limiting effect of den site availability with lower levels of forest cover.

Home range sizes are known to be smaller for female martens compared to males (Balharry 1993; Caryl *et al.* 2012ab). The ratio of males to females within a population, however, has not previously been examined. Although the proportions of forest and non-forest habitat within an area is an important determinant of habitat suitability, non-forested areas support different ecological function, such as foraging or resting, depending on their composition (Dunford & Freemark 2005; Caryl *et al.* 2012a), and so the high proportion of females at Abernethy may be due to the composition of non-forest habitat within the site, although testing at further forest sites is advised to confirm this.

The calibration of a traditional methods of marten population monitoring (scat counts), provides the first evidence that pine marten density could be inferred from scat abundance. For populations at relatively low density, indicated by larger home range sizes, a strong negative relationship exists between scat abundance and home range size. As scat counts increases above one per km this relationship markedly declines. This trend is, however, strongly dependent on one data point and should therefore be viewed with caution. Several factors, such as the decay rates of scats, season and field conditions, the diet of the animals and misidentification of samples can affect the number of samples found at any given time (see Davison *et al.* 2002; Birks *et al.* 2004). Unfortunately, limited data prevents a more detailed analysis of these factors, although following examples from Marques (2001), Webbon (2004) and Brodie (2006), a more detailed analysis of the factors affecting scat counts (such as degradation rates) and incorporation of these factors into future modelling attempts may provide an invaluable management tool and greatly improve the ability to monitor this species on a much larger scale.

As pine martens continue to reoccupy their former range in Scotland, close monitoring may reveal further differences between the behaviour exhibited by Scottish populations and those elsewhere in Europe. As a native mammalian predator, one of few remaining in Britain, conservation management should seek to attain a balance between enabling pine martens to continue the recovery of their former range with the need to protect vulnerable prey species. This will require detailed knowledge of habitat preferences and behaviour of this elusive species; the effects of management on these factors; as well as the impact of land use choices leading to changes in forest cover. Here we provide evidence that non-invasive hair samples can enable the cost effective, large scale monitoring that would enable this balance to be achieved.

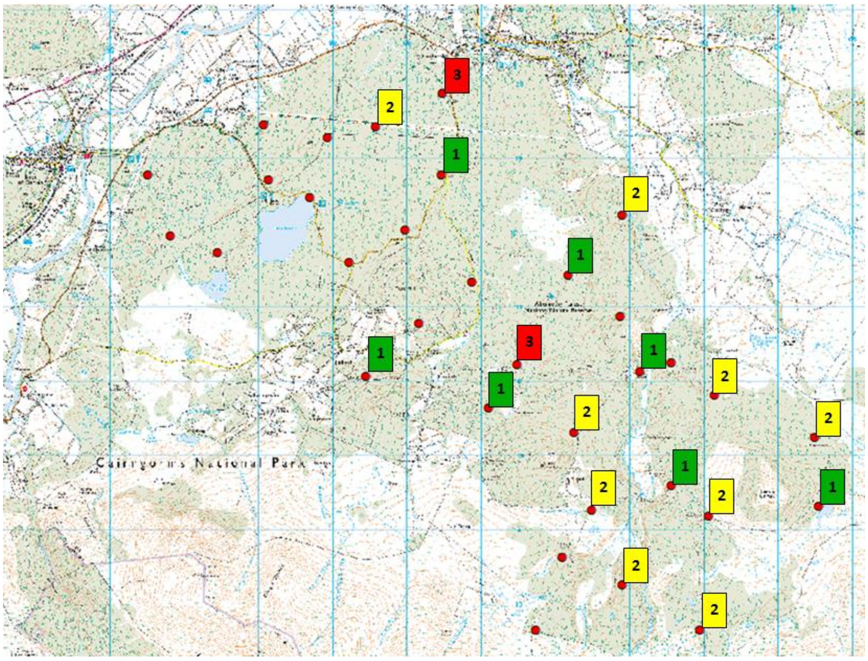
3.6 Acknowledgements

Thank you to David Bavin, Lizzie Croose, Tara Curry, Melissa Simmons and Kayleigh McCrory for field work; Stuart A'Hara, Bridget Lau and Catherine O'Reilly for advice on genetic analysis; Joan Cottrell and Hannah Feltham for comments on previous drafts; David Borchers for advice of SECR modelling.

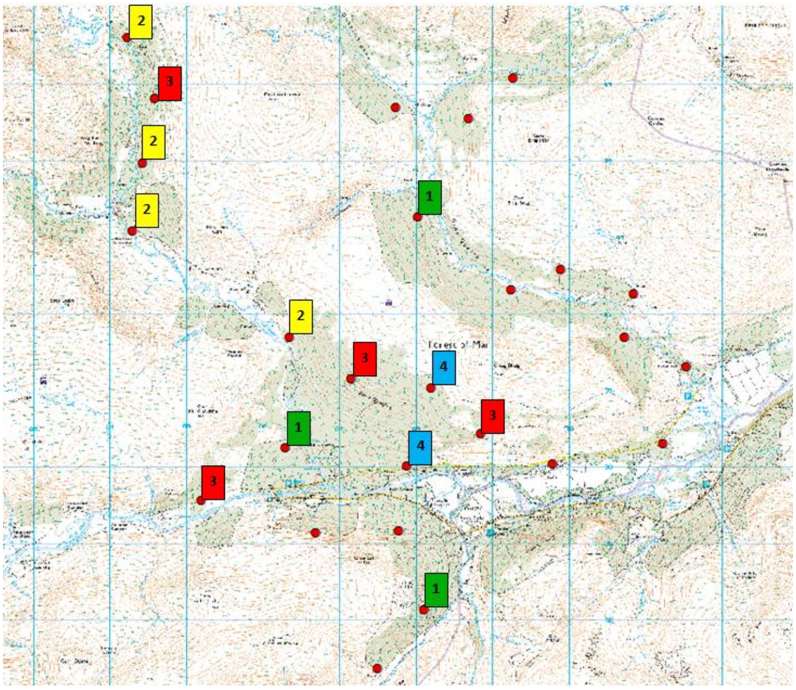
Appendix 3A

Hair tube placements within a) Abernethy b) Mar and c) Inshriach. Each red dot represents one hair tube. The coloured boxes indicate how many visits were received by each hair tube during the entire study period, where green = 1, yellow = 2 and red = 3 visits.

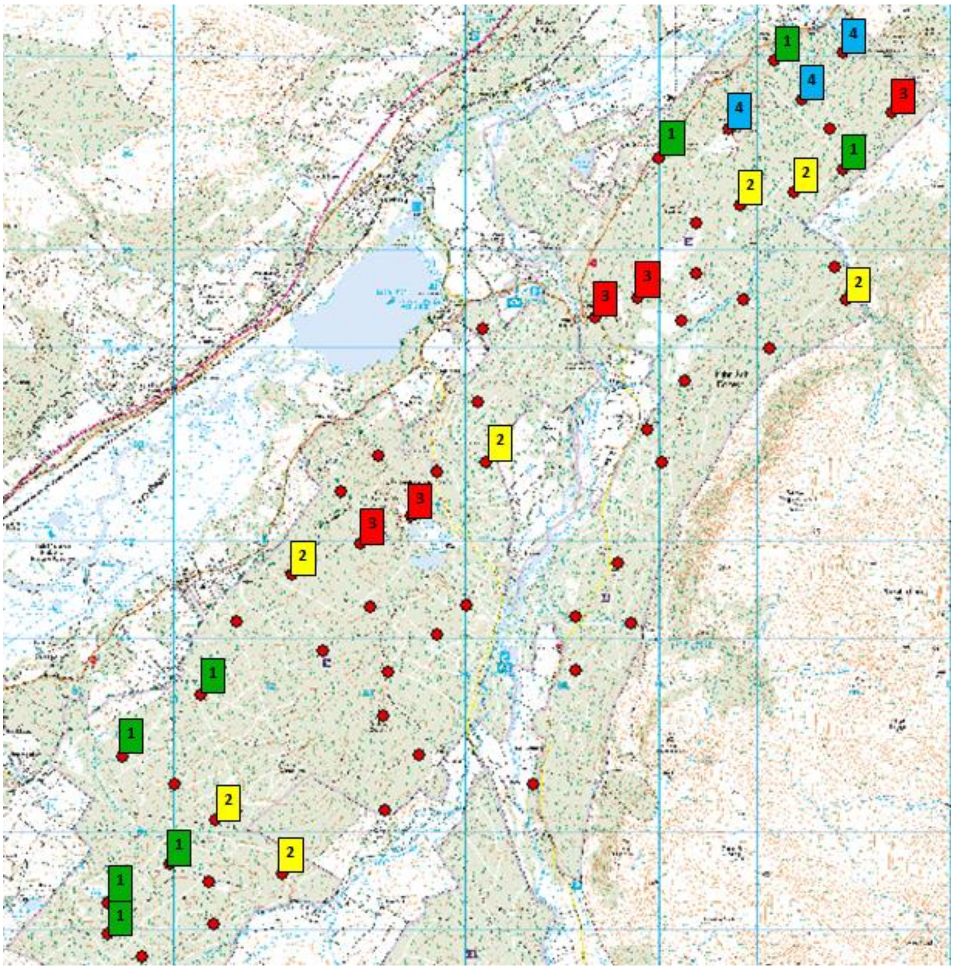
a)



b)



c)



Appendix 3B. Consensus genotypes and sex types of individual samples within Abernethy, Mar and Inshriach during autumn 2011 and 2012.

Site	Individual	Mar-56		Mar-21		Mar-64		Mar-08		Mar-58		Mar-43		Mar-53		Mar-36		P(gen)	Sex
Abernethy	AA	212	212	175	185	191	191	148	156	246	246	156	156	245	247	227	227	3.95×10^{-4}	--
	AB	212	212	175	185	183	191	148	156	246	246	156	156	245	247	227	227	1.32×10^{-3}	M
	AC	214	214	175	175	183	185	148	158	246	246	156	156	239	245	227	227	3.00×10^{-5}	--
	AD	212	214	175	185	183	185	148	156	246	246	156	156	245	245	227	227	4.72×10^{-4}	F
	AE	212	212	175	185	183	191	148	156	246	246	156	156	247	247	227	227	1.32×10^{-3}	--
	AF	212	212	175	185	183	191	156	156	246	246	156	156	245	247	227	227	6.58×10^{-4}	M
	AG	212	214	175	175	183	185	156	158	246	246	154	156	239	245	227	227	2.75×10^{-4}	F
	AH	214	214	175	175	183	191	148	148	246	246	156	156	245	247	227	227	2.04×10^{-4}	F
	AI	212	214	175	175	183	191	148	156	246	246	156	156	239	247	227	227	7.59×10^{-4}	--
	AK	210	214	175	175	183	191	148	156	246	246	156	156	239	247	227	227	5.80×10^{-5}	F
	AL	212	212	185	185	183	185	148	156	246	246	156	156	245	247	227	245	1.90×10^{-5}	F
Mar	MA	212	214	185	185	183	183	148	148	246	246	156	156	245	247	227	227	2.04×10^{-2}	F
	MB	212	214	185	185	183	185	148	148	246	246	156	156	245	247	227	227	2.72×10^{-2}	F
	MC	214	214	175	175	183	185	148	148	246	246	156	156	245	245	227	227	2.04×10^{-2}	M
	MD	212	212	175	175	183	185	148	150	246	246	156	156	245	247	227	227	2.48×10^{-3}	M
	ME	212	214	175	175	183	185	148	148	246	246	156	156	245	245	227	227	4.08×10^{-2}	--
	MF	212	214	175	175	183	185	148	148	246	246	154	156	245	245	227	227	9.08×10^{-3}	F
Inshraich	IA	212	214	175	185	183	191	146	148	246	246	156	156	245	245	227	227	2.17×10^{-4}	F
	IB	212	214	175	185	183	185	158	158	246	246	156	156	239	239	227	227	4.87×10^{-4}	F
	IC	212	214	175	185	171	183	158	158	246	246	156	156	239	247	227	227	9.27×10^{-4}	M
	ID	212	214	175	185	171	191	156	158	246	246	156	156	245	247	227	227	1.74×10^{-3}	M
	IE	212	214	175	185	185	191	156	158	246	246	156	156	239	247	227	227	9.13×10^{-4}	F
	IF	212	214	175	185	191	191	148	156	246	246	156	156	245	247	227	227	5.70×10^{-4}	M
	IG	212	214	175	185	171	183	148	158	246	246	156	156	239	245	227	227	1.52×10^{-3}	M
	IH	212	214	175	185	183	191	148	158	246	246	156	156	245	245	227	227	1.74×10^{-3}	F
	IJ	214	214	175	185	183	191	148	158	246	246	156	156	245	245	227	227	1.06×10^{-3}	F
	IK	212	214	175	185	171	183	156	158	246	246	156	156	239	245	227	227	2.78×10^{-3}	M
	IL	212	214	175	175	183	185	156	156	246	246	156	156	239	247	227	227	2.35×10^{-4}	F

Chapter 4

Dietary composition between the sexes for European pine martens in fragmented landscapes

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4.1 Abstract

Variation in diet between demographic groups within animal species can result from a number of physical and behavioural differences such as size dimorphism, variation in home range size or different energy requirements for breeding or rearing young. For species of conservation concern or those under management, knowledge of these differences may increase the efficacy of interventions; for example, where management involves the provision of food, efforts can be directed towards the relevant target groups. The pine marten is a generalist predator that largely preys on small mammals, particularly voles native to Europe, although populations in the UK are now largely restricted to Scotland. In contrast to the preference for *Myodes* voles found in mainland Europe, Scottish populations show a strong preference for *Microtus* voles and include open habitats within their home ranges. Despite this preference, there is some evidence for frugivory in Scottish populations which we theorise may increase in areas of more continuous forest cover due to the consumption of berries associated with forested habitats. Male martens are larger, have bigger home ranges and utilise more open spaces, which may lead to higher *Microtus* vole consumption. We examined the effect of forest cover and sex on autumn diet breadth and composition for pine marten populations in Scotland. We found a 5-fold increase in the percentage estimated weight intake of fruit in the diet (from 2% to 10%) as forest cover increased from 4% to 47%. There was no evidence of dietary niche partitioning between the sexes in terms of *Microtus* consumption. Detailed knowledge of diet composition will greatly improve the chances of effective management.

4.2 Introduction

Dietary niche partitioning between demographic groups within a species, such as between sexes or ages, can result from a number of behavioural and physical differences. Sexual size dimorphism is relatively common, particularly in mammals, with males often being larger than females (Trivers 1972; Isaac 2005; Zalewski 2007). This difference can lead to variation in diet as males can exploit larger prey than females (Tambling *et al.* 2014). Behavioural differences may also lead to variation in resource utilisation and this can be reflected in the diet. In territorial animals, for example, males may occupy larger home ranges than females and therefore have access to different food sources (Erlinge 1979; Moors 1980; Zalewski 2007). Restricted movement when rearing young may alter food availability depending on the nurturing role of each parent (e.g. Adélie penguins; *Pygoscelis adeliae*; Clarke *et al.* 1998). Solitary species with separate home ranges for animals of the same sex but overlapping ones between sexes promote inter-sexual competition and segregation of food resources (Zalewski 2007). For generalist predators, dietary profile may also be altered on both a local and landscape scale, as individuals switch to the most abundant or accessible prey given the time of year or their location (e.g. Wolf; *Canis lupus*; Capitani *et al.* 2004; European pine marten; *Martes martes*; Balestrieri *et al.* 2010).

Habitat alterations, during land management or harvesting, can have a profound effect on the diet (Hargis, Bissonette & Turner 1999). When forest fragmentation results in the loss of favourable habitat for a predatory species, the persistence of the population may rely upon its ability to adopt a more generalist diet to replace the potential loss of a favoured prey (Zalewski 2007), but differences in life history and behaviour make the likely adaptations of each species difficult to predict (Laurance 1991). Knowledge of the effect of habitat modifications on diet is important for conservation, as the results may have negative consequences for both the species affected and the wider ecosystem (i.e. higher predation rates of endangered species; Vilardell *et al.* 2012).

Dietary data for mammals is most often derived from looking at prey remains in faecal samples, and presented as the frequency of occurrence (i.e the frequency within which the remains of prey items appear in faeces). This measure, however, assigns the same importance to all items in the diet regardless of their size or energy content and therefore tends to overestimate the importance of items such as berries or invertebrates, which are small, but more likely to be consumed in high numbers (Lockie 1961; Balharry 1993). In contrast, indices based on the volume of prey consumed, such as the estimated weight intake, provide an estimate of the biomass consumed by multiplying each item found in the scat by a correction factor based on the weight of the item, derived from feeding trials with captive animals. Although this latter approach overcomes the problems of overestimation associated with frequency based methods, the captive populations used to derive correction factors live under different conditions than the wild populations under study and tend to have a narrower dietary profile, leading to potential differences in the identifiable material obtained in the scat (Klare, Kamler & Macdonald 2011). This method, however, delivers the most ecologically relevant method as it provides a realistic representation of the actual diet (Klare *et al.* 2011).

The pine marten is widely distributed across Europe, as well as parts of northern and central Asia (Balestrieri *et al.* 2010). Despite the species long-considered status as a closed-canopy forest specialist (Brainerd *et al.* 1995; Kurki *et al.* 1998), populations in Scotland display notable differences in habitat and dietary preference to those elsewhere, particularly in Eastern Europe. Previous research in Scotland suggests that population density peaks with moderate levels of forest fragmentation (i.e. increased forest edge density or decreased forest cover) and pine martens actively select areas of tussock grassland to include in their home ranges (Caryl *et al.* 2012a). This difference in habitat selection is reflected in their diet as, unlike the preference for bank voles (*Myodes glareolus*) found in populations in Eastern Europe, Scottish populations show a strong preference for field voles (*Microtus agrestis*; Putman 2000; Caryl *et al.* 2012b). *Microtus* voles become more prominent in the diet as forest fragmentation increases (Caryl *et al.* 2012b), although effects of fragmentation on other components of the diet remain unclear.

Whilst *Microtus* voles remain the most important item in the diet for much of the year, fruit forms a major part of the diet during the autumn and pine martens focus on this food source once availability increases in autumn, regardless of the high abundance of small mammals during this time of year (Rosellini, Barja & Pineiro 2008; Caryl *et al.* 2012b). Despite the small amount of energy provided per item, frugivory may be a more efficient foraging strategy than hunting when large amounts of fruit are easily accessible. The most common berries found in the pine martens diet are rowan (*Sorbus aucuparia*) and blaeberry (*Vaccinium myrtillus*; Caryl *et al*. 2012b), the latter being most prolific in woodland understories and heathland (Welch *et al.* 1994). As heath moorland (e.g. dominated by *Calluna vulgaris* or *Nardus carex*) is actively avoided by pine martens (Caryl *et al.* 2012a), the majority of berries are most likely consumed in forested habitats and frugivory may decline in areas with lower levels of forest cover.

Limited research exists on variation in the diet between male and female pine martens (Balharry 1993; Zalewski 2007), although some evidence suggests that food-niche partitioning results from behavioural, rather than physiological, differences (Zalewski 2007). In central and Eastern Europe, variation in the consumption of small mammals has been found between the sexes, but the dominant consumer varies between locations (Zalewski 2007). In Scotland, males and females vary in their habitat use, both at the landscape scale (relative proportions of habitats within the home range) and the local scale (i.e. the intensity of use of habitats within their home range; Caryl *et al.* 2012a). Male pine martens occupy home ranges on average three times larger than females (Balharry 1993; Bright & Smithson 1997; Halliwell

1997; Caryl *et al.* 2012a) and select open habitats, such as tussock grasslands, to a higher degree than females (Caryl *et al.* 2012a). Males may, therefore, encounter *Microtus* voles more often than females which may lead to higher consumption.

As a generalist predator, the diet of pine martens includes other items such as invertebrates, amphibians and birds depending on the season and the availability of prey (Caryl 2012b; Zalewski 2004). With the expansion of the pine martens range, there is concern by some stakeholders in Scotland about the depredation of chick of vulnerable ground-nesting bird species, such as capercaillie, by pine martens (Summers *et al.* 2009). Diversionary feeding of pine martens has been suggested as a management strategy to reduce this risk, either by altering the foraging range of pine martens, or diverting their attention from ground nests (Ron Summers pers. comm.). The efficacy of diversionary feeding varies greatly depending on the behaviour of the target species, particularly where this differs between demographic groups and different landscapes (Chapter 5). Diversionary feeding can be a relatively expensive management strategy, stakeholders, therefore, need to ensure that efforts are focused towards the demographic group causing the damage (Redpath 2001), or within the most sensitive areas. In the case of pine martens, knowledge of the conditions under which depredation of capercaillie nests is more likely (such as the level of forest cover) or whether a specific group within the population (such as one gender) depredate chicks at a higher rate is the first step to enabling this targeted approach. There is evidence to suggest that females may prey on birds more than males for populations in central and Eastern Europe (Zalewski 2007), although the habitat and behavioural differences for populations in Scotland mean that further investigation is required. Here, we test the effects of sex and forest cover on the autumn diet of pine martens populations in Scotland. We use two data sets for these analyses; the first includes four forests where scats were sex-typed using molecular techniques; the second includes an additional five forests, for which we do not have data on the sex of the martens, but enables a more thorough analysis of the effect of forest cover on the diet. Our aims are as follows:

1. Assess whether frugivory decreases in forests with lower levels of forest cover;
2. Test the hypothesis that an increased selection for tussock grasslands by males leads to a higher consumption of *Microtus* voles, and consequently a narrower niche breadth, compared to females.
3. Assess whether pine martens will experience a narrower dietary niche breadth with lower forest cover as hunting efforts are focused on utilising their preferred source of prey, the *Microtus* vole.
4. To further the understanding of the factors affecting ground-nesting bird predation. We test whether the sex and percentage forest cover affect the proportion of birds in the diet.

4.3 Methods

4.3.1 Study Sites

Scat samples were collected from four sites in Scotland during mid-September to mid-November 2011 and 2012. Abernethy (57°15′N, 3°40′W) is a Royal Society for the Protection of Birds reserve containing approx. 36km^2 of Scots pine (*Pinus sylvestris)* plantation and ancient native pinewood (Summers *et al.* 2010). Mar (57° 00 N, 3° 37 W) is a National Trust for Scotland site consisting of Caledonian pinewood located along Glen Lui and Glen Quioch. Inshriach (57° 06 N, 3° 56 W) is a Forestry Commission owned site in the North Cairngorms consisting of managed Scots pine plantation with some areas of Caledonian pinewood (Twiddle & Quine 2011). Darnaway (57° 33 N, 3° 45 W) is managed by Moray Development Company Ltd and contains commercial Scots pine, Sitka spruce (*Picea sitchensis*), Douglas fir (*Pseudotsuga menziesii*) plantation and some deciduous woodland.

4.3.2 Scat Sampling

Scat samples were collected from transects running along vehicle tracks and footpaths. To ensure that scats were as fresh as possible, all transects were cleared five days prior to sample collection. The total length of transects for each forest was 106 km (Abernethy), 74 km (Mar) 96 km (Inshriach) and 80 km (Darnaway). All transects were then checked once during each five day (Mar; Inshriach) or six day (Darnaway; Abernethy) sample collection session. The length of a sampling session was determined by the length of time taken to check all transects. Three consecutive sampling sessions were held in total for each forest.

Pine marten scats were determined by sight and scent (Corbet 1996). Although morphology can vary greatly between scats, a typical scat is black, 8 – 10 cm long with a tapered twist at the end; all surveyors were trained in scat identification by Rob Coope (Forest Research) or the author. A small sample was collected using a fresh toothpick and placed in a small sample tube and labelled for DNA analysis. The remaining scat was then collected in a small pot and labelled with the same identifier for diet analysis. All scats were photographed and location recorded (using a handheld GPS). All samples were frozen at -20 ˚C within 8 hrs of collection and transferred to -80 ˚C within three weeks to await DNA and dietary analysis.

4.3.3 Scat-content analysis

Scats were defrosted, placed in a Petri dish with 60% ethanol and examined using a dissecting microscope with 6.3 x magnification. Dietary items were identified to categories; small mammal, large mammal, bird, invertebrate, plant material, or miscellaneous using a reference collection. Within these broad categories, items were then identified to species level wherever possible (Appendix 4A). Time limitations meant that the identification of small mammal species from hair was not possible, therefore species identification was carried out using teeth where present (54% of small mammal occurrences). Carrion was identified by the presence of a dark, viscous substance (Balharry 1993) and identified to species level by the presence of hair where possible. Birds were identified from unique characteristics of the bones and from feathers. Although limited time prevented the identification of bird species, bird remains were, where feathers were obtained, recorded as ‘large’ or ‘small’ bird depending on quill diameter (large >2 mm, small ≤2 mm; Caryl 2012a).

Contents were recorded using both a frequency-based method and a volumetric (biomass) method (Klare *et al.* 2011). First, the frequency of occurrence of each item per scat (FO) was calculated, where the presence of an item was recorded for each scat, then the total number of each item divided by the number of scats. The estimated weight intake (EWI) was then recorded using the following method; the number of diet items within each taxa was counted within each scat; for berries, the number of seeds was divided by the average number of seeds for that species (see Coope 2007); for other taxa, a minimum number of items per taxa was obtained by counting legs (invertebrates) or teeth (small mammals). Large mammals and birds were counted as presence only. The number of items for each taxa was then multiplied by a correction factor to provide an estimate of the biomass consumed and, therefore, energy supplied by each diet item. Correction factors were taken from previously published feeding trials with captive pine martens (Balharry 1993). If a diet item did not fall into one of the categories for which a correction factor was available, a conservative estimate was provided by using the correction factor for the smallest diet item within the group of broad taxa to which the item of interest belonged (Appendix 4A).

In order to examine the association between percentage forest cover and diet, we included data from five forest sites used in previous analyses of pine marten diet in Scotland (Balharry 1993; Bright & Smithson 1997; Halliwell 1997; Caryl *et al.* 2012a), using measures of EWI for the main diet categories during autumn. EWI data was selected as it was the only common measure presented in all papers with the exception of Halliwell (1997). Here, FO (measured as the frequency of occurrence within all dietary items) was converted to EWI using the correction factors from Balharry (1993).

4.3.4 Sex and species identification

Genomic DNA was extracted from scat samples using the QIAamp DNA stool mini kit (Qiagen, #51504) with a negative control included in each round of extraction. Extractions were carried out in a designated DNA extraction area to avoid contamination. Template DNA was stored at -20 °C until needed for analysis. Species identification was verified by detection of at least one sex-typing primer (MMX, MMY; Mullins *et al.* 2009), or species specific primer (Mar08, Mar21, Mar36, Mar43, Mar53, Mar56, Mar58, Mar64; Natali *et al.* 2010) during attempted sex typing and individual identification as decribed in chapter 3.

4.3.5 Habitat analysis

Forest fragmentation was calculated for each site as percentage forest cover within a 9.77 km radius (300 km^2) from the centre of each forest (Caryl *et al.* 2012a), using data from the LCM 2007 land-use layer. A 9.77 km radius was selected by Caryl et al. (2012) as the minimum convex polygon containing all home ranges of radio-tracked female pine martens, including a buffer the length of one home range to ensure that the habitat composition represented that of the wider landscape. Measures of forest cover for five additional sites used in the analysis (Balharry 1993; Bright & Smithson 1997; Halliwell 1997; Caryl *et al.* 2012a) were taken from Caryl et al. (2012). Although forest edge density is often used as an index of fragmentation, a strong negative linear correlation exists between edge density and forest cover for the forests included in the current analysis (chapter 3), negating the need to include both.

4.3.6 Statistical analysis

Diet breadth was estimated using the standardized Levin's index (B_{sta}; Krebs 1989), using EWI data in the 'RInSp' package for R (version 1.0; Zaccarelli, Bolnick & Mancinelli 2013) in R version 3.1.0 (R core team 2014). Values are bound between 0 and 1, with larger values representing a wider niche breadth (Colwell & Futuyma 1971). Values were obtained for each sex (n=2; 134 scats) and each forest (n=4; 546 scats; Table 4.1), then used to examine differences between the sexes and between different levels of forest cover.

To determine whether lower forest cover is associated with lower levels of frugivory and altered consumption of birds, separate generalised linear models (GLMs) with a logit link function and binomial distribution were fitted. Data were included from previously published studies encompassing five forests (n=9 for each model). The %EWI (of plant material and birds respectively) was used as the response variable. A quadratic term for forest cover was included in the starting model and removed if a likelihood ratio test between models with and without the quadratic term was non-significant. To test whether lower forest cover results in narrower dietary niche breadth, the same method was used but with the standardized Levins indices as the response variable.

To test whether males consumed more *Microtus* voles than females, individual scats were scored for presence or absence of *Microtus* spp (response variable) in a GLM with binomial distribution. Three forest sites were included (n=130 scats per model; Mar was removed as only four scats were successfully sex-typed). 'Sex' and 'forest ID' were included as categorical variables. The analysis was then repeated with the presence or absence of bird remains as the response variable. Due to a high percentage of 'unknown' small mammal remains (46%), the analysis was repeated with this category as the response variable.

4.4 Results

4.4.1 Autumn diet

A total of 546 scats were analysed for diet content; 239 from Abernethy, 84 from Mar, 167 from Inshriach and 92 from Darnaway. Of the 54% of small mammal remains identified to species level, 86% were *Microtus* voles. In terms of energy intake, 40% EWI was provided by small mammals, 27% by birds and 24% by large mammals. Only 3% of total energy intake was provided by plant material (Fig 4.1; Table 4.1). Items with the highest frequency of occurrence were invertebrates (67%) and small mammals (55%), with plants being the third most frequent item, found in 39% of scats.

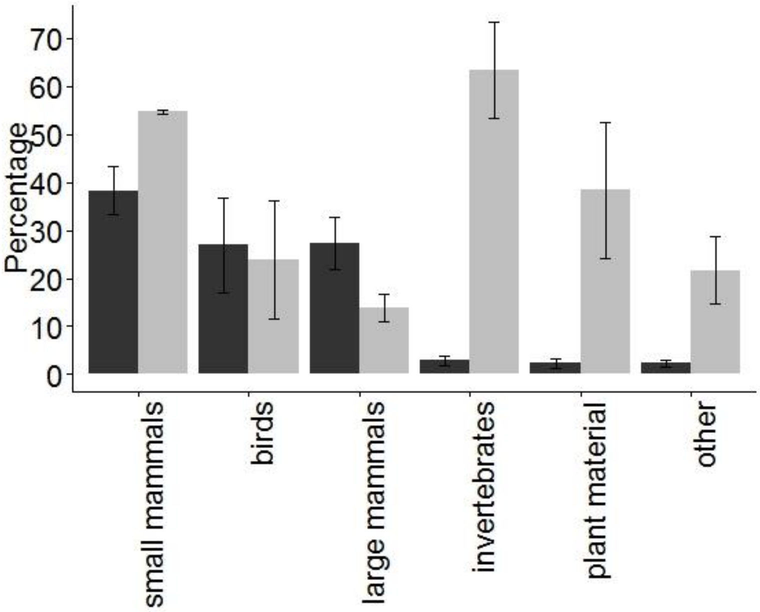

Figure 4.1. Diet composition for pine marten in Abernethy, Mar, Inshriach and Darnaway forests in Scotland. Figures are the mean values for all scats for all forests combined ± SE, presented as the % estimated weight intake (dark grey bars) and % frequency of occurrence (light grey bars) of diet items per scat.

Table 4.1. Composition of the pine marten diet for the main dietary categories in four forests where scats were sex-typed (n=134) and for all forests combined (n= 546). Data for *Microtus* voles (*Microtus agrestis*) are also provided due to the importance of this item in the diet. Data are presented as the percentage frequency of occurrence within scats (%FO), and percentage estimate weight intake (%EWI).

	All forests		Abernethy		Mar		Inshraich		Darnaway		Males		Females	
	%FO	**%EWI**	**%FO**	**%EWI**	**%FO**	**%EWI**	**%FO**	**%EWI**	**%FO**	**%EWI**	**%FO**	**%EWI**	**%FO**	**%EWI**
SMALL MAMMALS	54.58	39.59	54.30	46.01	55.84	39.03	53.89	44.14	54.76	23.78	40.48	39.85	52.17	34.42
Microtus agrestis	*24.91*	*21.08*	*28.51*	*35.27*	*16.88*	*22.0*	*24.55*	*30.10*	*23.81*	*17.7*	*30.95*	*31.28*	*16.30*	*19.76*
LARGE MAMMALS	11.90	23.96	7.24	24.51	20.78	42.31	11.98	25.91	15.48	16.40	9.52	22.86	9.78	18.50
BIRD	19.41	27.07	9.50	14.31	9.09	14.71	16.17	22.82	60.71	56.10	26.19	30.53	27.17	41.11
INVERTEBRATE	67.40	3.51	73.30	5.82	33.77	1.41	71.26	2.74	75.00	1.58	19.05	0.83	48.91	2.43
PLANT MATERIAL	39.01	3.02	18.10	4.80	12.99	0.52	73.65	2.86	48.81	1.03	57.14	1.95	54.35	2.18
OTHER	18.68	2.85	15.84	4.58	15.58	2.10	12.57	1.50	42.86	1.13	0.10	3.98	15.22	1.36
NO. SCATS	546		221		77		167		81		92		42	

4.4.2 Effect of forest cover

Diet breadth between the four forests in the current analysis ranged from the most specialised diet at a Levins index of 0.30 (Darnaway) to the most varied at 0.47 (Abernethy). For the analyses including all forests from the current and previous studies, there was no association between forest cover and diet breadth (χ^2 = 7.1, d.f = 8, p = 0.753), %EWI of birds in the diet (χ^2 = 1.03, df = 8, p = 0.685), or %EWI of plant material (χ^2 = 1.44, df = 8, p = 0.537).

4.4.3 Diet comparison between sexes

In total, 134 scats (25%) were successfully sex typed, of which 92 (69%) were from female pine martens and 42 (31%) were from males. Diet breadth was similar for both sexes (Table 4.2). *Microtus* voles were present in a significantly higher number of scats from males (0.33; 95% CI 0.20-0.50) than females (0.16; 95% CI 0.08-0.23); although the opposite trend was found for unknown small mammals, with a higher proportion in scats from females (0.29; 95% CI 0.20-0.39) than males (0.05; 95% CI 0.12-0.26; Fig 4.2, Table 4.3). *Microtus* vole consumption varied between forests, with scats from Abernethy containing *Microtus* vole remains three times higher than Inshriach, and four times higher than Darnaway (Fig 4.3; Table 4.3). The proportion of birds did not differ significantly between male and female scats, but differed significantly between forests, with bird consumption three times higher in Darnaway than the other sites (Fig 4.3; Table 4.3).

Table 4.2. Diet breadth, as the standardised Levins index, when the main diet categories of small mammals, large mammals, birds, invertebrates, plant material and other items are included, and when all sub-categories (species-specific identification) are included. Results are given for each forest separately (including scats that could not be sex-typed), and for males and females separately.

	Forest				**Sex**	
	Abernethy	**Mar**	**Inshriach**	**Darnaway**	**Male**	**Female**
Levins index	0.47	0.37	0.43	0.30	0.45	0.42

Table 4.3. Results of separate GLMs for the effect of sex on the proportion of pine marten scats containing *Microtus* voles, unknown small mammals and birds. Abernethy is the comparison site for the factor 'forest'. Estimates are for the full model. Log-likelihood χ^2 statistic, error degrees of freedom and associated p-values are for the deletion of each term from the full model.

***Microtus* voles**	**Estimate ± SE**	χ^2	**df**	**P**
Intercept	-0.692 ± 0.413			
Sex (males)	1.140 ± 0.482	5.707	1	0.017
Forest (Darnaway)	-1.857 ± 0.757	9.338	1	0.025
Forest (Inshriach)	-1.390 ± 0.526			
Unknown small mammals				
Intercept	-0.733 ± 0.430			
Sex (males)	-1.052 ± 0.535	4.457	1	0.035
Forest (Darnaway)	-0.413 ± 0.661	3.003	1	0.391
Forest (Inshriach)	-0.207 ± 0.511			
Birds				
Intercept	-1.486 ± 0.502			
Sex (males)	-0.197 ± 0.461	0.184	1	0.668
Forest (Darnaway)	2.025 ± 0.642	19.697	1	<0.001
Forest (Inshriach)	0.150 ± 0.575			

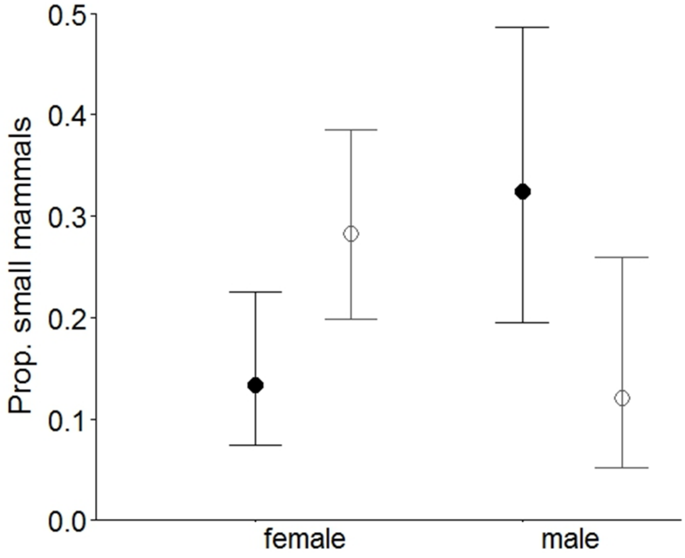

Figure 4.2. Proportion of scats from female and male pine marten that contain *Microtus* voles (black, closed points) and unknown small mammals (grey, open points). Data points represent predicted proportion from the GLM (Table 4.3) and error bars represent the 95% confidence intervals for the model from 1,000 repeated model simluations using random draws from the estimated parameter distributions.

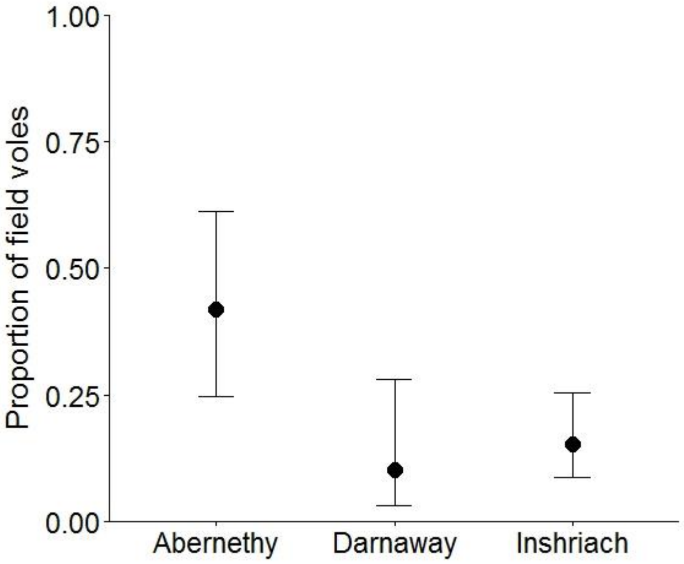

Figure 4.3. Proportion of scats per forest that contain *Microtus* voles. Data points represent predicted proportions from the GLM (Table 4.3), error bars represent the 95% confidence intervals for the model from 1,000 repeated model simulations using random draws from the estimated parameter distributions.

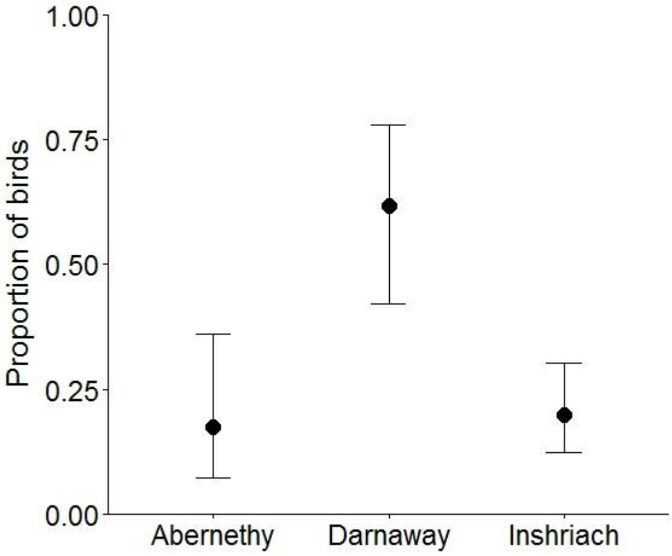

Figure 4.4 Proportion of scats per forest that contain bird remains. Data points represent predicted proportions from the GLMM (Table 4.3) and error bars represent the 95% confidence intervals for the model from 1,000 repeated model simluations using random draws from the estimated parameter distributions.

4.5 Discussion

Our data provide a detailed analysis of within- and between- population differences in the autumn diet of pine martens in Scotland. The data presented here agrees with previous findings that the most important dietary categories in autumn in terms of energy intake are mammals (Balharry 1993; Bright & Smithson 1997; Halliwell 1997; Caryl *et al.* 2012b), and provides evidence that birds also constitute a high proportion of biomass during autumn. Although our results support previous findings that fruit constitutes a large proportion of the diet as evaluated by frequency of occurrence, it contributes relatively little to overall energy intake. However, when abundant, fruit constitutes an easily accessible source of food without the expenditure of energy required for hunting small mammals or birds.

Although *Microtus* voles were present in twice as many scats from males than females, the opposite trend was found in the proportion of scats containing unknown small mammals.

Studies on captive red foxes show that the ratio of teeth from different small mammals species found in scats is a reliable measure of the ratio in which these species where consumed (Lockie 1959), justifying the re-allocation of unknown small mammals (i.e. those recovered without teeth) into species groups based on relative recovery rates of teeth (Lockie 1964; Balharry 1993; Caryl *et al.* 2012b). For our analysis, 86% of the small mammals identified to species level were *Microtus* voles, suggesting that 86% of the unknown small mammals in our samples are also *Microtus* voles. We cannot, therefore, conclude that there is any real difference in *Microtus* vole consumption between males and females, although it remains unclear why teeth were recovered less frequently from scats from females.

Increased forest fragmentation results in the support of higher population densities of pine martens in Scotland as it provides more opportunities to forage for *Microtus* voles (Caryl *et al.* 2012a). We theorised that this would cause a decrease in diet breadth as the preferred prey type increases in abundance, but did not find support for this. Pine martens do, however, increase their diet breadth during summer, when food sources become more abundant (Caryl *et al.* 2012b). Our analysis suggests that diet breadth is not, however, driven by the abundance of a preferred prey type. Although this prey type may constitute a large proportion of the diet, variation in the diet will be maintained.

We did not find support for the hypothesis that fruit consumption would increase in forests with higher levels of forest cover. In terms of managing forests for pine marten, this suggests that fruit would not need to be a major concern when selecting the composition of forest (rowan) or understory (blaeberry) habitats. The management of forest composition, at least in terms of what would be most beneficial for pine martens, can therefore focus on other aspects such as the inclusion of appropriate den sites (Birks *et al*. 2005). Pine marten density is highest as intermediate levels of forest cover (chapter 3; Caryl *et al*. 2012b); our result suggests that this increase in density is not driven by increased consumption of berries. As the conservation of capercaillie includes providing blaeberry rich habitat (Kortland 2003; Eaton *et al.* 2007), our result suggests that this action will not risk increasing pine marten density and therefore could alleviate this concern for those managing capercaillie habitats.

Management strategies aimed at reducing pine marten predation of vulnerable prey species, such as capercaillie, rely on detailed knowledge of predator-prey interactions and the relative predation rates of different demographic groups. This enables a targeted approach, increasing efficacy as well as reducing the costs of a wide spread intervention. Diversionary feeding has been suggested as a management option to reduce the impact of pine marten predation on capercaillie (Ron Summers, pers. comm.). We found no difference in consumption of birds between the sexes, or in areas with different levels of forest cover. This finding means that, within areas of priority for capercaillie conservation, there is no evidence that feeding efforts need to focus on particular areas or sub-groups within the pine marten population. However, as the most vulnerable period in terms of depredation of capercaillie by pine marten is during the nesting and chick rearing stages of the life cycle, and pine marten display seasonal changes in their diet and habitat use, further testing is advised to ensure that these findings hold during spring and early summer and are not limited to autumn, when sampling occurred.

4.6 Acknowledgements

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Appendix 4A

Correction factors and values for the estimated weight intake of all broad and fine scale taxa. Values are given for individual forests (sexes combined), as well as both sexes (all forests combined). Values for fine scale taxa are derived by multiplying each occurrence (based on count data) by the correction factor for that item. Broad taxa are the sum of all fine scale taxa within that category. Correction factors are taken from feeding trials with captive pine marten (Balharry 1993). Where a correction factor was not provided a conservative estimate was applied by using the correction factor for the smallest diet item within that category (italicized, bold).

	Correction factors	Abernethy	Mar	Inshriach	Darnaway	Females	Males
SMALL MAMMALS		46.0	39.0	42.4	25.1	34.4	39.8
Microtus agrestis	16	35.3	22.0	28.7	17.7	19.8	31.3
Apodemus sylvaticus	16	0.6	0.0	2.9	1.7	2.6	2.4
Myodes glareolus	16	0.6	1.7	0.7	0.0	1.3	2.4
***Sorex* sp.**	5	1.0	1.6	0.9	0.3	0.4	0.0
Unknown sp.	***5***	8.6	13.7	9.2	5.5	10.3	3.8
LARGE MAMMALS		24.5	42.3	24.8	11.8	18.5	22.9
Sciurus vulgaris	25	12.2	0.0	0.0	0.0	2.1	0.0
Cervidae	77	0.0	0.0	0.0	0.0	0.0	0.0
Ovis aries	77	0.0	0.0	3.5	0.0	0.0	11.6
Oryctolagus cuniculus	51	0.0	0.0	0.0	0.0	0.0	0.0
Carrion (unknown sp.)	***25***	12.2	42.3	21.3	11.8	16.5	11.3
BIRD		14.3	14.7	21.8	57.2	41.1	30.5
Bird	***16***	3.9	3.4	1.4	3.4	2.6	9.6
Small bird	16	3.4	3.4	13.6	11.8	15.8	9.6
Large bird	25	7.0	7.9	6.7	42.1	22.6	11.3
Egg shell	16	0.0	0.0	0.0	0.0	0.0	0.0
INVERTEBRATE		5.8	1.4	3.5	1.9	2.4	0.8
Coleoptera	0.5	1.7	0.7	0.8	0.6	1.0	0.4
***Abax* sp.**	0.5	0.2	0.0	1.2	0.4	0.4	0.0
***Geotropes* sp.**	0.5	0.7	0.5	1.0	0.8	0.6	0.3
***Vespa* sp.**	0.5	0.6	0.0	0.1	0.0	0.2	0.0
***Bombus* sp.**	0.5	0.6	0.1	0.2	0.1	0.0	0.1
Pollen / wax	25	1.7	0.0	0.0	0.0	0.0	0.0
Nest material	***0.5***	0.1	0.0	0.0	0.0	0.0	0.0
Unknown sp.	***0.5***	0.2	0.1	0.3	0.1	0.2	0.1
PLANT MATERIAL		4.8	0.5	5.7	1.7	2.2	2.0
Vaccinium myrtillus	0.5	0.2	0.2	0.0	0.2	0.0	0.0
***Rubus* sp.**	0.5	0.1	0.0	0.0	0.0	0.0	0.1
***Prunus* sp.**	0.5	2.1	0.0	0.0	0.0	0.0	0.0
***Sorbus* sp.**	0.5	0.0	0.0	5.1	1.0	1.8	1.7
Juniperus communis	0.5	0.0	0.0	0.0	0.2	0.0	0.0
Unknown sp.	0.5	1.9	0.2	0.2	0.0	0.0	0.1
Misc. vegetation	***0.5***	0.1	0.0	0.3	0.3	0.3	0.1
Garden berries	0.5	0.3	0.2	0.0	0.0	0.0	0.1
OTHER		4.6	2.1	1.8	2.2	1.4	4.0
Lacerta vivipara	5	1.4	1.6	1.1	0.3	0.8	1.5
***Bufo* sp. / *Rana* sp.**	16	2.8	0.0	0.0	0.0	0.0	2.4
Fungi	5	0.0	0.0	0.0	0.0	0.0	0.0
Peanut	***0.5***	0.4	0.3	0.6	0.3	0.4	0.1
Bird seed	***0.5***	0.0	0.2	0.0	1.6	0.1	0.0
Unknown sp.	***0.5***	0.0	0.0	0.0	0.0	0.0	0.0

Chapter 5

Diversionary feeding: An effective management strategy for conservation conflict?

5.1 Abstract

Human population growth has led to increased contact between people and wildlife, with adverse impacts such as damage to economic crops, depredation of livestock and threats to human safety. Conflicts arise where stakeholders with opposing interests disagree on how to alleviate problems. Diversionary feeding, where food is used to divert animals from activities causing a specific conflict, is sometimes proposed as a socially acceptable conservation action, but little information exists on how successful it is or what influences its efficacy. We review literature on diversionary feeding to identify factors contributing to success or failure. Of 30 trials presented in 19 experimental studies, 12 enabled assessment of the ultimate management objective (related to either increasing threatened prey density, crop yield or nuisance reports), and only 5 of these were considered successful, perhaps due to a focus on statistical significance rather than effect size. Studies often failed to report the results of feeding in sufficient detail to allow an evidence-based evaluation. Although diversionary feeding was considered expensive, cost-effectiveness analyses were rarely carried out, and only a third of studies reported information on costs and benefits that would allow future managers to evaluate their efforts. Based on the factors identified as contributors to success during the literature review, we propose a decision-making framework for planning and implementing diversionary feeding, which incorporates knowledge of the ecosystem and financial costs with evidence from previous studies in an adaptive format. Clear reporting of findings, costs and effort expended will allow the return-on-investment to be calculated for

different levels of management effort and future decisions to be driven by cost-effectiveness and efficiency of the action.

5.2 Introduction

As human populations encroach into natural habitats and modify large areas to provide food and other services, interactions between people and wild animals are increasingly common. These interactions can result in damage to economic crops (e.g. agriculture and forestry; Sullivan *et al*. 2008; Barrio *et al*. 2010), threats to human safety (Kaplan *et al.* 2011; Rogers 2011), and predation of commercially valuable species such as livestock (Smith *et al.* 2000) or game (Redpath 2001; Redpath & Thirgood 2009). Competition between humans and wildlife over resources is a common cause of these impacts (Kaplan *et al.* 2011) as abundant game, commercial crops or timber provide sources of food for some species, so the likelihood of damage is higher when the availability of natural food sources is low (Calenge *et al.* 2004; Ziegltrum 2004; Barrio *et al.* 2010). Habitat loss and changes in land use patterns can also lead to adverse wildlife-wildlife interactions and potentially increase predation of vulnerable species (Smart & Ratcliffe 2000). In response to these human-wildlife and wildlife-wildlife interactions, stakeholders (which may include environmentalists, resource managers, game keepers, land owners or members of the general public with an interest in the species or issue) often try to manage the landscape to mitigate adverse impacts either on biodiversity or on economic output. Where multiple land use objectives or differing values regarding the species involved lead to stakeholder disagreement on the best course of action to mitigate these impacts, conservation conflicts arise (Young *et al.* 2010; Redpath *et al.* 2013).

Methods for managing wildlife to increase or decrease populations are manifold. Culling predators for the benefit of prey species, for example, can result in demographic improvements for vulnerable bird species such as increased hatching and fledging success (Smith *et al.* 2010) and size of the breeding population (Fletcher *et al*. 2010). This, however, can depend on the ability to maintain a low-density predator population (Payton *et al.* 1997) and the strategy has shown a mixed and often low success rate for livestock protection (Conner *et al.* 1998; Berger 2006), or conserving bird populations (Cote & Sutherland 1997). Where the ethics of culling predators are questioned (Witmer *et al.* 2000; Ziegltrum 2008), translocation is often considered a humane alternative (Massei *et al.* 2011). Benefits to prey populations, however, may be unsustainable, with the movement of animals back into the area a recurring problem, particularly for large mammals (Rogers 1988; Linnell *et al.* 1997).

In cases where target species are protected or where public acceptance of the management action is paramount, it may not be possible or desirable to employ lethal control and other management options are required. Diversionary feeding is one such alternative. The terms 'diversionary' and 'supplementary' are sometimes used interchangeably in this context. For the purposes of this review, diversionary feeding is defined as the use of food to divert the activity or behaviour of a target species from an action that causes a negative impact, without the intention to improve or increase the density of the target population. In contrast, supplementary feeding is defined as the use of feeding as a conservation method to improve the population viability or density of a particular species or species group. Whilst diversionary feeding has been used in mitigation for a range of wildlife impacts (e.g. forest damage by black bears; *Ursus americanus*; Witmer *et al.* 2000; predation of game species by hen harrier; Redpath 2001; Ziegltrum 2008; raiding of human food stores by Baboons; *Papio ursinus*; Kaplan *et al.* 2011), to date, with an exception of evidence for strategies aimed at bird species (see Williams *et al.* 2013), there has been no synthesis of available evidence to assess the factors influencing success of the strategy.

The effects of diversionary feeding can be measured at three stages; the initial uptake of diversionary food; the 'output' or direct impact of the action on the problem (e.g. a reduction in predation or damage); and the 'outcome' or overall benefit relative to management objectives (e.g. increased crop yield; Walsh *et al.* 2012; Fig 5.1). Failure to reach desired management goals may, in part, be due to the underlying variability associated with natural systems. The application of any intervention to a stochastic natural system will alter its structure in potentially unpredictable ways, so predicted outcomes based on the original state of the ecosystem might no longer be valid (Walters & Holling 1990). Understanding the reasons for the success or failure of previous conservation actions and associated uncertainties can assist with deciding what outcomes could potentially be achieved given similar conditions. Uncertainty in the system and in the effectiveness of actions lends itself to an adaptive approach, whereby actions are continuously monitored and refined in an iterative process over time (Walters & Hilborn 1978). This is particularly relevant for conservation conflicts which incorporate the contrasting values of stakeholders and encompass changing political and social facets as well as ecological ones (Turnhout, Hisschemoller & Eijsackers 2007). A transparent planning process that incorporates stakeholder objectives, current knowledge of the ecosystem and an existing evidence-base on the effectiveness of the strategy (Sutherland *et al.* 2004), can greatly improve stakeholder participation, which is vital for successful management and conservation in systems where wildlife and people interact (Bunnefeld, Hoshino & Milner-Gulland 2011).

Limited funding requires that managers prioritise actions to achieve the greatest returns for a given level of effort (Murdoch *et al.* 2007; Auerbach, Tulloch & Possingham 2014). Decision analysis allows managers to compare alternative management actions and their likely benefits to decide, in this case, whether or not to carry out diversionary feeding. Informing decision analyses by incorporating costs and expected returns using economic techniques such as return on investment (ROI) has been particularly successful for conservation planning for protected areas, with an higher number of species protected compared to other priority-setting approaches (Murdoch *et al.* 2007; Underwood *et al.* 2008). Given the perceived expense of diversionary feeding (Conover 2002), and the uncertainty in the likely benefits, ROI analyses may be useful in comparing the cost-effectiveness of potential management options (Andreassen, Gundersen & Storaas 2005).

Whilst some diversionary feeding experiments have monitored progress and attempted to address the factors hindering success (Redpath 2001; Sullivan, Sullivan & Hogue 2001; Sullivan & Sullivan 2004; Sullivan & Sullivan 2008; New *et al.* 2012), most provide an *a posteriori* review of effectiveness without attempting to isolate causal factors. In this study, we review the literature on diversionary feeding to identify variables that influence its efficacy. We use these variables to develop a series of questions that can be used in a decision-making framework that helps managers develop an effective strategy. We structure the resulting framework into three components: the operating process, which looks at the effect of feeding on the wildlife population; the monitoring process focusing on how the response to, and success of, feeding can be observed; and the evaluation process to develop and evaluate indicators of success. We then attempt to use existing data within an ROI analysis to evaluate likely cost-effectiveness of management, based on the reported costs and resulting levels of success.

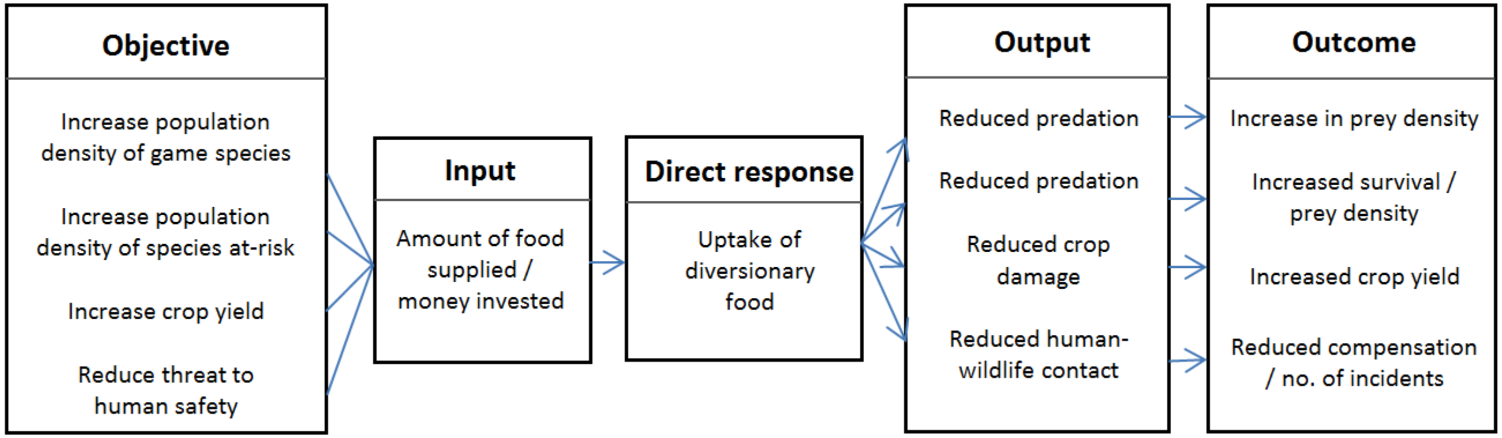


Figure 5.1. Example of the sequence of events required by stakeholders as a result of diversionary feeding for four categories of conflict; predation of game species; predation of vulnerable species; damage to crops; threats to human safety. In practice, stochastic influences on the system and unexpected behaviour of the target species make the effects of feeding more uncertain. Adapted from Walsh *et al*. 2012.

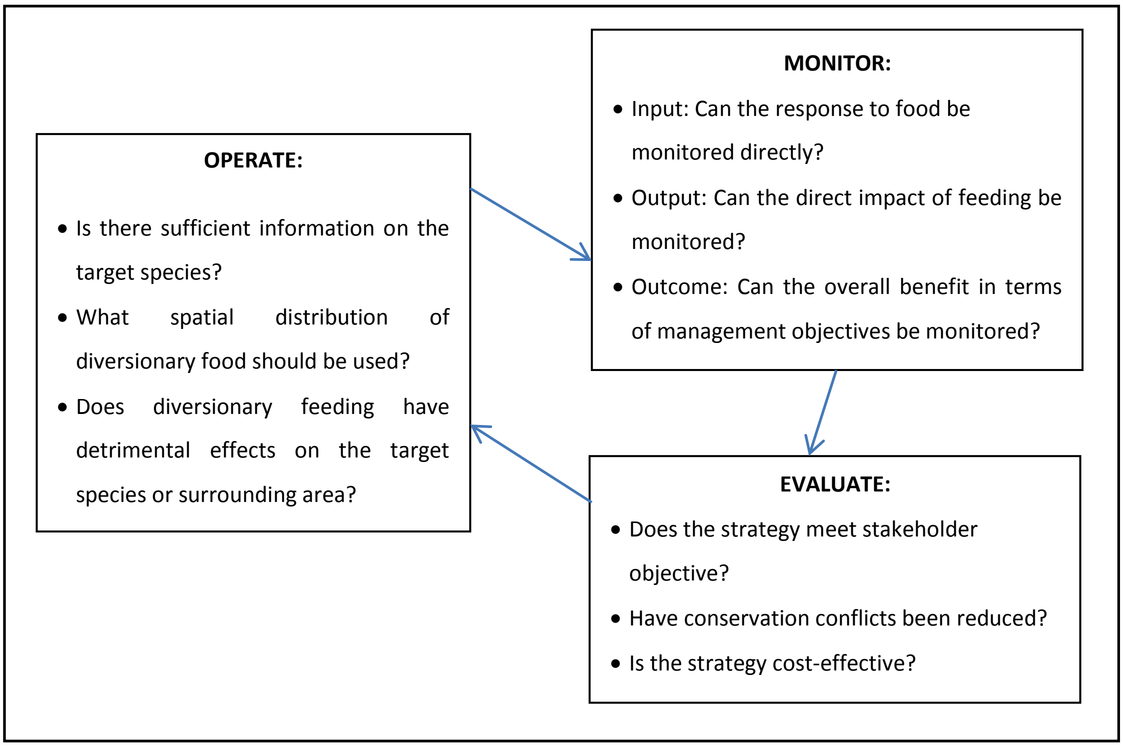
5.3 Methods

References to diversionary feeding were sourced within the title or keywords of papers using the databases ISI Web of Knowledge, Science Direct and Google Scholar with the words ‘diversionary’, ‘supplementary’ and ‘supplemental’ combined with ‘feeding’ or ‘food’ and one or more of the following: ‘conservation; conflict; mitigation; non-lethal; management’. For papers found using the term ‘supplementary’, the aims were checked to see whether they fell under our definition of supplementary or diversionary feeding. Whilst we refer to studies on supplementary feeding when details of the experimental design are of relevance to diversionary feeding, the outcomes of these studies were not included in this review. The references and citations of each paper found in the original searches were checked and sourced where applicable. A total of 52 papers were initially returned for ‘diversionary feeding’ and 1628 for ‘supplementary feeding’; 20 papers contained experimental data and one modelled the efficacy of diversionary feeding (New *et al.* 2012).

The experiments from each study were only included in a quantitative review of efficacy if the methods included at least one of the following criteria: target individuals or treatment sites replicated; control sites included; paired or randomised site selection; cross-over of treatment and control sites; monitoring conducted before or after treatment. The criteria followed by each study are presented in appendix 5A. Nineteen of these were used for the quantitative review of efficacy, whilst one (Amar *et al.* 2004) was excluded as direct effects of feeding were not quantified. Five studies were used in the ROI analysis described below.

Studies were grouped into four categories, based on initial review of their objectives; ‘increase population density of game species’, ‘increase population density of at-risk species’ ‘increase crop yield’ and ‘reduce threats to human safety’. To quantify results, studies were classified as either successful or unsuccessful based on the initial study objectives or on the effect size considered to represent a success by the author of each study. To provide the information necessary for a ROI analysis, we standardised results within each category as a percentage difference in the measured response variable before and after treatment – this value was the ‘return’, or benefit of management. Where costs were given, we calculated unit cost (US$) per hectare (ha), or per feeder where site size was not specified. ROI curves were produced from predicted values of a non-linear least squares regression, relating the dependent variable of management benefits to the costs of each feeding program. Analyses were conducted using 'nls' and 'predict' functions in R version 3.1.0 (R core team 2014).

Review of all relevant literature revealed seven common factors to be the most important influences on the success of management. We frame these as questions below, integrated into the three-part decision-making framework (operating, monitoring and evaluation; Fig 5.2), which we use to evaluate each of the diversionary feeding studies reviewed.



OPERATE:

- Is there sufficient information on the target species?
- What spatial distribution of diversionary food should be used?
- Does diversionary feeding have detrimental effects on the target species or surrounding area?

MONITOR:

- Input: Can the response to food be monitored directly?
- Output: Can the direct impact of feeding be monitored?
- Outcome: Can the overall benefit in terms of management objectives be monitored?

EVALUATE:

- Does the strategy meet stakeholder objective?
- Have conservation conflicts been reduced?
- Is the strategy cost-effective?

Figure 5.2. Decision making framework for planning and implementation of a diversionary feeding strategy. Questions are grouped into operating, monitoring and evaluation processes. These processes should be part of an adaptive system where the efficacy of the strategy is re-evaluated during each iteration of the cycle.

5.4 Operating process

The operating process requires knowledge of the ecology and behaviour of the species, and how this might change in response to alternative management actions. Knowledge of the ecological requirements of the target species will drive decisions regarding the type of food used in diversionary feeding, how and when it is distributed, and inform likely responses, which are needed when setting targets for evaluating the achievement of objectives (e.g. expectations of a 50% reduction in threats to human safety might be unrealistic, but an increase in crop yield of 20% might be expected if there is evidence of this being possible in previous experiments). There are three key questions, outlined below, that can be used to inform choices about where and how to employ diversionary feeding (Fig 5.2).

5.4.1 Is there sufficient information on the target species?

5.4.1.1 Is the population food limited?

It is important that diversionary food is only provided for short time periods as increased food resources could enhance survival leading to increased population density (Conover 2002). Where damage occurs to crops or timber, diversionary food needs to be more nutritionally appealing (e.g. higher calorific content) than the crop (Sullivan 1979; Sullivan & Sullivan 1982). In British Columbia, a short ‘pulse’ of sunflower seeds, which have a similar nutritional content to several conifer seeds, reduced tree damage by American red squirrels (*Tamiasciurus hudsonicus*) by 41% (Sullivan & Klenner 1993). In this case, damage occurred within a short time period (May - June), when natural food was limited. If, however, natural food limitation and subsequent damage are more sustained, providing diversionary food without risking an increase in population density is more difficult to balance. A short ‘pulse’ of diversionary food could be exhausted quickly with no significant long-term reduction in damage and, in some cases, may cause a short-term increase in population density as animals switch to the resource under protection once the diversionary food has been exhausted (Sullivan 2001; Sullivan 2004). Mammalian predators of vulnerable species are often generalists, consuming a wide diversity of prey. In these cases, opportunistic predation of vulnerable prey may occur in spite of diversionary food uptake. Managers may therefore aim to use diversionary feeding to shift the predator away from sensitive areas entirely. This may not be possible where natural food is abundant, as was speculated to be the cause of failed attempts to prevent depredation of ground-nesting birds by racoons (*Procyon lotor*) in Georgia, USA (Storey 1997).

5.4.1.2 Is damage caused by a sub-set of the population?

In some cases, a particular demographic group or individual may need to be targeted. In the UK, for instance, female hen harriers mated to monogamous males depredate fewer red grouse chicks than those mated to bigamous males. It may, therefore, be more efficient to target such females directly rather than attempting to supply diversionary food to all individuals (Redpath 2001); targeting selected individuals may also reduce the risk of increasing the population size (Sullivan *et al.* 2001; Massei *et al.* 2011). Although entire troops of baboons engage in activities which damage residential areas in South Africa, providing food for the alpha male alone can cause substantial changes in troop behaviour and a reduction in time spent by the troop in urban areas (Kaplan *et al.* 2011). Targeting specific animals, however, is not always straightforward; in the US Pacific North West, the majority of commercial timber debarking by black bears is carried out by small bears, most likely females raising cubs (Witmer *et al.* 2000). Targeting this sub-group would, however, be difficult due to the time needed to find and supply den sites.

5.4.1.3 How will the target species respond to diversionary food?

Where diversionary feeding is used to reduce predation, the target species are usually generalist predators (Storey 1997; Greenwood, Pietruszewski & Crawford 1998; Smart &

Ratcliffe 2000; Redpath 2001) which usually focus on the most abundant prey at the time (Smart & Ratcliffe 2000). As long as natural food is limited, this trait is favourable for diversionary feeding strategies because target animals will be more likely to switch quickly to the proffered food (Conover 2002). The quantity of food provided can affect how long it takes for the target species to utilise it. For example, the time taken for cinereous vultures (*Aegypius monachus*) to visit `vulture restaurants` is reduced, and the number of visiting vultures increased, with a higher quantity of food available (Moreno-Opo *et al.* 2010).

5.4.2 What spatial distribution of diversionary food should be used?

5.4.2.1 How will food be distributed?

The nature of the conflict, including social aspects of land ownership and multiple management objectives, as well as the ecology of the target species dictate the most appropriate distribution method of diversionary food. The use of feeding stations, where a concentrated amount of food is placed, is the standard method for feeding large mammals such as black bear or moose (*Alces alces*; Ziegltrum 2004; Sahlsten *et al.* 2010; Rogers 2011). For birds, previous studies have placed food near the nests of birds of prey (Smart & Ratcliffe 2000; Redpath 2001; New *et al.* 2012), used feeding stations (Hammond 1961; Knittle & Porter 1988) and sacrificial crop fields (Hammond 1961). Feeding stations offer flexibility as they can be moved, whereas sacrificial crop fields allow more animals to access the food source simultaneously, increasing effectiveness and reducing competition (Conover 2002). On a finer scale, presenting food in long strips rather than concentrated piles can prevent dominant animals controlling access to the food (Vassant, Jullien & Brandt 1992; Calenge *et al.* 2004).

5.4.2.2 Where should feeding stations be located?

The success of diversionary feeding can vary between locations and during different times of year, making it difficult for managers to make predictions based on results elsewhere. This could either be due to intrinsic differences in behaviour between populations, or differences in behaviour within a population during different phases of the life cycle. For instance, although feeders on migratory paths to over-wintering sites in south-eastern Norway prevented moose-traffic collisions (Andreassen *et al.* 2005), a study in northern Norway and Sweden found that moose ignored feeding stations along the migration paths, but heavily utilised those placed in wintering areas (Sahlsten *et al.* 2010). The density of feeders can also affect their attractiveness. A high density of feeders could cause more mobile, sociable species to aggregate. For solitary species such as the white-tailed deer, low feeder density may prove more effective as individuals will use the resources available in their home range (Miller *et al.* 2003), rather than relocate entirely.

5.4.3 Does diversionary feeding have detrimental effects on the target species or surrounding area?

5.4.3.1 Does feeding create dependence for the target species?

Diversionary feeding strategies may create dependence on food supplied by humans that cannot be sustained by natural resources once this food source is removed or exhausted. This can lead to increased damage, as seen for black bears, where damage to conifer stands increased almost 7-fold after feeding stations were removed (Ziegltrum 2004). Feeding should coincide with periods of food limitation, but cease when natural resources increase (Witmer *et al.* 2000). The choice of food is also important in avoiding dependence. Black bears strip bark from economically valuable trees immediately after they emerge from hibernation as other natural food sources are scarce (Flowers 1987). To prevent dependence on diversionary food once natural food abundance increased, Flowers (1987) developed a diversionary food which was more palatable than the sapwood at risk, but less nutritious than wild berries, causing a switch to the wild alternative once it became available.

5.4.3.2 Is feeding detrimental to the health of the target species?

Feeding stations facilitate contact between individuals, increasing the risk of infectious disease transmission (Miller *et al.* 2003; Castillo *et al.* 2011), and a greater quantity of food may exacerbate the problem as animals may spend longer feeding (Miller *et al.* 2003). Poor food quality can also promote disease occurrence; Spanish imperial eagle (*Aquila adalberti*) populations receive supplementary food to increase breeding productivity, but the use of domestic rabbits containing high levels of antibiotics and anti-parasitic drugs causes higher pathogen abundance and a depressed immune system compared to those fed with wild rabbits (Blanco, Lemus & Garcia-Montijano 2011).

Supplemental feeding is often used as a ‘quick fix’, with little attention to long-term consequences for the target species (Blanco 2006). Feeding may increase survival of genotypically ‘weaker’ individuals, retaining undesirable traits in the population as these individuals continue to breed which, in time, could reduce the population density; supplementary-fed bird populations, for example, show reduced clutch sizes for blue tits (*Cyanistes caeruleus*) and great tits (*Parus major;* Harrison *et al.* 2010). This is of particular importance where the target species are protected as, although practitioners may not want to increase population density, they must be careful not to decrease it either.

5.4.3.3 Has the effect on the surrounding landscape been evaluated?

Aggregations of social animals around a feeder can have a direct impact on the local ecosystem. Many ungulates continue to browse natural vegetation when receiving additional food and damage to flora by white-tailed deer (Cooper *et al.* 2005) and moose (Gundersen, Andreassen & Storaas 2004; vanBeest *et al.* 2010) occurs with increasing proximity to feeding stations. The effects on local fauna may be more complex; in areas with moose feeding stations, different passerines species vary in response to increased moose activity, apparently as a result of altered arthropod food availability, with great tits having lower fledging success and pied-flycatchers (*Ficedula hypoleuca*) showing the opposite trend (Mathisen *et al.* 2012).

Indirect effects on ecosystems can occur when non-target predatory species are attracted to feeding sites. Studies using artificial bird nests suggest increased predation of ground-nesting birds adjacent to 'vulture restaurants' (Cortés-Avizanda *et al.* 2009) and deer feeders (Cooper & Ginnett 2000), with a 30% increase in the predation of artificial ground nests in close proximity to deer feeders in Poland (Selva, Berezowska-Cnota & Elguero-Claramunt 2014). Trials to quantify predation of turtle nests showed that scavengers attracted to deer feeding stations resulted in a five-fold increase in predation rates on artificial nests (Hamilton, Freedman & Franz 2002). Predation of artificial ground-nests, however, often differs from that of natural ones (Burke *et al.* 2003) so confirmation by monitoring natural ground-nests is required.

5.5 Monitoring process

Whether or not diversionary feeding is considered successful will differ depending on stakeholder objectives. As such, indicators of success and suitable monitoring techniques need to be developed to suit each case. This will also allow knowledge transfer to potential future applications. There are three questions that managers can ask to determine how to monitor responses, outlined below.

5.5.1 Can the response to diversionary food be monitored directly?

Different sub-groups or individuals may vary in their response to diversionary food. For instance, only 50% of mountain hares (*Lepus timidus*) visited feeding stations in a population in Scotland (Newey *et al.* 2010), whilst female hen harriers take diversionary food at a higher rate than males (Redpath 2001). Direct monitoring of food sources will allow observations of this and alert managers if non-target animals or species deplete the food supply. Camera traps have been used successfully to monitor feeding stations (Ziegltrum 2008) although it is not always possible to identify individual animals. In some cases, individuals may be marked visually (Smart & Ratcliffe 2000), or tagged with Passive Integrated Transponders (PIT tags) and individual visitations recorded by a receiver on the feeding station (Newey *et al.* 2009), although methods also rely on a substantial proportion of the population being tagged to provide this information.

Choosing appropriate monitoring methods is essential to highlight areas of improvement for an efficient iterative management approach. For instance, pellet counts to assess moose distribution in relation to feeding sites at the population level initially suggested that moose used areas close to feeding sites more than the surrounding area. Tracking individuals fitted with GPS transmitters revealed, however, that only one of 15 animals used the feeding site regularly, and only three used the site at all (Sahlsten *et al.* 2010), indicating that very few of the moose responded to diversionary feeding.

5.5.2 Can the direct impact of feeding (the output) be monitored?

The potential for habitat damage following diversionary feeding can be monitored using pre- and post-treatment habitat surveys (Sullivan & Klenner 1993; Sullivan *et al.* 2001; Sullivan & Sullivan 2004; Ziegltrum 2004; Barrio *et al.* 2010) or, where problems are highlighted by landowners, pre- and post-treatment questionnaires (Calenge *et al.* 2004). For predation, monitoring is dependent upon prey type. Direct monitoring may be possible, such as radio tagging red grouse to quantify adult survival rates (Redpath 2001), or using camera traps to quantify nest predation (Summers *et al.* 2009). However, ground-nesting birds are often found by flushing incubating females (Greenwood *et al.* 1998; Redpath 2001), which may be too disruptive for vulnerable species. Camera traps can also increase predation rates by drawing attention to nests (Summers *et al.* 2009). Whilst the effort required to locate rare or elusive species may reduce the cost-effectiveness of diversionary feeding, any on-going monitoring (e.g. to assess population trends) could incorporate such management objectives.

5.5.3 Can the overall benefit in terms of management objectives (the outcomes) be monitored?

To measure whether a strategy has achieved objectives of increased densities of game or at-risk species, increased crop yield, or reductions in threats to human safety, managers need to relate the results of outcome monitoring to the management targets set during goal-setting (e.g. an objective of reducing threats by 30%, or increasing game populations by 20%). Achieving improvements in outputs (e.g. reduced predation) may not necessarily translate to positive outcomes such as increased prey abundance (Fig 5.1). For example, despite a significant reduction in damage by the European rabbit (*Oryctolagus cuniculus*) to vineyards after the application of diversionary food (Barrio *et al.* 2010), increased grape yield was not observed. Likewise, reduced predation of ground-nesting birds by hen harriers (Redpath 2001) and skunks (Greenwood *et al.* 1998) did not result in observable increases in prey survival. This could have resulted from compensatory predation or increased densities of other predators (Greenwood *et al.* 1998; Jackson 2001) due to the attraction of diversionary food (Redpath 2001). To enable targeted revisions within the management framework, success should be measured at each of these stages wherever possible.

Where field experiments are not feasible because of funding constraints or the scale of the problem but sufficient prior knowledge of the system exists, modelling the effects of feeding under different management and environmental conditions would allow the likely outcomes (New *et al.* 2012) or net costs (Ziegltrum 2006) to be assessed for each scenario. Use of experts to evaluate the options for monitoring performance of management options, or predicting expected performance, could also be useful here. Bayesian inference, as utilised by New et al (2012), may be invaluable in these cases (Ellison 2004) as each subsequent experiment builds upon previous knowledge, essentially creating a meta-analysis of findings, reducing uncertainty in the resulting trends or highlighting those that are unfounded.

5.6 Evaluation process

5.6.1 Does the strategy meet stakeholder objectives?

Despite some differences in the indicators used to measure success, diversionary feeding experiments all have a quantifiable measure. Our review of these shows mixed success (Table 5.1). At the output stage, 10 of 15 trials (67%) for crop protection were considered a success, whilst only one of three trials (33%) involving risks to human safety, and two of five trials (40%) to reduce predation of vulnerable or game species are reported as successful. Fewer studies report the ultimate outcomes of diversionary feeding trials and, although success is comparable to the output stage with four of eight (50%) successful trials to reduce crop damage and one of two for risks to human safety, neither of the two outcomes for predation reduction were considered a success. Due to small sample sizes, however, these results should be interpreted with caution.

The majority (75%) of reported outputs and outcomes for all categories included a statistical measure of support based around a predetermined significance level of 95% (i.e. $p<0.05$). As the p-value is the probability (under the assumption of a true null hypothesis) of obtaining a result equal or more extreme than the one observed, it merely provides a measure of certainty in the effect rather than an indication of the magnitude of that effect. Focusing only on statistical significance related to p-values to report management as successful, rather than reporting p-values in combination with an effect size, may lead to the dismissal of potentially promising results (Fidler *et al.* 2006; Nakagawa & Cuthill 2007). Whilst most studies present an effect size, measured as the observed magnitude of the difference between treatments, only two outputs (7% of all reported results) refer to a relatively large observed effect as successful, regardless of non-significance at the 95% confidence limit (Sullivan *et al.* 2001; Table 5.1). Providing a level of significance, regardless of the arbitrary and precautionary 'success' threshold of 95%, coupled with an effect size and associated confidence intervals, will allow practitioners to decide upon the level of certainty they are willing to accept (e.g. 90%, or potentially lower levels of confidence in the results) depending on the nature of the problem, the potential payoffs or effect size, and the available funds.

5.6.2 Have conservation conflicts been reduced?

A number of factors, such as the high perceived uncertainty associated with the strategy (New et al. 2012), may mean that meeting stakeholder objectives during trials does not guarantee that the strategy will be adopted in the long term, causing conflict to remain unresolved (Thirgood & Redpath 2008). Where the stakes invested in successful outcomes are high, such as those involving human livelihoods or safety, lack of trust in the strategy may allow political and social pressures to override scientific findings (Thirgood & Redpath 2008). For example, despite successful application of diversionary feeding to prevent crop damage from black bears (Witmer *et al.* 2000; Ziegltrum 2004; Ziegltrum 2008), there has been little interest in testing the efficacy of the strategy to mitigate safety risks from this species (Rogers 2011).

Management to reduce crop damage was the most successful of all categories reviewed, with efforts in the US to protect timber plantations from damage by black bears gaining acceptance from the public, a positive response from animal rights groups, satisfaction from timber managers (Ziegltrum 2006) and continued use of the strategy (Witmer *et al.* 2000; Ziegltrum 2008). The strategy has been implemented on a long term basis by collaboration between wine growers and hunters to alleviate wild boar (*Sus scrofa*) damage to vineyards (Calenge *et al.* 2004), and has resolved 147 complaints from land owners regarding waterfowl damage to crops (Fairaizl & Pfeifer 1987), securing future use.

Table 5.1. Results from diversionary feeding studies for cost-effectiveness analysis. Consecutive rows within a study indicate multiple experiments, or success measured at different stages. Output (e.g. reduction in predation or crop damage) and outcome (e.g. increase in prey density, crop yield or nuisance reports) are given as % change or US$ saved. Results were standardised as the percentage difference between control and treatment using (%treatment - %control) / %control). Costs were converted to US$ and increased by respective inflation rates since publication. Cost effectiveness was calculated using output or outcome / input (US$). The success of studies is evaluated against the indicators stated in each paper; success for those measuring outputs are given as Y or N and those measuring outcomes are given as Y* or N*. Further details of each study are given in appendix 5A. References: [a](New *et al.* 2012); [b((Redpath 2001); [c](Storey 1997); [d](Smart & Ratcliffe 2000); [e](Greenwood *et al.* 1998); [f](Sullivan & Sullivan 1984); [g](Sullivan & Sullivan 1988); [h](Sullivan & Klenner 1993); [i](Sullivan *et al.* 2001); [j](Sullivan & Sullivan 2004); [k](Sullivan & Sullivan 2008); [l](Barrio *et al.* 2010); [m](Ziegltrum 2004); [n](Ziegltrum 2006); [o](Calenge *et al.* 2004); [p](Fairaizl & Pfeifer 1987); [q](Geisser & Reyer 2004); [r](Rogers 2011); [s](Kaplan *et al.* 2011); [t](Sahlsten *et al.* 2010); [u](Andreassen *et al.* 2005); [v]Test statistic not provided

Objective	Species subject to diversionary feeding	Input (US$ per ha)	Output	Outcome	Significance	Considered a success
Increase population density of game	*Circus cyaneus*[a]	*unreported*	-5%	0%	-	N
	Circus cyaneus[b]	$54.17	-86%	44%	F$F_{1,4}$ =11·8, P=0·001 (output)	Y N*
Increase population density of at-risk species	*Procyon lotor*[c]	*unreported*	64%	-	$F_{1,10}$=6.85, P=0.03	N
	Falco tinnunculus[d]	*unreported*	3%	-	$F_{1,13}$=2.61, P=not significant	Inconclusive
	Mephitis mephitis[e]	$0.20	-54%	41%	χ^2=3.83, P=0.05 (output) $F_{1,16}$=1.32, P=0.27 (outcome)	Y N*
Increase crop yield	Rodents and birds [f]	$174.75	-51%	-	-	Y
		$87.51	-72%	-	-	Y
		$137.8	-65%	-	-	Y
	Microtus spp [g]	*unreported*	-12.5%	-	d.f = 2; P<0.05[v]	N
		unreported	-60%	-	d.f = 2; P<0.05[v]	Y
	Tamiasciurus hudsonicus[h]	*unreported*	-80%	-	$F_{1,38}$=62.2; P<0.01	Y
		unreported	-86%	-	$F_{1,4}$=16.7; P=0.02	Y

Table 5.1. Continued

Objective	Species	Input (US$ per ha)	Output	Outcome	Significance	Considered a success
Increase crop yield	*Microtus* sp.[i]	*unreported*	-14%	-	$F_{2,2}=1.35$; $P=0.43$	N
		unreported	-67%	-	$F_{3,6}=1.12$, $P=0.41$	Y
		unreported	-65%	-	$F_{2,4}=4.59$; $P=0.09$	Y
	Microtus montanus[j]	*unreported*	169%	-	$F_{1,5}=13.40$; $P=0.01$	N
	Microtus longicaudus[k]	*unreported*	-	18.70%	$F_{1,3}=3.02$, $P=0.18$	N*
		unreported	-	30%	$F_{1,4}=4.11$, $P=0.11$	N*
		unreported	-	14%	$F_{1,2}=1.22$, $P=0.39$	N*
		unreported	-	36%	$F_{1,2}=18.67$, $P=0.05$	Y*
	Oryctolagus cuniculus[l]	*unreported*	-91%	-2%	$F_{2,4}=32.5$, $P=0.003$ (output) $Z=-0.079$, $P=0.997$ (outcome)	Y N*
	Ursus americanus[m]	*unreported*	-72%	-	$F_{3,1}=16.98$, $P<0.001$	Y
	Ursus americanus[n]	$\$3.13$	-	31%	-	Y*
	Sus scrofa[o]	$\$42.85$	-18%	23%	$t_{287}=1.77$, $P=0.08$ (output)	N Y*
	Waterfowl sp.[p]	$\$16.9\text{x}10^{4}$	-	$\$34.2\text{x}10^{4}$	-	Y*
	Sus scrofa[q]	*unreported*	62%		$F_{1}=2.780$ $P=0.103$	N
Reduce threat to human safety	*Ursus americanus*[r]	*unreported*	-	88%	$t_{9} = 4.14$, $P<0.002$	Y*
	Papio ursinus[s]	*unreported*	-26%	-	$Z=1.13$; $P=0.26$	N
	Alces alces[t]	*unreported*	0%	-		N
	Alces alces[u]	$\$1000$	-235%	$-\$541$	$F_{1,4}=9.55$, $P=0.037$ (output)	Y N*

5.6.3 Is the strategy cost-effective?

When evaluating the efficiency of a wildlife management option, the potential benefits must be assessed carefully against the effort and investment that must be expended to achieve them. In traditional economic cost-benefit analysis, costs and benefits are reported in monetary amounts and are easily compared. In circumstances where the aim of diversionary feeding is to prevent or reduce damage, managers must ensure that the cost of the strategy is lower than the cost of repair, replacement or compensation for damage. Diversionary feeding for crop protection, for example, may only be justified for crops of high economic value (Conover 2002). In some cases, such as vulnerable species protection, the intrinsic value of the subject species may be the guiding factor and benefits cannot be quantified in monetary terms. This can complicate decisions made by multiple stakeholders due to the difficulty of agreeing on expected outcomes and target levels of 'return' (e.g. a particular growth rate or increase in productivity).

The likely effects of diversionary feeding regimes are not always intuitive, making the overall cost difficult to estimate during planning. Browsing of commercial timber by brown bears (*Ursus arctos*), for instance, may result in compensatory growth, effectively reducing timber loss and reducing the cost-effectiveness of mitigation (Helgenberg 1998). The amount of food required may be higher than expected if consumption by non-target animals occurs (Conover 2002), although exact figures for this would be difficult to estimate prior to implementation.

Despite cost being of key importance to decision making, cost-effectiveness analyses are rarely reported in the literature, and only 33% (n=7) of the studies we reviewed reported the overall costs of management and conservation actions (Table 5.1). Where analyses are provided, the cost-effectiveness of diversionary feeding receives mixed support. For the purpose of crop protection, a 2:1 benefit-cost ratio has been found for protection against waterfowl damage (Fairaizl & Pfeifer 1987), whereas for protection against wild boar damage, the cost of feeding was similar to that of replacing lost crops (Massei *et al.* 2011). Where possible, it is also useful to be able to compare the cost-effectiveness of diversionary feeding with other potential mitigation techniques. For example, research on reducing moose-train collisions indicated that vegetation clearance was more cost effective than diversionary feeding (Andreassen *et al.* 2005).

Return on investment analyses enable comparisons between different initial damage levels and timescales of diversionary feeding; for example, feeding to mitigate timber damage by black bears reduced income losses between 11% and 31% (Ziegltrum 2006). Using the information available from the studies in our review (Table 5.1), an attempted was made to estimate ROI curves that compared the outputs of diversionary feeding programs with different objectives to their total costs for those that reported both. This analysis, however, was based on very limited data, as only four studies (n=7 trials) present both outputs and costs, clearly demonstrating the need for studies to report the overall costs of management strategies, as well as standardized results. With caution, our analysis suggests that diminishing returns may be found with diversionary feeding (Fig 5.3), although this trend is driven by a single data point. This curve is presented to illustrate how the use of an ROI approach could inform management plans costs were more readily reported. This highlights the complex nature of diversionary feeding, and the importance of taking into account a wide range of factors such as those we have outlined in our framework, so that the most efficient and cost-effective strategy can be selected for management. An important observation is the tendency for unsuccessful studies to fail to report costs or some other comparable measure of effort expended (Table 5.1; Storey 1997; Smart & Ratcliffe 2000; Geisser & Reyer 2004; Sahlsten *et al.* 2010), and we would encourage researchers to report costs whatever the outcome of their experiment. One cost that was not reported in any studies and hence could not be evaluated here was the cost of the monitoring itself. In many cases this might be incorporated within the overall management costs, but in others this could be a separate and ongoing cost that extends beyond the lifetime of the on-ground management if species are predicted to respond with a time lag. Monitoring and its effectiveness can and should be scrutinized using decision analysis in the same way that we have evaluated management here (Nichols & Williams 2006).

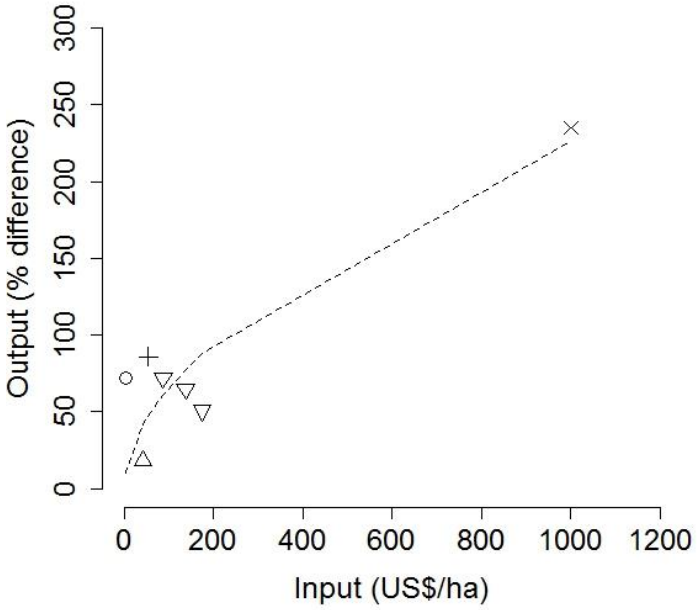


Figure 5.3. Return-on-investment curve for the outputs of diversionary feeding. Data were calculated from Sullivan *et al* 1984 (▽), Redpath *et al*. 2001 (+), Calenge *et al*. 2004 (Δ), Ziegltrum *et al*. 2004 (o) and Andreassen *et al*. 2005 (X). Results from Greenwood *et al* (1998) were excluded as operational costs were not provided. Return is given as % difference in output after application. Output from all sources was standardised as (%treatment - %control)/%control). Input (cost) was converted to US$ and increased by inflation rates since publication. The dashed line is the model prediction for a non-linear least squares regression of output on input (curve of best fit: Output = 5.4*(Input$^{0.5}$), R^2 =0.73).

5.7 Discussion

In theory, diversionary feeding provides an attractive alternative to more controversial measures such as wildlife culling or translocation for the mitigation of conservation conflicts. In reality, success varies greatly depending on the nature and location of the problem as well as target species behaviour. Successful uptake of diversionary food is most likely where populations are food limited (Witmer *et al.* 2000; Calenge *et al.* 2004; Ziegltrum 2004; Barrio *et al.* 2010), however care must be taken to avoid increasing population sizes of target species (Sullivan & Klenner 1993) or developing a dependence on the additional food source (Ziegltrum 2004). Overall, diversionary feeding trials to date appear to have been more effective for the mitigation of habitat damage or threats to human safety than for reducing predation of vulnerable prey (Table 5.1), although comparatively few examples of predation mitigation make a comprehensive evaluation difficult. The most successful use of diversionary feeding has been in combination with other management tools such as fencing (Kaplan *et al.* 2011), or scare devices (Conover 2002). In these cases, diversionary food provides an accessible alternative to food sources the animals have been excluded from, making them less likely to attempt to re-access the protected resource. The least successful programs result in increased damage or threats rather than a reduction, such as increased crop damage by voles (Sullivan & Sullivan 2004), or wild boar (Geisser & Reyer 2004). In these cases, the main reason for failure could be a lengthy period of damage over which protection is needed; providing diversionary food for a short period (in order to prevent an increase in population density) does not protect the resource for long enough and, for species with high reproduction rates, may lead to a temporary increase in numbers immediately after the provision of diversionary food, which then revert to the protected resource once diversionary food is depleted (Sullivan et al. 2004). This cascading effect highlights the need to consider the ecology and life history of the target species to ensure that the effects of food provision are fully understood.

By placing the results of previous studies of diversionary feeding into an adaptive decision-making framework that explicitly considers management objectives, it is possible to link objectives, inputs, outputs and outcomes (Fig 5.1), enabling predictions of the likely effectiveness of management actions and a solid evidence-base for efficient decision-making (Keith *et al*. 2011). Positive initial outputs, such as a reduction in damage or predation, often fail to translate into the outcomes desired by stakeholders, such as an increase in crop yield, or increased population density of the prey species. A number of factors may contribute to this, such as a compensatory response from other species (Greenwood *et al.* 1998; Jackson 2001), attraction of other animals to the area by the food source, response to feeding by only a sub-set of the population (Newey *et al.* 2010) or the presence of other strong influences on the problem such as disease or climate (Redpath 2001). Many diversionary feeding trials monitor only the initial response to feeding and, given the inconsistency in meeting the ultimate strategy goals, this may lead to unreasonable expectations as to its likely usefulness. By evaluating the objectives of monitoring as well as management using our framework, and choosing a monitoring method and timeframe that will efficiently measure likely outcomes relative to objectives, future studies can better place their results in the context of the effort expended.

Although diversionary feeding is considered an expensive option for management (Witmer *et al.* 2000; Mason & Bodenchuk 2002), detailed cost-effectiveness analyses are rarely conducted. Ideally, we would have liked to evaluate ROI for each objective so that outputs and outcomes were specific to the problem and provided more accurate predictions of returns. However, lack of published data means that this approach was not possible. With more reporting of results (even failures), future studies might be able to include predictor terms in ROI analyses, informed by the nine questions we have outlined in this review, that account for some of the variability in response (e.g. Walsh *et al.* 2012).

Decisions on the use of diversionary feeding as a tool for wildlife management and conservation are essentially a trade-off between the potential benefit of the action, as described by the magnitude of effect seen through observation or model prediction; the confidence in these findings, and the costs required to obtain these potential benefits. Many of the studies in the present review may have been considered successful if their effect size relative to effort expended had been evaluated instead of relying purely on the statistical significance of the result to categorise the management as successful. By providing transparent and unbiased information to stakeholders in each of these areas, with statistical significance presented without the constraints of an absolute threshold of perceived success (i.e. the 95% confidence limit), researchers transfer the power of decision making to the stakeholder by allowing them to set realistic targets for management and, when evaluating outcomes, to declare success or failure based on whether the results are within their own acceptable level of uncertainty.

5.8 Acknowledgements

This work was funded by the University of Stirling, Royal Society for the Protection of Birds, Forestry Commission Scotland and Forest Research and the Australian Research Council's Centre of Excellence for Environmental Decisions. Thank you to Nils Bunnefeld and Ayesha Tulloch for advice and support.

Appendix 5A. The measure or ‘indicator’ used to represent success and the outcomes of experimental trials of diversionary feeding. For each trial, the study was reviewed against the following criteria and the code (in brackets) recorded: target individuals or treatment sites replicated (rep) ; control sites included (cont); paired (pair) or randomised (ran) site selection; cross-over of treatment and control sites (c/o); monitoring conducted before or after treatment (b/a). Indicators of success were the main aim of the trial. Where more than one aim is stated, those relating most to the overall success of diversionary feeding were used. References: [a(]New et al. 2012)(New *et al.* 2012); [b(](Redpath 2001); [c(](Storey 1997); [d](Smart & Ratcliffe 2000); [e](Greenwood, Pietruszewski & Crawford 1998); [f](Sullivan & Klenner 1993); [g](Sullivan, Sullivan & Hogue 2001); [h](Sullivan & Sullivan 2004); [i](Sullivan & Sullivan 2008); [j](Barrio, Bueno & Tortosa 2010); [k](Ziegltrum 2004); [l](Ziegltrum 2006); [m](Calenge *et al.* 2004); [n](Fairaizl & Pfeifer 1987); [o](Geisser & Reyer 2004); [p](Rogers 2011); [q](Kaplan *et al.* 2011); [r](Sahlsten *et al.* 2010); [s](Andreassen, Gundersen & Storaas 2005)

Objective	**Species**	**Type of study:-**	**Indicators of success**	**Input (US$ per ha)**	**Output**	**Outcome**	**Significance**	**Notes**
Increase population density of game	Hen harrier [a]	Model	Decrease in provisioning of red grouse chicks to nests	*unreported*	-5%	0%	-	
	Hen harrier [b]	c/o; b/a cont; rep	10% increase in red grouse chick survival	$54.17	-86%	44%	$F_{1,4}$ =11·8, P=0·001 (output)	Costs per nest including food, transport, wages and logistics
Increase population density of at-risk species	Racoon [c]	c/o; cont; rep	Decreased red grouse chick predation Decreased red grouse adult mortality Increased red grouse density	*unreported*	64%	-	$F_{1,10}$=6.85, P=0.03	
	Kestrel [d]	b/o; rep	Reduction in movement (foraging) Reduction in little tern predation	*unreported*	3%	-	$F_{1,13}$=2.61, P=not significant	Overall result, some difference seen within time periods
	Skunk [e]	Pair;cont; rep; ran	Reduction in ground nesting bird predation Increased nest success	$0.20	-54%	41%	χ^2=3.83, P=0.05 (output) $F_{1,16}$=1.32, P=0.27 (outcome)	Costs for food only. Based on 18x applications of 95kg food @ $0.094/kg over 65ha
Increase crop yield	Red squirrel [f]	c/o; cont; rep	Reduced % pine trees with feeding damage Reduced feeding intensity (% stem girdled)	*unreported* *unreported*	-80% -86%	- -	$F_{1,38}$=62.2; P<0.01 $F_{1,4}$=16.7; P=0.02	Small scale application Large (operation) scale application
	Vole sp. [g]	c/o; cont; rep	Reduced seedling mortality in pine plantations	*unreported* *unreported* *unreported*	-14% -67% -65%	- - -	$F_{2,2}$=1.35; P=0.43 $F_{3,6}$=1.12, P=0.41 $F_{2,4}$=4.59; P=0.09	Expt A: Varied food types, output given for wood pellets Expt B: Pre-winter feeding, result for December Expt C: Alfalfa pellets applied to clear-cut sites
	Vole sp. [h]	c/o; cont	Reduced damage to commercial pine seedlings	*unreported*	169%	-	$F_{1,5}$=13.40; P=0.01	
	Vole sp. [i]	Pair; cont; rep	Increased survival of commercial conifer seedlings	*unreported* *unreported* *unreported* *unreported*	- - - -	18.7% 30% 14% 36%	$F_{1,3}$=3.02, P=0.18 $F_{1,4}$=4.11, P=0.11 $F_{1,2}$=1.22, P=0.39 $F_{1,2}$=18.67, P=0.05	Expt A: Initial study Expt B: Increased density of food/ha Expt C: Increased site size. Varied food/ha Expt D: Increased site size. Intermediate food/ha

Appendix 5A. Continued

Objective	Species	Type of study:-	Indicators of success	Input (US$ per ha)	Output	Outcome	Significance	Notes
Increase crop yield	Rabbit [j]	Pair; cont; rep	Reduced damage to grapevines Increase in grape yield	*unreported*	-91%	-2%	$F_{2,4}$=32.5, P=0.003 (output) Z=−0.079, P=0.997 (outcome)	Output for % reduction in severity of damage
	Black bear [k]	Pair; cont; rep	Reduction in damage to commercial timber	*unreported*	-72%	-	$F_{3,1}$=16.98, P<0.001	
	Black bear [l]	Model	Financial gain (i.e. reduction in income loss from timber)	$3.13	-	31%	-	Outcome = % saved income (11-31%). Input for current and future costs for 10yr feeding program. Based in simulation.
	Wild boar [m]	b/a	Reduction in damage to vineyards Financial gain (i.e. less compensation)	$42.85	-18%	23%	t_{287}=1.77, P=0.08 (output)	Costs include food, equipment and expenses. Output is % reduction in damage (kg/ha) Outcome is reduction in % compensation
	Waterfowl [n]	Rep; cont	Damage to lure crops	$16.9x10^4$		$34.2x10^4$		Input is savings due to feeding.
	Wild boar [o]	Rep	Reduction in damage to crops	*unreported*	62%		F_1=2.780 P=0.103	Output taken from plotted model results
Reduce threat to human safety	Black bear [p]	b/a	Reduction in reports of nuisance activity Reduction in bear removals	*unreported*	-	88%	t_9 = 4.14, P<0.002	Outcome based on no. of bears removed from study area
	Baboon [q]	b/a	Reduced time spent in urban areas Reduced no. of raided food items from humans Reduced use of urban sites	*unreported*	-26%	-	Z=1.13; P=0.26	Output for % time spent in urban areas
	Moose [r]	b/a; cont; rep	Fewer train-moose collisions Financial gain as a result of feeding	*unreported*	0%	-		Output for % time spent at feeders (winter sites)
	Moose [s]	Rep; cont	Divert moose away from problem areas: - within a 1000m buffer - within a 100m buffer	$1000	-235%	-$541	$F_{1,4}$=9.55, P=0.037 (output)	Cost per km per year

Chapter 6

The response of pine martens to diversionary food sources: Assessing the feasibility of diversionary feeding through a questionnaire study

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6.1 Abstract

Conservation aimed at protecting a vulnerable prey species by altering predator-prey interactions is especially complex when the predator is a protected species. Non-lethal management options include diversionary feeding, where an alternative food source is provided to divert the behaviour of the predator away from its prey. The efficacy of the strategy, however, depends of a range of variables, including the abundance of natural food, the distribution method and the ecology of the target species. A key component of success is the ability to quickly and reliably attract the predator to diversionary feeding sources. After severe persecution, the European pine marten is now protection under the Wildlife and Countryside Act 1981 and is recovering its former range in Scotland. There is concern from some stakeholders regarding predation by pine martens of the endangered capercaillie. We collected questionnaire data from people who have provided food for pine martens in Scotland. Fifty-eight percent of visits by pine martens to feeders were received within one week of food provision. Open-top feeders significantly decreased the time taken for an initial visit. Forty-six percent of subsequent visits occurred daily, with a combination of peanuts, jam and chicken resulting in a higher frequency of visits. Forty-eight percent of visits continued for at least two months. These findings can be integrated into the decision making process by

stakeholders in order to identify the conditions under which diversionary feeding is most likely to be effective and to ensure that implementation is as efficient as possible.

6.2 Introduction

Conservation management of vulnerable species is difficult when threats to the species include depredation (Woodroffe *et al.* 2005), as the predator may be of conservation concern which could lead to conflict between opposing stakeholders. In this case, management options may be limited to non-lethal methods which limit the impact on predators as well as prevent any detrimental effect on the prey (Witmer *et al.* 2000; Ziegltrum 2008). Predators, including mesocarnivores such as the pine marten, have a pivotal role in the ecosystem (Roemer, Gompper & Van Valkenburgh 2009) and the removal of a predator can have substantial cascading effects on the food chain (Polis *et al.* 2000) as prey populations may no longer be maintained. Reintroduction or recovery after a period of absence may have an equally substantial effect and, although the pine marten is native to Scotland, the recent reclamation of its former range will involve a period of re-adjustment.

Due to persecution and subsequent population decline in the late 19th century, pine martens were absent from most of the UK for the majority of the last century, with only a few remaining in North West Scotland (Lockie 1964). After designation as a protected species in the Wildlife and Countryside act (1981), the population has begun to recover, with expansion into their former range and presence found as far south as Stirlingshire, the Kintyre peninsula and some areas of central Scotland (Croose *et al.* 2013; Croose *et al.* 2014). Pine martens now overlap the range of the capercaillie in Scotland. The capercaillie is classified as a bird of conservation concern in the UK (Eaton *et al.* 2005), with 1,980 (95%CI 1,284-2,758) individuals remaining in Scotland in 2003/4 (Eaton, Marshall & Gregory 2007; Summers *et al.* 2010). In order to maintain the capercaillie population, breeding hens need to fledge between 0.6 and 1.1 chicks annually (Moss *et al.* 2000; Summers *et al.* 2010). Factors such as low temperature in April, high rainfall in June and predation pressure have contributed to nest failure and low population growth or population declines (Baines, Moss & Dugan 2004; Summers *et al.* 2004; Summers *et al.* 2009). In one study in Abernethy Forest, Scotland, pine martens were shown to depredate 33% of 20 monitored capercaillie nests (Summers *et al.* 2009), but their protected status means that any management to reduce predation requires a non-invasive approach.

Due to the effort required to locate capercaillie nests and the risk of disturbance of such action (Summers *et al.* 2009), management aimed at diverting the foraging behaviour of pine martens, rather than any action to directly protect nests, is considered the most feasible option to reduce pine marten depredation of capercaillie. Diversionary feeding, where food is provided to divert the damaging behaviour of the predator from the prey species of concern, has been suggested as one such option (Summers pers. comm.). This management technique has been found to be effective in diverting generalist predators (chapter 5) and may, therefore, be an option for pine marten management. The effects, however, are highly variable and depend on a suite of factors, including the response of the target species to the proffered food.

Knowledge of how pine martens respond to diversionary food sources, as well as practical details such as the most effective type and presentation method for food are essential to enable managers to plan an effective strategy. Diversionary feeding operations for other species have found the presentation method to affect the time taken for animals to utilise it (chapter 5). If the lag time between food placement and visitation is too long, food may be taken by non-target animals, increasing costs and potentially altering ecosystem dynamics by increasing the abundance or altering the behaviour of the non-target species (Cooper & Ginnett 2000; Cortés-Avizanda *et al.* 2009; Selva *et al.* 2014). Pine martens respond to olfactory cues for communication (Demonte & Roeder 1990) and scent lures have proven to be effective attractants in previous experiments (Chandrasekhar 2005; Roche 2008; Burki *et al.*

2009). Food presented on a feeding platform or other open-style feeder may, therefore, be the most effective as they would enable the scent to travel further. After the initial attraction to and use of feeders, animals must utilise diversionary food frequently enough to alleviate pressure on the vulnerable species. As protection is required during the nesting and chick rearing stages of the capercaillie life cycle, diversionary feeding is likely to be most effective over the breeding season during spring and early summer.

As a generalist and facultative predator, the diet of pine martens varies seasonally as different food sources become available (Caryl *et al.* 2012b) and as energy requirements vary with different stages of the life cycle (Zalewski 2007). This may lead to seasonal variation in the frequency at which martens utilise diversionary food, as well as which food types result in regular visits to feeders. Pine martens display increased diet breadth in response to an increase in available food sources, with a significantly higher diet breadth during summer (Caryl *et al.* 2012b). This may mean that pine martens would visit feeding stations less frequently during this time of year as other food sources are readily available. Further, during times of abundant natural food, pine martens are not likely to alter their regular foraging habits unless the proffered food is particularly attractive, so investigation into the most attractive type of diversionary food would be of benefit.

In order to assess the potential of using diversionary feeding for pine marten management aimed at reducing predation on capercaillie during the breeding season, as well as to provide guidance for effective application, we used questionnaires to collect data from people who have provided food for wildlife within the pine martens' current range in Scotland. Specifically, we aimed to:

1. Determine whether open-style feeders or feeding platforms will result in a shorter lag time than closed, box-style feeders as they will enable the scent of food to carry further.

2. Test whether the efficacy of diversionary feeding, as reflected in the frequency of visits, is lowest during the summer due to the abundance of natural food.
3. Assess whether food type has a significant effect on visit frequency and to identify the most effective sources of food.
4. Determine whether utilisation of diversionary food will continue for long enough to cover the full period when capercaillie are most vulnerable to predation, in this case, during incubation and chick fledging.

6.3 Methods

The questionnaire (Appendix 6A) was created using Bristol Online Surveys (University of Bristol) and distributed through the Forestry Commission Scotland website, the RSPB bulletin, via an email to volunteers within those organisations, and through the Stirling University website. The questionnaire was live from August 2011 to October 2013. Multiple choice questions were used throughout, with the exception of one question (8a), which asked for details of how visits were observed or recorded by the respondent; these responses were later categorised as ‘direct observation’, ‘camera trap’ or ‘other’. An open text box for further details was included within each question to enable responses that did not fit with one of the closed options, and allow the validation of responses by the authors. Where a response was given as ‘other’ and a comment provided, comments were checked and the response allocated to one of the options where possible. Out of a total of 70 replies, two surveys were removed from analysis as feeding took place outside of Scotland and a further two surveys were removed as respondents stated that feeding took place at holiday homes but did not know how long food had been provided before their arrival. All survey responses are provided in appendix 6A.

6.3.1 Statistical analysis

To test the effect of feeder type on the time taken for the initial visit by a pine marten, a proportional odds logistic regression model was fitted with ‘time to first visit’ as an ordered factor, with the levels ‘more than 6 months’, ‘3 to 6 months’, ‘1 to 3 months’, ‘less than one month’ and ‘less than one week’ coded as 1 to 5 respectively. Food placement was included as a factor with levels ‘open-top’, ‘closed’ and ‘ground’. The effect of season and food on the frequency of visits was tested using the a proportional odds logistic regression model with ‘frequency of visits’ included as an ordered factor with levels ‘irregular’, ‘more than once a month’, ‘more than once a week’ and ‘every night’ coded as one to four respectively. Responses of ‘unsure’ were removed from the analysis. Due to the high number of different combinations of food, the categories ‘jam’ and ‘honey’; as well as ‘peanuts’ and ‘peanut butter’ were combined for statistical analysis. An interaction term for season and food was included in the initial model, but removed if a likelihood ratio test between models with and without the interaction term non-significant. Analyses were performed in R version 3.1.0 (R core team 2014).

6.4 Results

In total, 66 verified responses were received between August 2011 and October 2013. Seventy one percent of respondents (47) stated that they provided food with the main purpose of attracting pine martens; of these, 15% (7) included other birds or other wildlife in their response. Thirty percent of respondents observed these visits using camera traps, and 28% through direct observation; the remaining 41% did not provide information on the method of observation. The majority of respondents placed food on an open-top platform feeder (32%), on the ground (28%) or in closed-style feeders (18%). The most popular choice of food was peanuts (49%), or a combination of peanuts and jam (23%). Items other than peanuts, jam or chicken were reported by 15% of respondents and included items such as eggs, cake and fish.

6.4.1 *Time between placement and first visit*

After the first placement of food, 58% of initial visits by a pine marten were received within one week, with a further 12% within one month and a further 15% within 3 months (fig 6.1). The type of feeder had a significant effect on time taken for the initial visit by a pine marten (χ^2=8.12, df=1, p=0.017). When food was placed in open-top feeders or on the ground, 65% and 63% of respondents, respectively, reported that visits began within one week, whilst only 18% of respondents reported that visits began within one week when food was placed in closed top feeders (Fig 6.2).

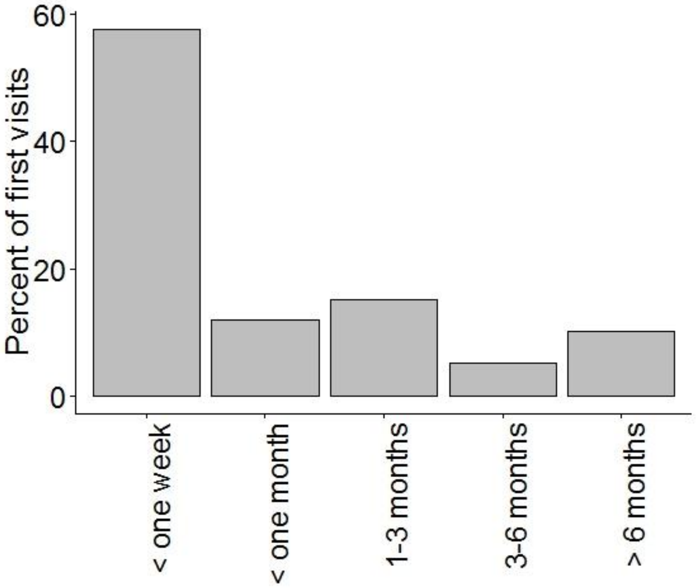

Figure 6.1. Percentage of initial visits by pine marten to feeders received during each time period, from less than one week to more than six months (n = 67).

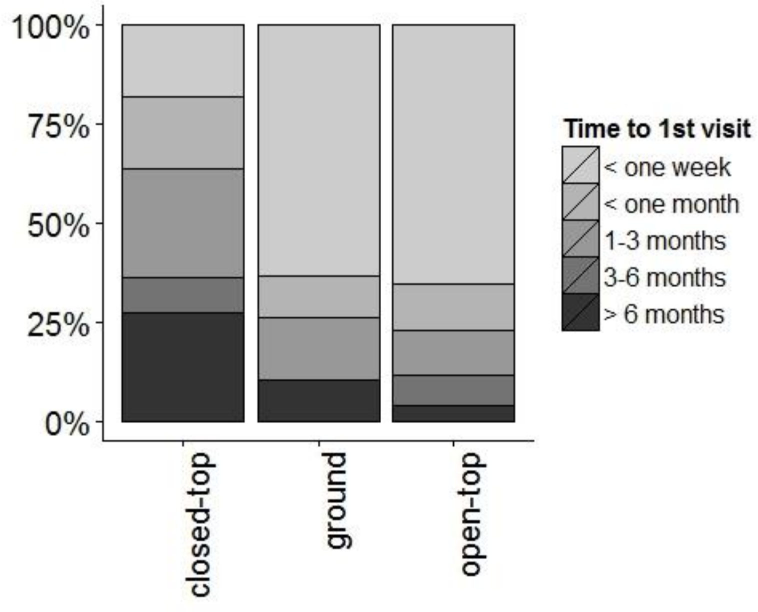

Figure 6.2. The time taken for an initial visit to a feeder by pine marten, grouped by feeder type. Responses are presented at the percentage of total responses attributed to each time period.

6.4.3 Frequency of visits

After the initial observed visit, 46% of respondents reported that subsequent visits were received daily, with a further 17% reporting that visits were received at least once per week and 2% reporting visits at least once per month (Fig 6.3); 20% of respondents were unsure of the frequency of visits. The interaction term for season and food placement did not improve model fit for the frequency of visits (χ^2=14.6, df=9, p=0.10). There was no effect of season, but a significant effect of food type on the frequency of visits, with a combination of peanuts, jam and chicken resulting in visits every night (63% of responses) or more than one per week (36% of responses). Peanuts alone were the least effective, resulting in visits ranging from every night (40% of responses) to infrequent visits (35% or respondents; Table 6.1; Fig 6.4).

6.4.4 Continuation of visits

The majority of visits continued for more than two months (48%); continuation of visits for up to one month were reported by 12.5% of respondents, although 57% of these had only provided food for one month at the time of response. Visits of up to one week were reported by 23%; of these, 46% had only been providing food for one week (Fig 6.5).

Table 6.1. Results of a proportional odds logistic regression model for the effect of season and food type on the frequency of visits by pine marten to feeders. Estimates are for the full model. Log-likelihood χ^2 statistic, degrees of freedom and associated p-values are for the log likelihood tests between full models and models without each term.

	Estimate	SE	χ^2_{df}	P
Season (spring)	-0.7495	0.5065		
Season (summer)	0.5713	0.5513	6.36_1	0.095
Season (winter)	-0.2360	0.5189		
Food (jam)	0.1160	0.6566		
Food (Peanuts, jam)	1.4041	0.4548	12.40_1	0.006
Food (Peanuts, jam, chicken)	1.2440	0.6574		

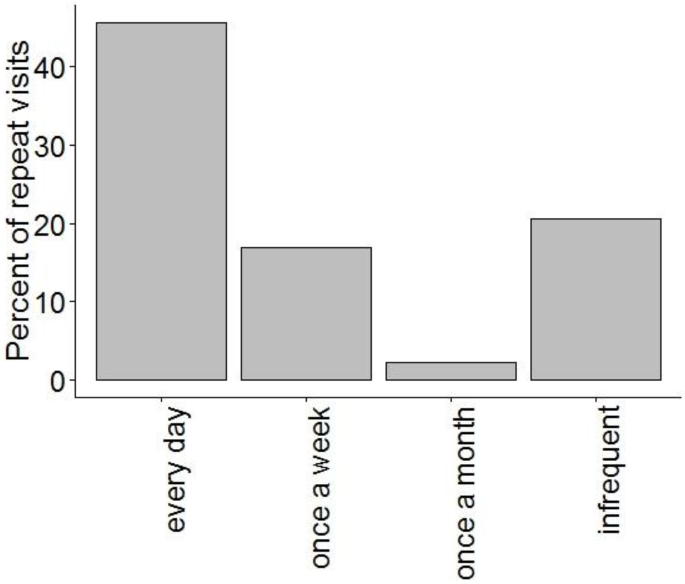

Figure 6.3. Percentage of repeated visits by pine marten to feeders grouped by visit frequency (n=67).

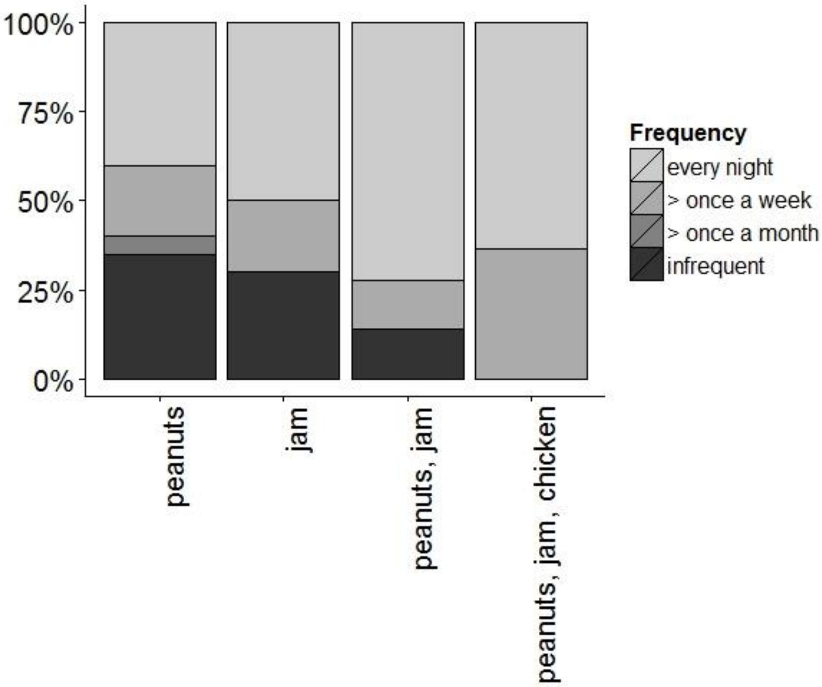

Figure 6.4. Frequency of visits to feeders by pine marten, grouped by type of food provided (n=67). Responses are presented as the percentage of total responses attributed to each time period.

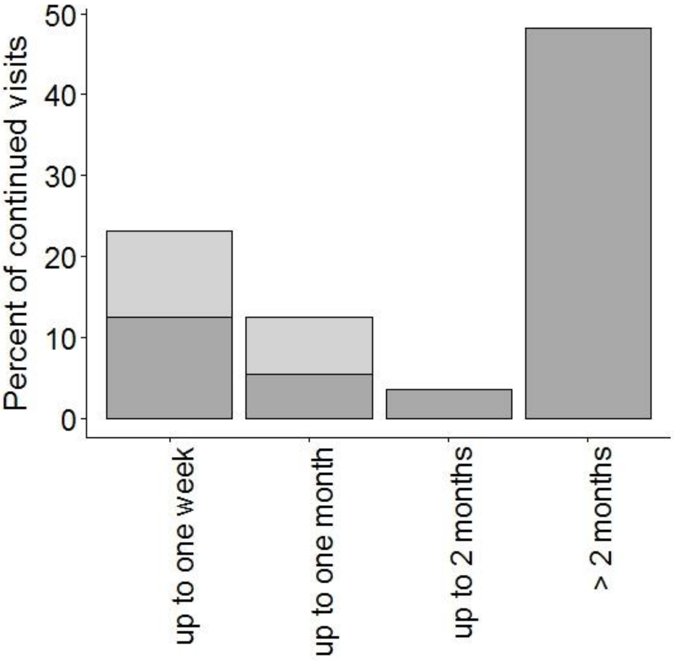

Figure 6.5. The reported period of time that visits continued for. The lighter sections represent responses that had only been feeding for a maximum of the time period given (n=67).

6.5 Discussion

Diversionary feeding has the potential to provide a non-invasive option to alleviate depredation of a vulnerable species where the predator is also of conservation concern. Stakeholder concern about the depredation of vulnerable birds such as the capercaillie by pine martens is one such instance. The efficacy of the action depends on success during several stages (chapter 5), from the initial response (i.e. utilisation of food by the target species), the 'output' or impact on depredation rates, to the 'outcome' i.e. whether management objectives are met (in this case, a recovery of capercaillie population). We assessed the initial response to diversionary feeding, and found that food used to attract pine martens to feeders in Scotland was successful. Fifty-eight percent of respondents reported visits within one week of food placement, 46% of respondents received daily visits thereafter, and 48% of visits continued for at least two months. This rapid response provides support that diversionary feeding may be a viable option for pine martens, at least in terms of how quickly they initially respond to the proffered food. After one week, there was a steep decline in the number of respondents that report that pine martens begin to utilise the feeder, with only 11% and 15% of feeders receiving a visit within one month and between one and three months respectively. This suggests that if a visit is not received quickly, the probability of a pine marten utilising the food source dramatically declines. In terms of management, a pilot study to test the time taken to attract pine martens in the area of interest would be quick and cost effective, as lack of interest in the feeders after one week suggests that the strategy is unlikely to work in that area at that time.

The use of open-top feeders or placing food on the ground reduced the time taken to attract pine martens to feeders, in line with our prediction that open sources of food would be the most effective. Lure sticks have previously been used to attract pine martens, with Hawbakers marten lure (S. Stanley Hawbaker and Sons, Fort Loudon, Pennsylvania, USA) resulting in the most visits (Burki *et al.* 2009). The use of a scent lure may further improve the reliability of diversionary food by increasing the proportion of feeders found within a short space of time.

As 46% of respondents reported that subsequent visits were received daily once feeders had been located, with a further 17% received weekly, diversionary feeding attracts pine marten regularly and therefore has the potential to alter the animals feeding behaviour, although further testing would be required to confirm this. Diversionary feeding is considered an expensive management option (Witmer *et al.* 2000; Mason & Bodenchuk 2002), although details of the costs are rarely reported (chapter 5). Depending on the number of feeding stations, layout and duration of deployment, daily visits would incur a relatively high cost compared to less frequent ones, as well as demand substantial labour time, to supply and replace food. These details should be included in any cost-benefit analyses, as well as reported with the results where diversionary feeding is carried out in order to inform future attempts on the potential return on investment.

Despite a wider dietary niche breadth during the summer (Caryl *et al.* 2012b), and possible food limitation during other times of the year, season had no significant effect on the frequency of visits to feeders. Pine martens are considered to be opportunistic generalists (Clevenger 1994), and add food items to the diet as they become available rather than replace existing ones (Caryl *et al.* 2012b). Diversionary food tends to be more effective, in terms of the utilisation of food sources, where populations are food limited (Witmer *et al.* 2000; Calenge *et al.* 2004; Ziegltrum 2004; Barrio *et al.* 2010). This may not be the case for pine martens, as the current results suggest a positive response to food regardless of natural availability. Conversely, the output of diversionary feeding, in terms of its impact on depredation rates may be limited, as pine marten may add the diversionary food to the diet, rather than replace existing food sources such as ground-nesting birds, although this would need to be tested directly.

Although the results presented in this study are promising, there are several caveats regarding the reliability of data. The anonymity of respondents (Hunter, Alabri & van Ingen 2013), as well as lack of investment by participants in the results of the research, may lead to misleading or inaccurate data. It is possible that more responses than those excluded were from holiday makers. Therefore, although a high proportion of visits were received within one week, food may have been placed in this location prior to the respondents' arrival. Almost half (46%) of the respondents that reported that visits were restricted to one week (10.7% of responses overall) also stated that they had been feeding pine martens for one week only; this suggests that the data provide a conservative estimate of visit continuation, as visits may have continued for longer if the provision of food had not stopped. Despite this concern, this study remains the first assessment of the potential of diversionary feeding for pine marten management, providing clear operational guidance for application in Scotland. We suggest that the initial response to diversionary food by pine martens would be positive, although the feasibility of successful outputs and outcomes would require further testing.

6.6 Acknowledgements

Thank you to Cerian Tatchley and Paul Lintott for comments on previous drafts.

Appendix 6A. Questionnaire responses from 67 respondents, regarding food placed for to attract wildlife, including pine marten. Figures are presented as the percentage of total responses. Responses to questions 2 and 4 do not total 100% as they provided the option to select more than one response.

1	**Do you currently, or have you in the past, left food out which attracted (or intended to attract) pine marten?**	**Yes, currently**	**Yes, in the past**			
		75	25			
2	**What was / is the main purpose of this food?**	**To attract birds**	**To attract pine marten**	**To attract other wildlife**		
		30	61	21		
3	**What did you place the food on?**	**Open-top feeder**	**Closed feeder**	**Ground**	**Other**	
		32	29	18	21	
4	**What type of food did you use?**	**Peanuts**	**Jam**	**Chicken**	**Other**	
	Spring	75	28	3	42	
	Summer	63	31	3	42	
	Autumn	66	34	4	37	
	Winter	63	31	7	34	
5	**How long after the placement of food did you receive the first visit by pine marten?**	**Less than one week**	**Less than one month**	**1 to 3 months**	**3 to 6 months**	**Other**
		58	12	15	5	10
6	**How did you observe pine marten visits?**	**Camera trap**	**Direct observation**	**Other / none**		
		30	28	42		
7	**How often did you receive visits by pine marten?**	**Every night**	**More than once a week**	**More than once a month**	**Irregular**	**Other/ unsure**
	Spring	18	32	0	18	32
	Summer	19	16	0	8	57
	Autumn	38	24	10	29	0
	Winter	21	18	3	21	38
8	**How long did these visits continue for?**	**Up to one week**	**Up to one month**	**Up to two months**	**More than two months**	**Other / unsure**
		23	13	4	48	13

Chapter 7

Discussion

Monitoring the status of populations is particularly difficult when the species is rare or elusive. Innovative methods are often required to ensure that decisions regarding conservation and management are based on a sound understanding of a species behaviour and demography. The European pine marten is one such species, and making conservation decisions based on its ecology and behaviour is particularly complicated given that it exhibits different habitat and dietary requirements across its range (i.e. Scotland; Caryl *et al.* 2012a; Caryl *et al.* 2012b). Despite the coexistence of pine martens and capercaillie in mainland Europe, capercaillie populations in Scotland are currently endangered and continue to decline (Ewing 2012). As pine martens in Scotland continue to recover their former range following their persecution and decline in the early 19th century, there has been some concern regarding their depredation of capercaillie. This highlights the need for accurate and up to date knowledge of the status of pine martens throughout their range in order to inform research and management. We attempted to provide an insight into the population status of pine martens in Scotland, assess the dietary profiles of males and females and investigated the effects of forest fragmentation on both density and diet. We then evaluated the feasibility of diversionary feeding to provide a non-lethal management option for the management of this native predator.

7.1 Non-invasive genetics for conservation

The benefits of non-invasive genetic sampling are manifold, with the lack of disturbance for vulnerable species and ease of data collection from elusive species two of the most important (Taberlet *et al.* 1996; Taberlet & Luikart 1999). The use of DNA extracted from samples such as hair, faeces, feathers, urine and egg shells is becoming commonly used in ecology (Beja-Pereira *et al.* 2009; Goossens & Salgado-Lynn 2013). Measures of presence, distribution and population density, as well as the genetic diversity (Napolitano *et al.* 2008), phylogeography (Sharma *et al.* 2009), population structure and dispersal patterns (Rodgers & Janecka 2013) can be gained without having to trap or disturb the target species. However, despite significant recent advances (Goossens & Salgado-Lynn 2013; Lampa *et al.* 2013), non-invasive genetic sampling has been hindered by poor quality DNA since its inception (Taberlet *et al.* 1996), with faecal sampling presenting a particular problem (Lucchini *et al.* 2002; Brinkman *et al.* 2010; Panasci *et al.* 2011; Demay *et al.* 2013).

In chapter two, we took the first steps to refining the process for pine martens by quantifying the effects of time and rainfall on the quality of DNA from scats, and the number of hair follicles used on DNA quantity obtained from hair samples. For scats, we found a 38% reduction in DNA amplification success over a period of 16 days, but only where scats were exposed to rainfall. These findings suggest that the time between sample deposition and collection should be kept to a minimum during periods of rainfall. Where longer periods may be required to allow sufficient samples to accumulate, sampling sessions should be carried out during drier periods only. For hair samples, increasing the number of hair follicles in each reaction significantly increased success rate and reduced the occurrence of errors during DNA amplification. Where funding is not sufficient for processing all samples, those with 13 hair follicles or more should be given priority. The rate of DNA degradation and effect of environmental conditions on DNA quality have not previously been quantified for pine martens, making these results the first to refine sample collection and processing methods in order to reduce the high errors associated with scat sampling. Our results will help enable the wealth of information available through non-invasive genetic sampling (e.g. genetic diversity, kinship, dispersal rates; Selkoe & Toonen 2006) to be obtained and allow a reliable approach to estimating population densities.

By using a combination of individual genotyping of hair samples and spatially explicit capture recapture modelling in chapter three, we provided the first estimates of population density for three forests in Scotland through non-invasive sampling. Although 71% of samples were successfully genotyped, associated errors were relatively high, with 7% of amplifications containing false alleles and 6% containing allelic drop-out. An investigation into the effect of environmental factors on DNA quality from hair samples would be of benefit here. Hair samples are protected from rainfall in the hair tubes used for our study, but factors such as temperature and humidity may adversely affect DNA quality and knowledge of this could guide future use of hair samples for genetic analysis. Despite attempts to ascertain individual identity from scat samples, success rates were poor at 7.9% for 579 scats. To our knowledge, only one previous attempt has been made to individually identify pine marten from faecal DNA (Ruiz-Gonzalez *et al.* 2013). Success rates were substantially higher for this attempt (61.9%), although genotyping was only attempted for 21 samples in total. Due to time constraints for data collection, we were not able to restrict sampling to dry periods only, although relatively short collection sessions of four to five days were used to prevent the degradation of DNA. Shorter sessions may have violated assumptions of sample independence. The ease of collection of a large number of scat samples makes refining this process valuable, and the evaluation of further variables, including storage and extraction techniques and elimination of PCR inhibitors, is an important area to focus on for future research. Until these refinements have been made, we would recommend the use of hair samples for population density monitoring.

Non-invasive genetic techniques are among the most rapidly advancing areas of genetics and, although microsatellites are currently the standard marker for individual level analyses (Broquet *et al.* 2007) alternative analysis methods may present a more reliable option. Fragment length has been found to correlate with genotyping error rates from degraded DNA (Demay *et al.* 2013). Single nucleotide polymorphisms (SNPs) may, therefore, be preferable as they do not require amplification of the relatively long DNA sequences of microsatellites. The initial identification of SNPs in new organisms remains expensive and requires the screening of a large number of individuals (Ojeda *et al.* 2014). Cost-effective methods for screening existing SNPs, however, have been developed (Ellegren 2008), and the development of these markers for pine martens is likely in the near future (Schwartz *et al.* 2012).

When faecal abundance (i.e. the number of faeces found within a designated area, usually a length of forest track) proportionally relates to the abundance of animals, faecal counts can be used as an index of population density. Scat-based indices have been used successfully for several species (sika deer; Marques *et al.* 2001; red fox; Webbon *et al.* 2004; sambar; Brodie 2006). However, variation in scat decomposition rate depending on weather conditions (Brodie 2006) as well as the influence of diet on defecation rates (Panasci *et al.* 2011; Vynne *et al.* 2012), need to be taken into account. In chapter three, we present the first attempt to quantify this relationship for pine martens in Scotland. We used home range size as a proxy for density as a strong negative correlation exists between the two. There was a significant non-linear negative relationship between home range size and the number of scats, although this equates to a relatively minor increase in scat counts (0.4 km^{-1}) for a large decrease in home range size (17km^2). Additional data is needed to clarify the relationship where home ranges are small as very few data exist at this end of the scale. Advancements in the ability to individually genotype scats, as discussed in chapter two, would be of value here as it would enable the additional measures of population density, along with scat count data, needed to increase confidence in this relationship. The addition of data regarding the effects of environmental variables on scat and DNA degradation will further strengthen confidence in scat-based indices. This could eventually enable the creation of a cost- and time- effective monitoring method, negating the need for expensive genetic analyses.

7.2 Ecological divergence between the sexes

Resource partitioning between demographic groups within a species is well reported in the literature (Main 2008; Phillips *et al.* 2011; Macho & Lee-Thorp 2014). Several theories exist on the mechanisms driving resource partitioning in mammalian species, including differential predation risk, foraging selection and levels of activity (Wearmouth & Sims 2008), as well as innate physical differences between demographic groups (Hillen *et al.* 2011; Levin *et al.* 2013). Pine martens display significant sexual size dimorphism with males being larger than females (Rossolimo & Pavlinov 1974; Zalewski 2007). Males have larger home ranges of, on average, three times larger than females (Balharry 1993; Halliwell 1997; Bright & Halliwell 1999; Caryl *et al.* 2012a) and select tussock grasslands and heath to a higher degree than females (Caryl *et al.* 2012a).

In chapter four, we assessed sex-related differences in diet. We found no significant differences in the consumption of small mammals or birds between males and females, although the high proportion of unidentified small mammals means that further analysis would be required for confirmation. In terms of habitat utilisation (chapter two), no significant difference was found between the sexes in the use of hair tubes on the edge of forest tracks. Previous evidence that females avoid open areas more than males, although the habitat types referenced were heathland and tussock grassland (Caryl *et al.* 2012a), rather than small scale features such as tracks. Differences in the behaviour, diet and habitat use between demographic groups of a population can greatly influence the effect of species management as actions targeted towards specific habitats or altering specific behaviours may only be required for a sub-set of the population (Redpath 2001; Newey *et al.* 2010). Although sex-related differences do exist for pine martens, the results of the spatial sampling regime in chapter two and diet analysis in chapters four, suggests that these differences may not affect the species response to management, particularly where actions involve the provision of diversionary food (chapters five and six).

An uneven sex ratio was found in one of the three forests tested, with eight males to two females in Abernethy. Although inference cannot be drawn about population-wide differences from the results of one forest, diverse habitat associations between males and females mean that biased sex-ratios depending on the habitat at a particular site is possible. Examination of further forest sites would enable a statistically robust analysis of whether the trends showing differential habitat preferences between the sexes were a true indication of sexual segregation.

7.3 Management implications

Due to the reclamation of their former range in Scotland, the pine martens range now overlaps considerably with that of the capercaillie, which is threatened in Scotland (Eaton *et al*. 2005). Capercaillie populations are thought to be in decline, although imprecision in population estimates has impeded precise monitoring of population trends (Ewing *et al*. 2012). There is concern amongst stakeholders such as the Scottish Game Keepers Association and the Game and Wildlife Conservation Trust that pine martens represent a depredation risk to capercaillie and that this may add to the drivers of their decline. To alleviate this risk, there has been call for Scottish Natural Heritage to consider a cull (The mammal society, 2013). The main contributors to capercaillie decline are high rainfall in June, a large change in April temperature and high predator activity (Moss, Oswald & Baines 2001; Baines *et al.* 2004; Summers *et al.* 2009). Predators have been identified as red foxes, crows and raptors as well as pine martens, with scat count indices representing predator abundance for red foxes and pine martens for all studies. Pine martens have been found to depredate 33% of capercaillie clutches at Abernethy, as measured via camera trap data (Summers *et al.* 2009), although this remains the only direct assessment of depredation on capercaillie in Scotland. Pine martens may reduce the number of chicks and broods per capercaillie (Baines *et al.* 2011a), however this only appears to be the case when weather variables (temperature and rainfall) are included in the analyses. A parallel report using the same data for predator indices and capercaillie productivity, but excluding weather data, found no correlation between indices of pine martens and any of the measures of capercaillie productivity (chicks per hen, broods per hen, brood size; Baines *et al.* 2011b).

During 2002 to 2009, red foxes were controlled across 34,000ha of capercaillie habitat, including many of the forests included in the above analyses, as part of the capercaillie LIFE project (Summers *et al.* 2009; Baines *et al.* 2011a; Baines *et al.* 2011b). Despite this, red fox indices increased by 2.7 fold between 1995 and 2009. Scat count indices are influenced by several other environmental variables such as temperature and rainfall (Brodie 2006; Panasci *et al.* 2011; Vynne *et al.* 2012) and it may be that the indices used do not reflect relative changes in red fox abundance. If this is the case, the indices used for pine martens may also be unreliable. This highlights the need for refinements to the scat count index presented in chapter 3. It also suggests that current assessments of the link between pine marten density and poor productivity for capercaillie are not based on sound data. Alternatively, the increase in red fox indices may reflect the failure of control measures. In this case, there is no evidence to suggest that a cull of pine martens would be any more effective. Any management aimed at removing pine martens from areas of importance for capercaillie would need to ensure their continued absence, although there is no current information on the required quota for predator removal. The rapid reclamation of much of their range in Scotland over a relatively short timescale suggests that this is likely to require a sustained management effort and encur considerable expense. Given that the effect of depredation by pine martens has not been reliably quantfied, but is certainly only one of a suite of factors contributing to the decline of capercailllie, pine marten removal is unlikely to yield any substantial increase in capercaillie populations and is not justifiable. This, coupled with the protected status of pine martens in

Scotland means that non-lethal management techniques are the only viable option. These techniques, along with more robust measures of the effect of depredation should be further explored.

Diversionary feeding has been suggested as a management technique to reduce the depredation of capercaillie by pine martens, although use of the strategy has seen highly variable results. Chapter five represents the first attempt to quantify the success and cost-efficiency of the strategy across 30 experimental trials with mammalian and avian species. Positive initial outputs, such as a reduction in predation, often fail to follow through to outcomes that meet stakeholder objectives, such as increased population density of the prey species (Redpath 2001). Diversionary feeding proved more effective for the mitigation of habitat damage or risks to human safety than for the reduction of depredation; when targeted towards food-limited populations (Witmer *et al.* 2000; Calenge *et al.* 2004; Ziegltrum 2004; Barrio *et al.* 2010); and when used in combination with other deterrents such as scare devices (Conover 2002). These factors were integrated into a novel decision-making framework to aid the initial planning of the strategy and its subsequent implementation within an adaptive format. Whilst a return-on-investment analysis predicted high diminishing returns for diversionary feeding, researchers rarely provide information on the costs of the operation so the analysis was based on very limited data resulting in a high level of uncertainty. Disclosure of these costs, as well the detailed reporting of effect sizes would enable future decisions to be driven by cost-effectiveness, which is vital when funding is limited.

The details of pine marten life history provided throughout this thesis can be used to guide conservation management decisions by incorporation, for example, into the decision making framework outlined in chapter five for diversionary feeding. As a protected generalist predator, the pine marten is a good candidate for diversionary strategies. Pine martens are solitary animals, suggesting that diversionary feeding is likely to reduce their foraging range if food is placed within a home range, rather than cause animals to relocate and aggregate at a particular source of food (Miller *et al.* 2003). As an opportunist feeder, however, pine martens increase their dietary niche breadth as food items become available (Caryl *et al*. 2012a) and although they may focus foraging efforts on a preferred food type, do not exclude other dietary items completely (chapter 4). This may reduce the effectiveness of diversionary feeding in terms of a reduction in predation. The results in chapter two indicate that there is no significant difference in the number of male and female visitors to hair tubes with varying proximity to forest tracks. This suggest that the placement of feeding stations close to tracks will not result in a difference in attraction between the sexes, improving the ease of access and reducing effort for food placement. The spatial distribution of feeders may not be affected by distance to tracks, but other aspects of pine marten habitat use should also be taken into account here (Caryl *et al.* 2012a). Similarity, differences in the consumption of birds between the sexes demonstrated in chapter four suggests that depredation may not be caused by a subset of the population, although more direct observation of the interaction between pine martens and capercaillie would be of benefit.

The response of target species to diversionary food is key to the success of this strategy. Chapter six provides the first assessment of this response for pine martens via questionnaire responses from people who have provided food for wildlife throughout Scotland. Pine martens appear to react positively to anthropogenic food sources, with 58% of respondents reporting that initial visits to feeders were received within one week of placement, and 46% of respondents reporting that subsequent visits were received daily. The distribution method of food that proved most effective in terms of the time taken to attract pine martens was open-top feeders and a combination of peanut butter, jam and chicken resulted in the most frequent visits. This guidance should be incorporated into the operating process outlined in chapter 5 to ensure an efficient and effective management process.

Forest management in Scotland is a dynamic process, with an increase in woodland cover from 5% to 18% since the start of the 20th century (Sing *et al.* 2013; Woodland advisory group 2013). The current goal is to further increase this to 25% by the latter half of the century (Forestry Commission Scotland 2009). On a landscape scale, this woodland expansion may benefit pine martens as, despite selection for open habitats, mature forests remain a key component of their range. On a finer scale, consideration must be given to the structure of these forest expansions as the inclusion of other matrix habitats, such as grasslands, are vital to provide foraging opportunities for the population as a whole. The current study suggests that forests with 20-35% forest cover are most beneficial for pine martens and this information should be integrated into future forestry plans. The scale of woodland expansion in Scotland means habitat alterations need to accommodate the ecosystem functions already in existence. As a top predator in these ecosystems, pine martens play a pivotal role (Roemer *et al.* 2009) and their persistence should factor highly in any management actions that alter the forested environment. This is especially important at the edge of their current range to ensure that the recovery of this charismatic native species continues to be a success.

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