Behavioural and breeding ecology of a population of European nightjar *Caprimulgus europaeus*

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Thesis submitted for the degree of Master of Research

April 2016

Acknowledgements

For their expertise, tireless assistance, and seemingly endless patience and hospitality during fieldwork, I thank Andy Lowe and Ann Ward, without whom none of the work presented here would have been possible. I am grateful to my primary supervisor Kate Durrant for similarly patient assistance at each stage of fieldwork and labwork, and for providing helpful feedback on an early draft, along with my secondary supervisor Francis Gilbert. I owe thanks also to members of the Birklands Ringing Group and Georgie Bray for assistance during fieldwork; to Sara Goodacre for helpful advice during labwork; to Alastair Feather for guidance in creating habitat maps; and to Tom Reader for advice in data analysis. Finally, I thank each of the Forestry Commission, the Heritage Lottery Fund and the University of Nottingham for funding contributions.

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Abstract

A breeding population of European nightjar *Caprimulgus europaeus* in Nottinghamshire was monitored over the course of the 2015 breeding season, and analysed alongside data recorded in previous seasons in order to investigate features of the species' behavioural, breeding and population ecology. Of particular interest were the details of the nightjar mating system, about which relatively little is known, and potential effects of nearby recreational human disturbance, which are often suggested to have a negative impact upon nightjar nest abundance and success.

Results from the study into the effects of human recreational disturbance upon breeding nightjars and nest success rates suggested that differing forms of human site visitation may have contrasting effects. Particularly, numbers of walkers and dogs were generally negatively associated with habitat productivity measured as the number of fledglings produced, and site occupancy by paired males, while relatively non-invasive visitation by cyclists appeared positively associated with these same measures.

With regard to the nightjar mating system more generally, results from GPS tracking of individuals demonstrated significant differences in nightly range sizes and distances from roosts over the course of the night between males of paired and unpaired breeding statuses, with unpaired males tending to exhibit a greater range of movement, and appeared to indicate a likelihood of extra-pair copulations. Larger area of sexually dimorphic white spots on the male plumage was found to be strongly and positively related with muscle score, which was considered an honest indication of male quality, and it is suggested that these white spots may play a role in courting and dominance interactions.

Results from genetic analyses provided some support for the hypothesis that mate switching by females may occur between broods, while geographically nearby populations were not genetically differentiated. There were significant genetic differences among breeding populations between seasons, and the population did not appear to be at Hardy-Weinberg equilibrium. It is suggested that this could provide an indication of movement between breeding sites by migratory individuals.

Overall, the results of this study of nightjar ecology provide insight into the effects upon breeding nightjar of nearby human recreational disturbance, the nightjar's breeding ecology more generally, and some elements of nightjar population ecology. The results suggest that the impacts of human recreational disturbance may not

necessarily be negative so long as the disturbance is non-invasive, and also that males appear to compete for females, with both sexes potentially mating with multiple partners. Recommendations are made for future research in a number of areas, with a suggested focus upon migratory movements following the results of the genetic analyses, with applications in the conservation and understanding of the habits of this cryptic species.

Chapter 1

1. Introduction

1.1 Conservation and the importance of behavioural ecology

Species conservation and the preservation of biodiversity is an area of widespread and growing concern to land managers, biologists and the general public alike. Earth as a whole is widely regarded as undergoing a period of rapid and unprecedented ecological and environmental change as a result of human activity, in an epoch sometimes referred to as the 'Anthropocene' (Goymer 2014). In addition to the importance of wildlife in tourism, and of the intrinsic value of preserving biological diversity and functioning ecosystems, there is increasingly an appreciation for the importance of wildlife to human wellbeing and enjoyment (Miller 2005).

Conservation biology is a branch of biology concerned primarily with the study of species and ecosystems that are impacted directly or indirectly by human activities (Soulé 1985). Often, primary focuses in approaching and informing conservation measures are placed upon questions of habitat availability (Wichmann 2004), the availability of suitable foraging or prey species (Sharps 2014), introduced predators and competitors (Lowe et al. 2000), and anthropogenic pollution and over-exploitation (Frankham 2003). A number of factors besides these are likely to influence a habitat's suitability for a given species and whether or not it is likely to become colonised, but may often be neglected. Questions of behavioural and breeding ecology have important applications in biological conservation and management (Caro 1999; Linklater 2004), and the importance of an in-depth understanding of animal behaviour in conservation is widely recognised (Berger-Tal et al. 2010). Considerations of behavioural ecology may be particularly pertinent where issues of nearby urban development and human disturbance are considered.

While there is a wide appreciation for the intrinsic and ethical value of conserving populations, and by extension species and ecosystems, conservation efforts and practices often hinge upon financial resources and providing recreational enjoyment for humans (Soulé 1985). If species may be negatively impacted by human site use and disturbance, the latter could potentially lead to contradictory scenarios, where

conservation efforts become reliant upon supporting activities that may be detrimental to their success (Gill 2001). For example, otherwise suitable habitat may remain uncolonised or sustain fewer individuals of a species than expected if individuals are deterred by high rates of human disturbance (Beale & Monaghan 2004). Additionally, the geospatial availability of similarly suitable habitat can have important effects not only upon the chances of an area of habitat becoming colonised, but also upon behavioural and population ecology. This may be particularly important where areas of suitable habitat are fragmented and geographically distant (Caro 1999), with potential implications for conspecific interactions (Harris & Reed 2002) and breeding and molecular ecology (Frankham et al. 2002).

The value of behavioural ecology in conservation is in understanding how individuals and populations may react to anthropological and environmental changes. In order to confidently propose measures for the conservation of a species, a strong understanding of that species' habits and interactions between both individuals and their environment must be obtained. Otherwise, conservation efforts risk failure, and could even negatively impact target species or ecosystems (Haukos & Boal 2016). This can present a problematic scenario, whereby in order to address species and population declines and becomes increasingly rare, an in-depth knowledge of that species' habits and interactions becomes necessary. However, recent advances in technology have meant this may often present less of a challenge than it had previously, for example through the deployment of lightweight GPS-enabled tags to record an individual animal's location over brief or extended durations without the need for direct observation. Utilisation of tracking-enabled tags over short and longer intervals has subsequently become extremely popular in studies of behavioural ecology and conservation biology for a wide range of taxa, including birds (Burger and Shaffer 2008), marine reptiles (Schofield et al. 2007), fish (Sims et al. 2009) and mammals (Bandeira de Melo et al., 2006).

1.2 Molecular ecology in conservation

Interdisciplinary approaches to conservation biology increasingly emphasise the valuable role of molecular ecology (Lande 1988). It is well established that while populations may recover from long-term and rapid numerical declines, especially when the focus of targeted conservation efforts, such declines can have lasting and

negative impacts upon their molecular ecology. Specifically, populations of a reduced size are likely to suffer both from reduced genetic diversity, and the concomitant reduction in their capacity to evolve (Frankham 2003), and an increased rate of inbreeding depression (Lande 1988) which is known to have strongly negative impacts upon individual fitness (Ralls et al. 1983). Consequently, the majority of threatened species show signs of genetic deterioration (Frankham 2003), and rapid but temporary declines can have long-lasting or even irreversible negative impacts upon populations.

One of the major issues of population genetics with regard to conservation biology is that of reduced gene flow, often as a direct result of populations becoming fragmented following habitat loss (Frankham et al. 2002). Further to applications in assessing genetic diversity, approaches of molecular ecology can provide vital insight into the population dynamics of species, such as the degree of population mixing, or otherwise the genetic differentiation of populations (Frankham 2003). Such insights may be particularly important where populations of a species are geographically fragmented, and so may become isolated; or if individuals are capable of travelling large distances, raising the possibility of movement between populations geographically distant from one another (Haig et al. 2011).

An important concept in studies of molecular ecology is that of Hardy-Weinberg equilibrium. If a population is at Hardy-Weinberg equilibrium, allelic and genotypic frequencies will not differ significantly over generations (Haig et al. 2011). For a population to be at Hardy-Weinberg equilibrium at a given locus, a number of criteria must be met. Notably, there must be no selection at that locus, no migration between populations, and mating must be random (see: Waples 2015 for a discussion of the assumptions of Hardy-Weinberg equilibrium). A significant deviation from Hardy-Weinberg equilibrium is therefore often used as an indication of evolutionarily significant processes, such as gene flow (e.g. migration of individuals between populations) or inbreeding depression (Haig et al. 2011), that warrant further investigation (Waples 2015).

1.3 The Nightjar family and European nightjar *Caprimulgus europaeus*

The Nightjars (Family: Caprimulgidae) are a family of approximately 120 species of nocturnal or crepuscular, medium-sized insectivorous birds within the order Capimulgiformes (del Hoyo et al. 1999). Species tend to be inactive and appear to exhibit a degree of torpor during the day (Smit 2011), and forage as well as court throughout the night, particularly following dusk and before dawn (Holyoak 2001; Martin 2010). Most if not all species are extremely difficult to observe in the wild due to their inconspicuousness in flight and nocturnal or crepuscular habits, and this has posed a significant challenge for their study as well as understanding their conservation status (see: Conway et al. 2007; Sharps 2014).

Perhaps the best-known species of nightjar, and the first identified, is the European nightjar (*Caprimulgus europaeus*, henceforth 'nightjar'); a seasonal Afro-Palearctic migrant with breeding populations ranging geographically from western Europe to central Asia (Holyoak 2001; Martin 2010). Active primarily from shortly after dusk until shortly before dawn, nightjar populations are identifiable by the distinctive territorial 'churring' call of the male (Reino et al. 2015), besides which individuals are generally silent in flight unless courting, when contact calls and wing-clapping are also often audible. Despite their nocturnal foraging activity and insectivorous diet, individuals are thought to forage by eye (Cramp 1985), and physiological characteristics such as light-reflecting *tapetum lucida* behind their retina suggest they are well-adapted for doing so (Martin 2010).

During the day, female nightjars nest on the ground in large open areas separately from males, who often nest in similar habitat or on a tree perch (Martin 2010), with each so cryptically camouflaged as to be generally indistinguishable from their surroundings (Troscianko et al. 2016) unless 'flushed' — caused to leave the nest or roost by the approach of an individual or potential predator. Nests tend not to have any discernible physical structure, and clutches usually consist of two eggs. Females frequently double-brood, with the second nest laid after or shortly prior to the fledging of the first, and generally nearby within the same area of habitat (Lack 1957).

Nightjar populations have historically inhabited large areas of heathland suitable for ground-nesting females, although over the last half-century areas of clearfell or 'restock' forest within commercial plantations appear to have created similarly suitable habitat (Alexander & Cresswell 1990). Nests and territories are widely associated with

the presence of bracken *Pteridium aquilinum*, sparse ground cover, and juvenile silver birch *Betula pendula* (Dolman 2010; Wichmann 2004).

1.4 Conservation status and paucity of knowledge

Populations of nightjar experienced large-scale declines during the twentieth century, particularly in western Europe (Gregory et al. 2002). In the United Kingdom, rapid declines are thought to have been the result of widespread habitat loss, with large areas of heathland replaced by agricultural land or commercial forest plantations. Nationwide population numbers are thought to have partially recovered towards the end of the century, with the maturation of commercial forest leading to the creation of suitable habitat by clearfelling (Conway et al. 2007). Nonetheless, the species in the UK is thought, following the most recent census in 2004 (see: Conway et al. 2007), to be in gradual decline and retains only a fraction of its previously extensive range; having suffered breeding range declines of more than 50% between 1972 and 1992 (Gregory et al. 2002), and with populations often fragmented and distant from one another. Consequently, the species is included on the IUCN 'Red list' of threatened species (Del Hoyo et al. 2014), and the 'Amber' list of British Species of Conservation Concern — having been downgraded from the Red list in December 2015 due to the apparent success of targeted conservation efforts (Eaton et al. 2015). Europe is thought to constitute between 50 and 74% of the species' total breeding range, and has an estimated population size of 470,000 - 1,000,000 breeding pairs (BirdLife International, 2012). UK populations account for approximately 1% of the total population, with an estimated 4,600 males temporarily resident over summer (Conway et al. 2007).

In some respects, measures that could be taken in order to avoid future declines are clear. For example, nightjars are insectivorous and rely on a reliable food source of invertebrates, many species of which suffered declines as a result of widespread pesticide use (BirdLife International 2012). The creation and maintenance of suitable habitat of heath and restock too is almost certain to have a positive effect upon population numbers in the UK. Forestry clearfelling is known to sustain produce sites with relatively high abundance of invertebrates (Atkinson et al. 2015), and so should also provide suitable foraging habitat. In other respects, such as with regard to the

effects of human recreational site use, the measures that should be taken are less clear, and require further research.

Despite being arguably the member of the Caprimulgidae about which the most is known, many of the habits of the nightjar remain unclear (Holyoak 2001), which as well as being a point of scientific interest has led to a degree of uncertainty in approaching its conservation. The nightjar is a species that exhibits a number of highly specific traits and habits (Martin 2010), certainly with respect to other birds common in the UK and much of Western Europe. For example, nightjars are the only known group of insectivorous birds known that appear to forage nocturnally by eye (Mills 1986), and one of very few nocturnally active birds to nest on the ground in open areas, with no discernible structure to the nest itself. Moreover, breeding populations migrate to and overwinter in sub-Saharan Africa (Cresswell & Edwards 2013), with little known of the location of wintering grounds or the degree of population mixing. As a result of its unique set of traits, the nightjar is in the UK a species with few if any ecological analogs with which comparisons can be reliably made. An in-depth understanding of its habits at both individual and population levels is of great importance both in considering potential measures for its conservation, and considering the impacts of environmental and anthropogenic perturbation.

1.5 Population ecology

The nightjar is one of a number of Afro-Palearctic migratory birds that travel annually between summer breeding grounds in Northern Europe and Asia to wintering sites in sub-Saharan Africa (Sanderson et al. 2006). Because of the large areas and distances involved in the migration, little is known of the migratory route, location of wintering grounds, or the degree of connectivity of this migratory system for many species. Furthermore, a large proportion of Afro-Palearctic migrants have suffered recent declines (Sanderson et al. 2006), with one in ten species considered at threat of extinction (BirdLife International 2005), while the causes as well as species' capacity for recovery are mostly unclear. A widely speculated cause for the recent numerical declines of a number of these species is the loss of suitable habitat, at both wintering grounds and 'stopover' sites throughout the African continent, as a result of widespread agricultural intensification (Tilman et al. 2002).

This migratory life-history strategy raises a number of questions regarding the nightjar's life history and conservation status; in particular, whether discrete and geographically distant breeding populations migrate to nearby areas or along similar routes, potentially leading to population mixing, or remain largely separated. The latter scenario would raise serious concerns over the conservation status of populations individually and the species as a whole. While the extensive geographic range of the species has often led researchers to conclude the species is not at threat of extinction (Eaton et al. 2015), individual populations have become increasingly fragmented due to a reduction in widespread heathland habitat. A subsequent lack of population mixing may render populations vulnerable to declines and damage the chances and sustainability of future recoveries (Finch et al. 2015). While the impact of widespread agricultural intensification in Africa upon UK breeding populations of nightjar may have been 'buffered' in some respects by the increased proportion of suitable habitat at breeding grounds (Morrison et al. 2013), genetic isolation as a result of continuing habitat loss at breeding and wintering grounds would provide cause for concern.

1.6 Recent research

In recent decades, much of the concern regarding threats posed to breeding nightjar populations has focused on the effects of human recreational disturbance at close proximity, and of nearby urban development. As a result of nightjars and in particular females ground-nesting in open areas, the use of nearby paths by walkers, particularly those with dogs, and cyclists has been thought likely to discourage individuals from nesting at sites (Feather 2015; Langston et al. 2007a), and often to present direct threats to nest survival (Murison 2002). Comparative studies of sites with contrasting rates of recreational visitation appear often to support a role of recreational disturbance in detrimentally impacting population numbers and subsequent breeding output (Langston et al. 2007a; Lowe et al. 2014). Evidence for any impact of recreational human disturbance upon nest survival are less clear. The nature of the relationship between human disturbance and the distribution of nesting females is likely to be highly important in informing conservation and forestry management practice.

Given its highly cryptic and nocturnal nature, the nightjar is a species that has been difficult to study in situ. Consequently, much of the knowledge and understanding of

the habits of the species remain largely anecdotal (Martin 2010). Recent advances in technology, such as the availability of lightweight GPS loggers, can be utilised to gain important insights in individual, population and species-level nightjar ecology. The use of radio and GPS tracking technology in nightjar research has subsequently become widespread in recent years. A large amount of subsequent research into nightjar ecology has sought to address questions of foraging suitability and habitat selection (Alexander & Cresswell 1990; Feather 2015), however these technologies can also provide important insights into behavioural and breeding ecology, which are likely to be similarly important determinants of the success and preservation of breeding populations. An appreciation of the factors that may influence nightjar breeding success is likely to be important in addressing current and future population declines of the species, while also shedding light on the behaviour and ecology of this elusive and poorly understood species.

Recent studies of nightjar movements have illustrated the importance of understanding the nightjar's behavioural and breeding ecology in order to reliably assess population numbers, and therefore conservation status. Surveys of nightjar population numbers have generally been conducted by counting numbers of churring males at different patches of suitable territory within a larger landscape (Conway et al. 2007). Habitats surveyed in these censuses are often visited sequentially. The total number of churring males observed has then been used as an indication of male population size, from which corresponding numbers of females are commonly inferred. These population surveys are then used to evaluate population trends, and as an indication of the species' conservation status in the UK. However, Sharps (2014) and Feather (2015) observed from radio-telemetry and GPS-tracking data that the range sizes of 'paired' and unpaired males (males with and without an apparent breeding partner, respectively, as inferred from field observations of individual behaviour) appeared to differ markedly, with unpaired males exhibiting larger nightly range sizes. This finding appears to suggest that traditional survey methods for this species may be unsuitable, and could lead to an overestimate of male and overall population sizes if males moving between habitats are observed more than once. However, conclusions from these studies have tended to rely on small sample sizes, and recorded tracking data over single nights, which may provide unreliable range size estimates. The importance of the independence of observations and the potential for movement between sites in wildlife surveys are widely appreciated, particularly in species of seabirds and marine mammals (Buckland et al. 2010; Glennie et al. 2015).

In general, the majority of recent research into nightjar ecology appears to have focussed upon their conservation, and providing recommendations for land management (see: Murison 2002; Sharps 2014; Wichmann 2004). In contrast, much earlier research had focussed upon characteristics such as the influence of lunar stages upon individual behaviour (Mills 1986), diurnal heterothermy (Peiponen 1965) and waking time (Wynne-Edwards 1930), and breeding ecology (Lack 1957). While such considerations have, perhaps understandably, taken a backseat in recent nightjar studies, many of the habits and traits of nightjars remain unclear, and a great deal of knowledge remains largely anecdotal (Martin 2010).

1.7 The Nottinghamshire nightjar population

The Nottinghamshire nightjar population is thought to sustain approximately 66 males, as of the 2004 UK nightjar census, accounting for approximately 2% of the total UK population (Conway et al. 2007). At that time, the population was reported to be in decline, although it is not clear whether this remains the case. The Nottinghamshire population is constituted by a large number of nearby but geographically separate commercially forested sites, broadly referred to as Sherwood Forest (Feather 2015), which appear due to frequent movements of individuals between sites both within and between seasons to constitute a single population (Feather 2015) (A. Lowe pers. comm.).

1.8 Aims

In the current study, a number of aspects of nightjar ecology are investigated within the Nottinghamshire population. The population is spread over a number of commercial coniferous forest habitats, many of which are operated by the Forestry Commission (FC), for whom the protection of threatened species such as, and specifically, the nightjar is a stated aim. These habitats are nonetheless subject to high levels of visitation by humans and therefore recreational disturbance. The aims of this thesis are to elucidate upon both the behavioural and breeding ecology of this breeding nightjar population, and also to investigate the effects that human disturbance and habitat fragmentation are likely to have upon nightjar populations. In

doing so, the results of this thesis are likely to have useful applications in both conservation strategy and methodologies of ascertaining the species' conservation status, while also shedding light upon the behaviour, ecology and habits of this popular but highly cryptic and poorly understood species.

This thesis has three main sections, each addressing aspects of nightjar ecology, with applications in the species' conservation and understanding. The first focuses on the nesting ecology of the Nottinghamshire nightjar population, with particular regard for the effects of human disturbance upon nesting distribution and success. The second section addresses nightjar breeding and behavioural ecology with regard to the mating system more generally, investigating potential interactions between male and female individuals. Of particular interest are whether apparently paired and unpaired males have differing range sizes and patterns of nightly movements, and whether sexually dimorphic white spots on male feathers might provide an indication of male quality. The third section addresses questions of relatedness between individuals, population genetics and molecular ecology; such as whether mate switching by females appears frequently to occur between consecutive broods (see: Cresswell & Alexander 1990), and whether there is significant genetic differentiation between populations that might indicate a degree of geographic isolation. Altogether, this thesis seeks to investigate the breeding behaviour of nightjars, as well as factors that may influence the viability of habitat for breeding populations, and to elucidate where possible upon the genetic structure of breeding populations and possible movement of individuals between them.

Chapter 2

2. General methods and procedures

Fieldwork took place throughout the 2015 breeding season, beginning on 30/05/2015 and finishing on 15/08/2015. Data from the 2015 season were pooled with data collected in 2013 and 2014 breeding seasons where indicated, with fieldwork in those seasons having been conducted following the same methodology outline below. British Trust for Ornithology (BTO) licensed bird ringers with many years of experience in observing and surveying nightjar nests and territories were present for and directed all of the fieldwork procedures outlined below, with the exception of disturbance surveys for which they were not present.

2.1 Field sites

Fieldwork took place at five non-contiguous, predominantly coniferous and commercially managed forests located in northern Nottinghamshire: Clipstone Forest (53°090’08.7”N 1°050’14.7”W, 1489 ha), Birklands Forest (53°120’28.1”N 1°050’41.7”W, 882 ha), Thieves and Harlow Woods (53°060’27.5”N 1°100’54.3”W, 423 ha), Park Forest (53°030’01.6”N 1°140’46.7”W, 179 ha) and Rainworth (53°133’39”N 1°130’70”W, 159 ha) (Figure 2.1). With the exception of Park Forest, each of these sites are managed under a 99-year lease by the Forestry Commission (FC), publicly accessible, and are subject to high levels of recreational visitation by walkers, including those with dogs, and cyclists. Nest location, monitoring and mist-netting were carried out with equal observer effort among sites within each of the separate forests, while disturbance surveys and artificial nest experiments were restricted to Clipstone Forest. Large areas of Clipstone Forest are subject to particularly high levels of human recreational disturbance, with recreational site visitation having increased steadily from 2000 to 2010 (Lowe et al. 2014), and since plateaued (A. Lowe pers. comm.).

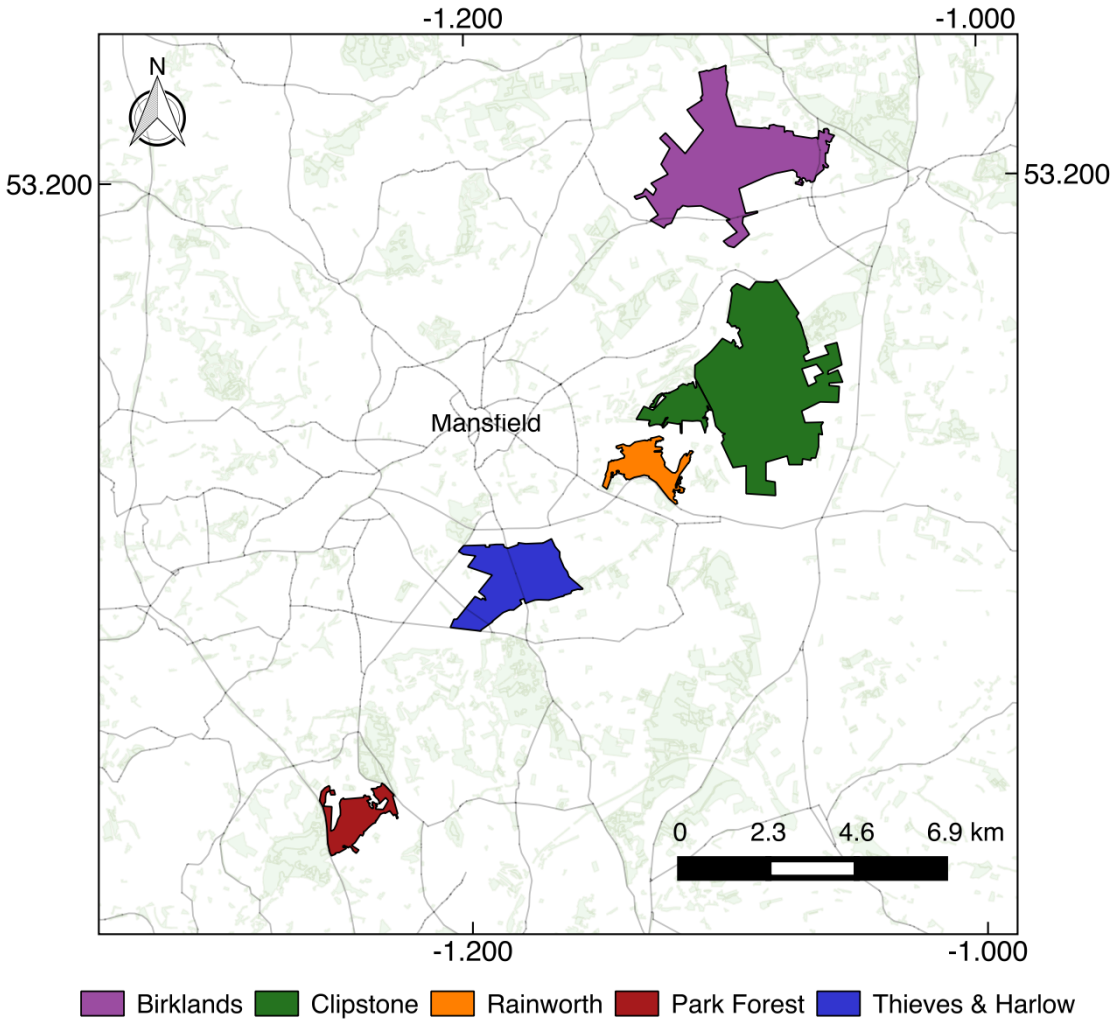


Figure 2.1: Map of the five forests at which fieldwork was carried out. North to South: Birklands Forest, Clipstone Forest, Rainworth, Thieves and Harlow Woods, Park Forest.

2.2 Location of territories

Areas were identified as suitable for male occupancy by the presence of habitat with which they are commonly associated (Alexander and Cresswell 1990; Dolman 2010; Sharps 2014; Verstraten et al. 2011), such as lowland heath, restock or pre-thicket (see: Hemani et al. 2004). Each site identified as a potential male territory was subsequently observed for approximately 2 hours between 21:00 and 23:00 on a night with negligible wind, and no or little rain. Where a male was observed or heard making the churring call within the confines of the habitat, the corresponding habitat was considered to constitute a male territory (Holyoak 2001), and was revisited at least twice more over the remainder of the season.

Once a male territory was located, the site was then surveyed for the presence of a female. These surveys were conducted by observing the site for approximately two hours between 21:00 and 23:00, either on the same or a subsequent and similarly suitable night, listening for contact calls between interacting male and females (Martin 2010), and watching for females flying over the territory or flushing from the ground within the habitat.

For each of the territories located, it was inferred whether or not the male was paired using criteria described by Sharps (2014) and used by Lowe et al. (2014). Males were classified as paired if they were observed to practice churring calls only intermittently and to frequently interact with females at the nest, or appeared to engage in the provisioning or incubation of a nest (Holyoak 2001). Results of nightjar tracking studies have previously indicated that these criteria are suitable and that classification appears accurate (Feather 2015; Sharps 2014), though it is important in interpreting results to consider that this classification is inferred through behavioural observation.

2.3 Nest searching and monitoring

All male territories and sites at which females were observed were subsequently surveyed for nests. Sites were monitored during nighttime activity for either individual seeming to vacate or return to a specific location on the ground within the habitat, that may be indicative of a nest in the area, or for nightjar activity seeming to be focussed around any given area of the larger habitat. Any such sites or wider areas were revisited during daylight hours, and the area around the suspected nest searched

systematically by teams of BTO-licensed experts and trained volunteers, each tapping surrounding vegetation with a metre-long stick in order to flush any nesting females in the immediate vicinity. Where the activity of interacting males and females provided no specific clues as to the location of the nest within the habitat, areas of that habitat deemed by the presence of commonly associated vegetation such as sparse bracken *Pteridium aquilinum* and juvenile silver birch *Betula pendula* to be most suitable for nesting sites (Alexander & Cresswell 1990; Verheyen et al. 2011) were searched in the same manner.

Once an active nest was located, if a nesting female was found to be incubating eggs an *iButton DS1921G-F5* (Maxim, USA) thermologger, covered with black tape to reduce its conspicuousness and glued to a nail to hold it in place, was pushed into the earth directly beneath one of the two eggs. The thermologger, accurate to within ± 0.5°C, was set to record the temperature of the eggs once every eight minutes, with the intention of monitoring for daily nest absences. The nest site was subsequently revisited between seven and eleven days afterwards in order to collect the thermologger, which could store up to eleven days' data (2048 readings). If the female was still incubating, the thermologger was replaced following the same methodology.

Where eggs had hatched and chicks were present at the nest, their age in days was approximated by developmental stage and the hatch date back-calculated. Hatching dates as well as field observations during the incubation stage were then used to infer approximate dates of egg laying, following the generally observed incubation time of 18 days (Holyoak 2001; Langston et al. 2007a). Chicks located at nests were ringed according to BTO procedure. All active nests with chicks were visited every seven to ten days until the chicks had fledged, and fledging success recorded.

Nightjar females are known commonly to double-brood (Lack 1957; Holyoak 2001). Where multiple nests were located at a single site, a common maternal parent could be inferred through a combination of the number of females observed at that site, inferred nest initiation and hatch dates of each nest, and proximity between nests. Often, further cues were available, such as the presence of ringed chicks from a previously located nest sharing or being observed in the immediate vicinity of the nest site of a female incubating eggs.

If a nest was found upon visiting to have failed, the stage of failure (i.e. whether chicks had hatched prior to failure) was recorded through a combination of earlier field observations from the nest and cues such as egg fragments at the nest site. Nests at which eggs remained but were no longer incubated, as judged by the absence of a female on subsequent daytime visits, were classified as abandoned.

2.4 Capture and GPS tagging

Adult individuals were captured using mist nets in order to record ring numbers for BTO records, ring newly caught individuals, record morphometric measurements and apply GPS tags. Capture of males was prioritised, as it was considered to interfere less with the incubation and provisioning of nests which are known or thought to be performed primarily by the female (Sharps 2014). Mist-netting was performed only when weather was considered suitable and not to pose a threat to individuals caught in the net, or offspring at the nest. Specifically, nights with strong winds and persistent rainfall were avoided as the nets were likely to be rendered conspicuous, and because caught individuals risked becoming hypothermic through exposure to rain.

Where male territories were identified, between 20 to 25m of mist nets, according to the size and shape of the habitat, were set at a suitable location. Suitable locations for the net were usually identified by the presence of concealing vegetation, such as juvenile silver birch *Betula pendula* which were considered to render the nets less visually conspicuous. A tape lure was placed in the vegetation below the centre of nets, with recordings of male nightjar churring and wing-clapping played periodically. The habitat area was then vacated, and nets were checked a minimum of once every 10 minutes.

Once captured, individuals were removed from the net by a fully trained and BTO licensed professional, and were placed into a cloth bag. Birds were weighed while inside the bag using a 0.5-graduated Pesola balance with a maximum capacity of 100g, then removed and the weight of the bag subtracted from the previous measurement. Thereafter, pectoral muscle (1 to 3), fat (1 to 8), stomach size (1 to 3) and, if female, brood patch (1 to 5) were all scored by licensed ringing experts according to standardised BTO protocol, and outside wing and tail primary feather lengths measured using a 300mm metal ruler. If a bird was an adult male,

photographs were taken of the left wing and left side of the tail with a ruler placed equidistant from the camera for scale, so that areas of white spots characteristic of the male plumage (Cramp 1985) could be measured (see: Chapter 4).

NanoFix (Pathtrack, UK) GPS data loggers were applied to individuals following methods approved by the BTO, similar to Sharps (2014) and as described by Feather (2015). Tags were applied to the base of the two central tail feathers using adhesive tape, and further secured with dental floss and cyanoacrylate adhesive. For analysis, data from these tags were pooled with data from PinPoint (BioTrack, UK) GPS data loggers that had been applied to individuals in 2013 and 2014 breeding seasons following the same methods. All GPS tags weighed less than 2% of the body weight of the individual to which they were applied, with 2-3% of an individual's weight widely considered the threshold for tags in order to avoid impacting individual survival (Whitworth et al, 2007). Tags fitted in all years were programmed to begin recording locations a minimum of two nights after the fitting of the GPS tag, in order to avoid potential effects of stress from capture upon the data retrieved. To retrieve data, individuals had to be recaptured, and tags removed. Because nightjars moult seasonally (Cramp 1985), it was considered that any tags not removed would be shed during the overwintering period.

2.5 DNA sampling

Once individuals were captured or located as chicks at the nest, DNA samples were collected. Buccal swabs were taken using rayon-tipped Sterilin swabs (ThermoFisher, UK), which represent a useful and relatively non-invasive method of obtaining samples from individuals without posing a significant risk to individual health (Wellbrock et al. 2012). The inner buccal cavity was gently brushed with the swab, which was then removed and placed in a sterile tube. Once collected, samples were stored at -20°C and left until extraction in order to avoid repeated thawing, known to negatively impact the quality of the DNA extraction product (Shao et al. 2011). Extracted samples that were not used were then frozen at -80°C along with un-extracted, unused samples.

2.6 Habitat classification and selection

Forestry habitat was classified according to dominant species and the stage of development following the criteria of Hemami et al. (2014), and as used in the study of nightjar habitat preference by Sharps (2014) and Feather (2015). Sites were classified as: agricultural, broadleaf, heathland, improved grassland, mature conifer, pole, pre-thicket, restock, thicket, urban and water habitats (see Table 2.1 and Table 2.2 for corresponding criteria). A vegetation raster including habitat classifications at all of the sites was constructed in QGIS (Quantum GIS Development Team, 2016) using publicly available maps and shapefiles for each of the field sites, freely obtainable from the Forestry Commission (FC), and existing data for previous years (Feather 2015). Habitats within the study area had been surveyed in summers of 2013 and 2014, and records from these years were used for data from the corresponding breeding seasons. Sites of suitable nesting habitat were visited in the 2015 season, and an updated shapefile for the 2015 season constructed by modifying habitat classifications from 2014 where appreciable change had occurred (e.g. through clearfelling of mature forest) for analysis of data collected during the corresponding 2015 breeding season. This was done by adding to the existing shapefile an attribute for 2015 habitat classification, and copying habitat classifications from 2014 in all habitats but those that had changed appreciably (following personal observations and guided by the advice of local fieldworkers), which were classified individually according to the criteria in tables 2.1 and 2.2.

Habitat selection analyses followed the methods outlined by Calenge (2016a) and performed in the corresponding R extension package *AdehabitatHS*. Manly selection ratios (Manly 2002) quantify disproportionate use of habitats with regard to their respective availabilities. There are three orders of Manly selection statistics that are commonly identified and utilised in habitat selection studies, described by Thomas and Taylor (1993). Design I studies do not differentiate between individuals, but instead quantify habitat selection ratios at the scale of the population. Design II studies do differentiate between individual habitat use, but again consider the availability of habitat to be the total proportion of habitat available to the population. Design III studies differentiate between individual habitat use, and also define proportional availability relative to each individual — often by including only habitat within a given distance of an area of primary activity such as a primary roost, or within the total range of individual movement. (Calenge 2007)

In the current study, design I Manly selection ratios are used to investigate habitat selection with regard to nightjar nests, while design III selection ratios are used to investigate the factors influencing nightly movements. Used nesting habitat is in this study defined by habitat classification at the location of the nest, while used habitat with regard to nightly ranges is defined by all GPS relocations over the course of the night. Available nesting habitat is defined based on the total proportion of each habitat classification within all of the five forests, as individuals have been commonly observed from ringing records to move between forests during a single breeding season. Habitat availability with regard to individuals' nightly movements is defined as the proportion of each habitat available within the total range of movement for each night.

2.7 Disturbance surveys

Throughout July 2015, 30-minute daytime visitation surveys were carried out at discontiguous areas of habitat identified as suitable for nesting nightjar throughout Clipstone Forest. Areas of heath and restock were considered as sites suitable for sustaining nesting female nightjars. Eighteen such habitat sites were identified, and each of these habitats were then surveyed for human disturbance in order to investigate any effects of recreational site use upon nesting nightjars.

Surveys of human recreational use were conducted for 30-minute periods at randomly generated points on a main path at each habitat site between 11 and 15 times over the 31 days of July 2015. At least one survey was conducted at each habitat site within each of the following time designations: early morning (07:00 to 8:59); mid-morning (09:00 to 10:29); late morning (10:30 to 11:59); midday (12:00 to 13:29pm); afternoon (13:30 to 14:59); late afternoon (15:00 to 16:59); evening (17:00 to 19:59). Surveys were scheduled randomly using random number generators. No surveys were conducted overnight, as the site is only accessible by gate to visitors between the hours 7am and 9pm — walkers and dog walkers may have been able to access some areas of the site overnight, but were considered unlikely to do so, as parking access was not available during these hours and there were no immediately neighbouring urban areas.

A one-minute acclimation period was allowed between arriving at the habitat and the start of each survey. Each visitor to pass the point on the path at which the survey was carried out was classified as a pedestrian or a cyclist, with numbers of dogs and vehicles such as cars and forestry vehicles also recorded. Numbers of birds of prey and corvids, which are often suggested to predate eggs at nests (Dolman 2010), to pass over the confines of the habitat were recorded, as well as their estimated distance from the observer. Observed numbers of deer and small mammalian potential nest predators (see: Dolman 2010) such as weasels observed within the habitat boundaries were recorded likewise. Each survey was carried out by two fieldworkers, with each surveying either human visitation or wildlife.

2.8 Ethical notes

A number of precautions were taken to avoid causing stress to captured birds or create unnecessary disturbance within or in the vicinity of suitable habitat. While performing fieldwork, workers were as quiet as practically feasible, and torches were always turned off until necessary. All handling of birds and nest-searching was carried out or supervised by BTO-licensed officials, with considerable experience handling and observing nightjars, following official BTO procedures and protocols. Access to field sites was granted by the FC.

Neither nest checks nor mist-netting were performed in unsuitable weather that may pose a threat to the birds or their offspring. Nest checks and handling of captured individuals were always performed over as short a period as possible to avoid causing unnecessary stress to individuals, or lengthy absences that may interfere with egg incubation or leave chicks vulnerable to predation. Where necessary, for example if weather was observed to change dramatically, individuals were released despite incomplete data collection. Nests were not checked within seven days of the last check, in order to avoid the creation of paths or cues that could draw potential predators to the nest.

2.9 Statistical analyses

All statistical analyses were conducted in R version 3.2.2 (R Core Development Team) unless otherwise stated. Mean averages are given ± one standard error. Figures were created using R packages *ggplot2* (Wickham & Chang 2016) and, for habitat selection graphs, *AdehabitatHS* (Calenge 2016a).

2.10 Tables

Table 2.1: Criteria used for habitat classification of coniferous forest growth stages, adapted from Sharps (2014).

Habitat	Age (years)	Mean tree height (m)	Mean canopy cover (%)	Tree density (m^{-2})	Ground cover
Restock	0-4	<1	0	0	Predominantly bare ground/moss with some herbs (~20%) and grasses
Pre-thicket	5-10	3	20	0.3	Increased grass cover (>50%), with bushes (eg. broom *Sarothamnus scoparius*, gorse *Ulex europaeus*, 12%) and herbs (12%)
Thicket	11-20	12	90	0.3	Increased grass cover (>50%), with bushes (eg. broom *Sarothamnus scoparius*, gorse *Ulex europaeus*, 12%) and herbs (12%)
Pole	21-44	18	90	0.15	Bare ground/moss dominant (>50%), some grasses (25%), with bramble (*Rubus fruticosa*, 8%) and bushes (5%)
Mature	>45	30	80	0.07	Increased ground cover, with grasses (27%), bracken (*Pteridium aquilinum*, 15%), herbs (15%), bushes (12%), bramble (5%)

Table 2.2: Criteria used for classification of areas of non-conifer forest habitat, adapted from Sharps (2014).

Habitat	Description
Broadleaf woodland	Broadleaf woodland, >30 years since planting
Improved grassland	Areas of grassland improved by farming practices, and amenity grassland such as picnic areas.
Agricultural	Farming and arable land situated outside of forest boundaries
Urban	Areas with >50% houses or open areas covered by concrete or gravel
Water	Rivers, reservoirs, ponds and lakes

Chapter 3

3. Nesting ecology

Migratory breeding grounds are unique within the life cycle of a population in the prioritisation of individual breeding success in addition to, or even at the expense of, individual health and survival (Ockendon et al. 2013). In addition to the suitability of habitat for sustaining breeding populations, its inhabitation and the subsequent survival of offspring is an important determinant of population health and sustainability. Questions of habitat suitability, nest success and offspring survival are therefore frequently the focus of conservation efforts and ecological research (Morris 2003).

A range of factors are likely to influence the suitability of habitat for a given animal species. Two of the most immediately obvious factors in determining viability with regard to nightjars are the availability of suitable nesting habitat and prey species (Larson et al. 2004). The widespread availability of restock and pre-thicket habitats resulting from the maturation and subsequent clearfelling of commercial forest is widely considered to have compensated for the widespread loss and fragmentation of heathland habitat that predominated in the early part of the 20th century (Verstraeten et al. 2011). Moreover, areas of forestry restock and heathland appear to provide ample foraging grounds, sustaining a large number of species of suitable invertebrate prey (Atkinson et al. 2015; Sharps 2015). It is less clear however whether heathland may nonetheless remain the preferred nightjar habitat, or if habitat resulting from clearfelling is just as viable for females choosing nest sites.

In addition to the presence of suitable nesting and foraging habitat, levels of human disturbance and, or relating to, urban development in surrounding areas appear likely to be similarly important for a number of species and for ground-nesting birds in particular (Showler et al. 2010). The effects of human disturbance upon vertebrate species is a contentious point of conservation biology and land management policy, and one of increasing importance as recreational use of the countryside has become increasingly popular (Gill et al. 1996). While public access and use of sites of conservation importance is generally considered beneficial in increasing their value

and public support, there have frequently been suggestions that it could also have strongly negative effects upon wildlife, and upon ground-nesting birds in particular (Gill 2001). Studies into the effects of human disturbance upon nesting birds have often recommended the creation and prioritisation of 'buffer' zones and set-back distances of paths from suitable nesting habitat (Showler et al. 2010).

Much of the research and conservation effort with regard to breeding nightjars and ground-nesting bird populations has focussed upon the effects of human site use (Langston et al. 2007a; Lowe et al. 2014; Murison 2002). It has been suggested that recreational use of paths neighbouring or passing through suitable habitat will have the effect of discouraging nesting females from occupying sites in areas with high levels of visitation, and that nest survival may be lower at frequently disturbed sites (Murison 2002). Negative effects of human visitation upon nest hatching success rates have been reported for a range of ground-nesting bird species, including the common ringed plover *Charadrius hiaticula* (Pienkowski 1984; Liley 1999) and hooded dotterel *Thinornis rubricollis* (Dowling & Weston 1999), and upon post-hatch fledging rates among adelie penguins *Pygoscelis adeliae* (Geise 1996). However, studies have also reported no effect of increased rates of human visitation upon hatching success in studies of ground-nesting black grouse *Lyrurus tetrix* (Baines & Richardson 2007) and common sandpiper *Actitis hypoleucos* (Yalden 1992), nor upon post-hatch fledging rates of gentoo penguins *Pygoscelis papua* (Holmes et al. 2006). Overall, the effects of human disturbance upon nest success rates and habitat productivity in terms of fledglings for ground-nesting birds remain unclear (see Showler et al. 2010 for a review).

As nightjars are nocturnal and appear to exhibit a degree of torpor during daytime (Peiponen 1965), it seems likely that this species will be susceptible to predation during this period, and may therefore be especially vigilant in selecting nest sites. Comparative studies between sites with differing levels of public accessibility, or at varying distances from urban developments, have supported a role of human recreational disturbance in nightjar breeding population declines (Langston et al. 2007a; Lowe et al. 2014). Lowe et al. (2014) investigated the effects of increased recreational site use in Clipstone Forest upon the distribution of breeding nightjars, and demonstrated a general movement of breeding pairs from the busy northern area of the forest to the less frequently visited southern area throughout a period of increasing recreational site use between 2000 and 2010. Despite this, there appears

to be relatively little evidence of any impact of human disturbance upon the survival of nightjar nests; Dolman (2010) and Lowe et al. (2014) reported no such trend, while Murison (2002) and Langston et al. (2007a) reported evidence that higher rates of human disturbance had a negative impact upon nest success rates.

Existing studies into the effects of human site use upon breeding nightjar populations have tended to rely upon inferred visitation rates based on path area (Murison 2002), accessibility of the site (Langston et al. 2007a), or the proximity to popular local amenities (Lowe et al. 2014; Liley & Clarke 2002; Liley & Clark 2003). These studies fail therefore to quantify visitation rates in order to demonstrate that human visitation itself appears to be the cause of lower occupancy rates, or to distinguish between the effects of differing forms of recreational visitation. It seems likely for example that the effects of frequent site visitation by walkers with dogs may have a more strongly negative impact upon nesting birds than frequent visitation by cyclists that rarely leave designated paths (Liley & Clark 2002). In the current study, an attempt is made to quantify visitation rates of differing forms of human recreational site use for a number of suitable habitat sites, and to investigate the proposed effects of increased site use upon both the occupancy by nightjar and their subsequent nesting success. Corvids and small mammals have been regarded as potential nest predators (Dolman 2010), and so numbers observed for each are included in analyses to account for possible non-human effects.

Previous studies have often suggested that nightjar nests nearer to paths suffer higher rates of nest failure (Feather 2015, Murison 2002), and so too nests in habitats that are more frequently visited (Langston et al. 2007a). Artificial nests are often utilised in studies with a focus upon avian conservation in order to investigate differences in predation rates between habitats or at varying distances from features such as footpaths (Purger et al. 2012). The use of artificial nests may therefore permit investigation into whether nest-predation rates in general appear higher in areas nearer to paths or in busier areas of habitat. Alternatively, an absence of any effect of site use or distance from paths upon predation rates may suggest that increased rates of nest failure nearer to paths or at busier sites are more likely related to female nest abandonment as a result of frequent disturbance, or that conspicuous flushing may attract predators (Dolman 2010).

The loss of widespread heath habitat has meant that habitat considered suitable for nesting nightjars has become largely concentrated within areas of publicly owned and commercially operated forest (Spencer & Haworth 2005). Although studies have suggested that areas of habitat more frequently visited by humans exhibit lower rates of occupancy by nightjar, it is clear that breeding nightjar must nonetheless often occupy habitats that are subject to human visitation, and that this is likely to be important if UK populations are to avoid further declines. In addition to avoiding especially busy areas of habitat, it has been suggested that nightjars, and females in particular, may to some extent compensate for the potential effects of human visitation by nesting further from paths than expected by chance (Murison 2002). Furthermore, it may be that females are able to adjust their behaviour at the nest to reduce their conspicuousness; for example, birds that are frequently disturbed are often observed to adjust the distance at which they practice evasive behaviour relating to the approach of a potential predator, measures of which have been considered an indication of species' likely tolerance to urban disturbance (Lin et al. 2011).

Finally, as a nocturnally active, insectivorous bird that is prone to predation both at adult, egg and chick stages, the lunar cycle is often thought to be important in influencing activity levels and clutch initiation of nightjars (Mills 1986; Perrins & Crick, 1996). It has been suggested previously that adults may be able to hunt more efficiently, i.e. with smaller ranges of movement, on nights of high lunar luminosity (Mills 1986; Holyoak 2001), with evidence of strong influences upon behaviour having been reported for the related species standard-winged nightjars *Macrodipteryx longipennis* and long-tailed nightjars *Caprimulgus climacurus* (Jetz et al. 2003). This has led to the suggestion that females may be more likely to lay clutches that will hatch either on nights of high lunar luminosity, or so that chick development stages coincide with periods of high lunar luminosity in order that parents might provision them with greater efficiency (Perrins & Crick 1996).

The primary aims of this chapter are to observe for effects of human recreational disturbance upon nest distribution and survival within publicly accessible forest habitats, investigate possible compensatory nesting behaviour, and to further investigate the factors influencing the location and timing of nesting events. The results of this chapter are likely to be useful in contexts of nightjar conservation, land

management, and more generally in understanding the nesting habits of a cryptic ground-nesting nocturnal bird species.

3.1 Methods

Analyses include data collected from all field sites unless otherwise stated. GPS co-ordinates of nests were recorded in years of 2014 and 2015, while nest numbers and records for individual areas of habitat within sites were available for breeding seasons from 2010.

3.1.1 Habitat selection

Habitat selection of nesting nightjars was investigated by referencing all nests from 2015 for which GPS co-ordinates had been recorded with a vegetation raster produced from shapefiles created in QGIS, using the R extension packages *Raster* (Hijmans 2015) and *rgdal* (Bivand et al. 2016a). Using the R package *adehabitatHS* (Calenge 2011), type I Manly selection ratios (Manly et al. 2002) were calculated for habitats of agricultural land, mature broadleaf forest, conifer forest, heath, pole, pre-thicket, thicket, and restock, classified following the methods of Hemami et al. (2004). Used habitat was defined as the habitat classification at the location of the nest, while available habitat was considered to be the total proportion of each of the habitats available within all forests. This classification of habitat availability was considered suitable as individuals are commonly captured within habitats in different forests during a single breeding season. Because of the large number of habitats included in comparisons, P-Values for selection ratios for each habitat classification were Bonferroni-corrected to provide a threshold for significance of $P < 0.005$.

3.1.2 Disturbance analyses

Surveys were conducted at areas of suitable nesting habitat in Clipstone forest between 01/07/2015 and 01/08/2015 following the methods described in Chapter 2. Mean averages of different survey measurements were taken for each of the time designations, which were subsequently averaged for each site to produce an generalised average rate of visitation per 30 minute survey by each of pedestrians,

cyclists and dogs, as well as observed numbers of potential predators of corvids, birds of prey, deer and small mammals such as grey squirrels *Sciurus carolinensis* and red foxes *Vulpes vulpes* (Holyoak 2001).

Numbers of nesting females per site rather than numbers of nests were preferred as a measure of site preference among nesting Nightjars, due to the species' tendency to produce two broods (Lack 1957; Berry 1979) which would violate the independence of nest numbers as a response variable. Ringing records suggest that nightjars do not appear to exhibit fine-scale habitat fidelity between seasons - that is, ringed individuals have not been observed to tend to return to the same patch of habitat within a given forest - and so independence of occupancy between years was not considered an issue. Records from the site for previous years (A. Lowe, unpublished data) showed that nests were frequently located in patches of habitat where paired males were absent. It was not thought that this discredited the classification of males as paired or unpaired, as males were suspected from field observations to potentially mate with females outside of their own territory. Moreover, it was considered possible that females may move to another area of suitable nesting habitat prior to clutch initiation, particularly if disturbed (Lowe et al. 2014). Given the usual dispersal of male nightjars throughout areas the forest (see: Lowe et al. 2014), numbers of nesting females were considered to provide a measure of female habitat preference independent of male occupancy. Rates of habitat occupancy by males and by paired are also investigated, following the suggestion that high rates of disturbance may decreased a male's likelihood of being paired (Lowe et al. 2014).

Because of the large number of variables recorded during disturbance and wildlife surveys, a Principal Component Analysis (PCA) was used in order to explain as much variance as possible while avoiding the issue of over-fitting models. Generalised Linear Models (GLMs) were subsequently used to investigate possible effects of survey measurements upon Poisson-distributed response variables of numbers of nesting females and fledgling productivity (number of nests to produce at least one fledgeling), and binomially distributed response variables of nest success and male occupancy for each area of habitat. Maximal models for each response variable included principal components of survey measurements, as well as habitat area and classification (heath or restock).

Relative levels of visitation by cyclists and walkers with and without dogs were for each area of habitat considered the result of its position along commonly used paths and relative to popular site entry points. Paths and entrances were unchanged between 2014 and 2015 breeding seasons, and visitor numbers both overall and between sites were suggested to have been similar within each of the two seasons (A. Lowe, pers. comm.). Subsequently, each of the areas of habitat were categorised from survey averages as being subject to relatively 'low' (none observed), 'medium' (visitors observed, but fewer than one per 30 minutes), 'high' (one or more per 30 minutes) or 'very high' (10 or more per 30 minutes) rates of visitation by each of walkers and cyclists. Generalised Linear Mixed Models (GLMMs) with Poisson-distributed response variables were then used to investigate possible effects of site visitation upon Poisson-distributed response variables of numbers of nesting females and fledging productivity, incorporating data from 2014 and 2015 breeding seasons. GLMMs were also used to investigate effects of visitation upon binomially-distributed response variables of nest success and the presence of male and paired male territories. As before, variables of habitat area and classification were included in maximal models, with year and habitat ID incorporated as random effects.

In order to investigate the effects of disturbance upon nightjars between 2000 and 2010, Lowe et al. (2014) partitioned Clipstone forest into north and south sites, either side of an intersecting road (Figure 3.1). In their study, the north site was said to be subject to considerably higher rates of visitation, following personal observations and the location of a visitor centre and other popular facilities at the north-end of the forest. The suggested higher rate of visitation for habitats in the northern area of the forest was investigated in the current study using a two-tailed Mann-Whitney rank sum test with a response variable of average numbers of human visitors per habitat area, and habitat areas grouped as being in north or south areas of the forest. A two-tailed Mann-Whitney test was also used to investigate whether nests in northern site experienced lower success rates than did nests in the southern area including nests over all breeding seasons from 2010 to 2015.

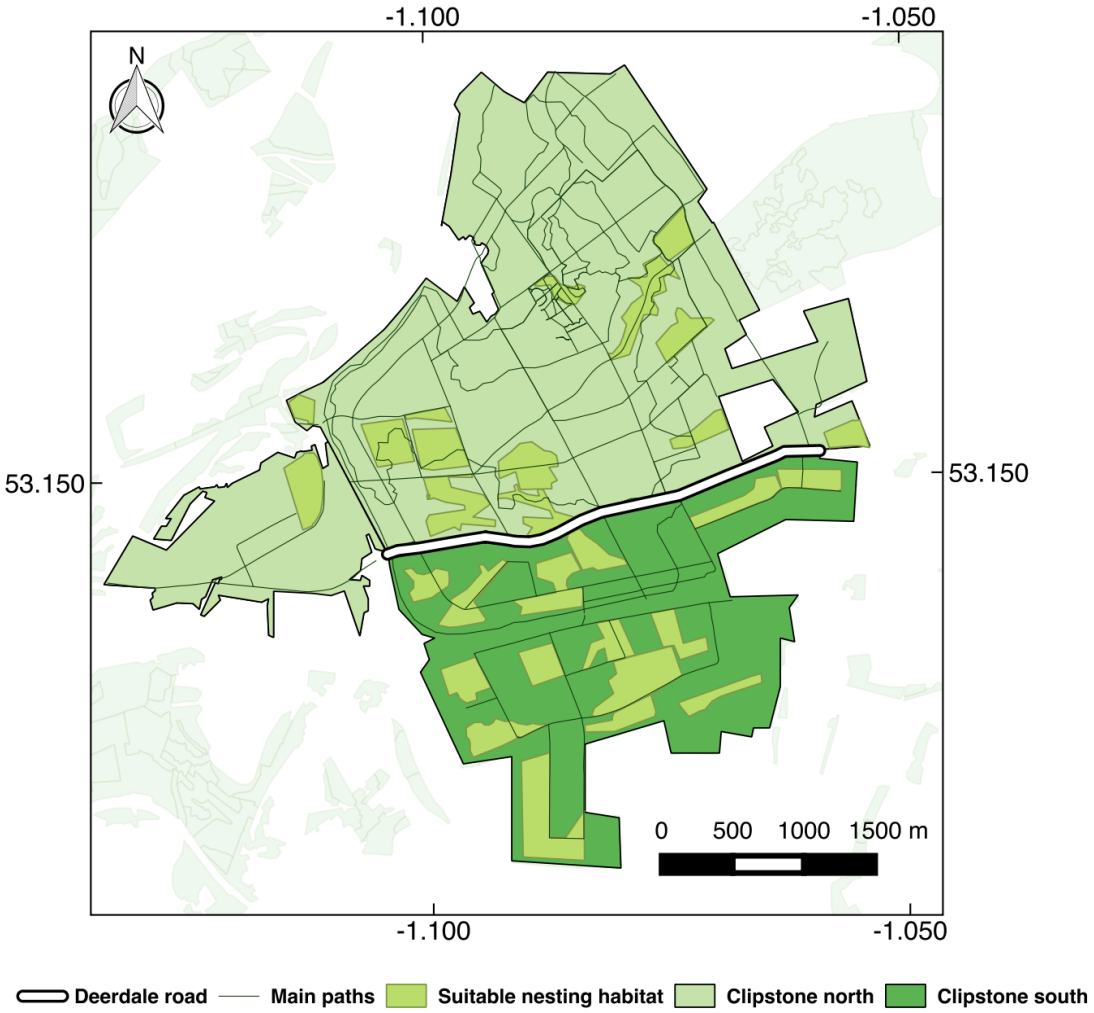


Figure 3.1: Map of Clipstone forest, with the intersecting Deerdale road that was used to partition the forest into north and south sites. Areas of heath and restock habitat are shown, although not all of these habitats will have been suitable for all six breeding seasons, nor necessarily contemporaneously.

3.1.3 Distribution of nests

Where nests were located in the 2015 breeding season, the GPS co-ordinates of the nest site were recorded and referenced with habitat maps produced following the methods outlined in Chapter 2. In addition to maps of forest habitats, shapefiles of recreational paths running through the site were obtained from the Forestry Commission.

For each of the nests from 2014 and 2015 for which GPS co-ordinates had been recorded a random point was generated within the the same area of habitat in QGIS. The distances of both randomly generated points and nest locations from the nearest point on an active path were measured by plotting a line between the two points in QGIS, and measuring its length in metres. The distances of generated points and nests from the nearest habitat edge were measured likewise. Any tendency for nesting females to avoid areas nearer paths or habitat edges, also suggested to influence nest location (Wichmann 2004), was subsequently analysed by performing two-tailed Welch paired t-tests for each, between distances recorded for located nests and for random points generated within the same area of habitat. Distances were normalised through square root transformation. Any relationship between distances of nests from the nearest active path and nest survival was investigated using a two-tailed Mann-Whitney test.

A possible tendency of females to nest further from paths in busier areas was investigated by subtracting the distance from the nearest path of nightjar nests from the distance from the nearest path of randomly generated points at the same area of habitat, and performing a two-tailed t-test for the resulting differences between northern and southern sites of Clipstone forest. The northern site of Clipstone forest was suggested to be the busier of the two, and so it was anticipated that there may be a greater tendency for nightjar nests to be located further from paths in the north site compared with the south.

Where two nest locations for a single female within a season were recorded, the distance between these two nests was measured in QGIS. A possible effect of failure of the first nest upon the distance of the second nest from that first nest was then

investigated using one-tailed Mann-Whitney tests, following the hypothesis that distances between broods would be larger where the first nest had failed.

3.1.4 Survival rates and artificial nests

A total of 28 artificial nests were placed at 14 areas of low lying heath or restock habitat, with two nests at each area of habitat: one placed at 10 metres from the nearest footpath, the other at 50 metres, with the intention of comparing predation rates at varying distances from paths. Points on the footpath from which nests were placed were 75 metres apart, and all artificial nests were placed a minimum of 150 metres from known nightjar nests, and a minimum of 50 metres from the nearest habitat edge.

Artificial nests were composed of one fresh quail (*Coturnix coturnix*) egg and an artificial egg of the same shape and size sculpted from white plasticine (Newplast, UK), as in Miller and Hobbs (2000), and covered with a layer of sealant (Plasti Dip, USA) following the advice of Purger (2012) in order to avoid un-representatively high predation rates. The plasticine egg was secured in the ground using a blunted toothpick in order that it would not be removed from the nest by a predator, and that marks left upon it may provide clues as to the species of predator. No attempt was made to conceal the nest, though nests were always placed in areas that appeared consistent with field observations of nightjar nests. Specifically, nests were placed at areas with visible soil substrate, and nearby bracken *Pteridium aquilinum* no more than 75cm in height. Special care was taken to ensure that nests placed in the same area of habitat shared superficially similar nest sites and levels of conspicuousness. Nests were checked once every two days, for a total of 18 days (12/07/2016 to 30/07/2016); widely considered the average duration of egg incubation by nightjar (Lack 1957; Holyoak 2001).

For comparison and quantification of survival, survival rates of nests were calculated using Mayfield survival estimates (Mayfield 1975; Johnson 1979). By taking into account the duration for which nests are monitored, Mayfield survival estimates control for the likelihood that observed survival rates will be positively biased by the increased probability of locating successful nests in their later stages. Differences in

success rates of artificial nests at varying distance from paths were investigated using a binomial sign test.

3.1.5 Nesting behaviour

Flight initiation distance (FID) is commonly used as a measure of the distance at which individuals evade an approaching threat (Blumstein 2003). This measure is usually obtained by recording the distance at which an individual abandons its nest following the approach of a fieldworker (Lin et al. 2012).

Over the course of 2014 and 2015 breeding seasons, FIDs of females were recorded at 32 nightjar nests. FIDs were measured by a single fieldworker approaching a nest, starting at a distance from which they would not have been visible to the nesting female, and stopping their approach at the point of the nesting female leaving the nest. The distance from the fieldworker to the nest, determined as the location of the nearest egg or chick, was then measured to the nearest 0.5m using a one-metre length stick. For each of the nest locations at which FID was recorded, it was also noted whether any vegetation within one metre of the nest was more than 0.5m high.

FIDs were normalised by square-root transformation. Any effects of distance from the nearest path, surrounding vegetation height and nesting female ID upon FID were investigated using multiple linear regression, by constructing a maximal model and performing stepwise model reduction until the model fit reached significance ($p < 0.05$).

3.1.6 Nest data loggers

Where a nest was discovered at the incubation stage, with a female incubating two eggs, a data logger (Maxim, USA) programmed to record temperature every eight minutes was placed under one of the two eggs (see: Libsch et al., 2008). The data logger's memory capacity allowed up to 11 full days' readings (2048 temperature recordings), and loggers were replaced every 7 to 10 days until hatching or nest failure.

Data from the temperature loggers was often difficult to interpret, and the initial intention of quantifying daily nest absences was not possible due to the subjectivity involved in defining the corresponding criteria. However, the timing of female absences in the evening and early morning were easily discernible by rapid drops in nest temperature, and were compared with changes in times of sunset and sunrise respectively, and analysed for individual repeatability.

ANCOVAs were used to investigate whether nest ID and changes in the timing of sunset and sunrise had significant effects upon timings of morning and evening absences. ANCOVAs included continuous variables of the timing of sunset or sunrise for evening and morning absences respectively, and a categorical variable of nest ID. Repeatability of absences with respect to nest ID were investigated using REML LMMs in the R package *rptR* (Nakagawa & Schielzith 2010). Correlations between timings of nest absences and times of sunset and sunrise were tested using Spearman's rank correlations.

3.1.7 Effects of lunar cycle

A possible effect of the lunar cycle upon hatching date for nests located and recorded from 2010 to 2015 was investigated by referencing inferred hatch dates with the lunar calendar. It was considered likely that nest initiation dates early and late in this season were less likely influenced by lunar stages due to alternative pressures to breed early or prior to migration (see: Perrins & Crick 1996), and so nests hatching in months of May and August were removed from the sample, leaving only nests hatching in months of June and July.

Records of lunar stages for each night were retrieved from the U.S. Naval Observatory (USNO). Lunar luminosity was categorised as being low (luminosity of 0 - 24.9%), medium (25 - 74.9%) or high (75 - 100%). Numbers of nests expected to hatch within each classification were calculated by dividing the total number of nests for which hatch-date was inferred by the proportion of nights within each classification during months of June and July over all six breeding seasons. A Pearson's Chi-squared test was then used to determine whether hatch dates for located nests differed significantly in their distribution between categories of lunar luminosity from

that expected by chance. A Mann-Whitney test was used to investigate whether lunar luminosity at hatch-date differed significantly between nests that fledged successfully and nests that failed to.

3.1.8 Analysis

GLMs and GLMMs were constructed by including the variables predicted to have an impact upon the response variable to produce a maximal model, after which stepwise model reduction was carried out by selecting for the lowest Akaike Information Criterion (AIC) score. Models with Poisson-distributed response variables were log-linked, while binomially distributed response variables were logit-linked. GLMMs were produced using Laplace approximation. Where ANOVA, linear regression and t-tests were used, assumptions of normality and homoscedasticity were satisfied by inspection of QQ-norm and fitted vs residual error diagnostic plots in R. The R extension package *lme4* (Bates 2015) was used for mixed models, and *lmerTest* (Kuznetsova 2016) used to produce corresponding P-values. REML LMMs used to investigate repeatabilities were conducted in the R package *rptR* (Nakagawa & Schielzith 2010).

3.2 Results

3.2.1 Habitat selection

Of the 21 nests for which GPS co-ordinates were recorded, eight were in areas of heath habitat, 12 in restock, and one in mature conifer. Manly selection ratios demonstrated significant selection with regard to all habitat classifications except for mature conifer (Table 3.1). Of the two positively selected for habitats of heath and restock, the Manly selection statistic was strongest for areas of restock habitat (heath: $Wi = 10.639$, $p = 0.001$, restock: $Wi = 54.167$, $p < 0.001$) (Figure 3.1). Overall, the results reported strong and significant habitat selection ratios for nesting nightjars ($X^2 = 132.209$, $df = 10$, $p < 0.001$).

3.2.2 Effects of disturbance

During the 2015 breeding season, 12 nesting females were located at areas of habitat that had been surveyed for site visitation, with at least one nesting female recorded at seven of the 18 sites. Seventeen nests were located, of which 12 were successful in producing at least one fledgling. Including data from 2014 and 2015 breeding seasons, 23 nesting females had been recorded at sites for which site visitation data were available, with a total of 33 nests, 22 of which had been successful in producing at least one fledgling. Over the course of the two seasons, 18 male territories were located, males at 13 of which were judged to be paired.

PCA of the variables recorded in the surveys illustrated that numbers of walkers and dogs appeared related, as was expected, but that numbers of cyclists did not appear to covary with numbers of walkers and dogs (Figure 3.2). The PCA produced the principal components displayed in Table 3.2, with four principal components explaining 99.9% of the total variance observed. *PC1* (63.5 of variance) was strongly influenced by visitation by cyclists, *PC2* (29.4% of variance) by numbers of corvids observed, *PC3* (0.68% of variance) by visitation by walkers and dogs, and *PC4* (0.02% of variance) by numbers of deer and small mammals observed.

Using the results from the PCA, the best-fitting GLM for numbers of nesting females in 2015 incorporated predictor variables of habitat area and principal components describing visitation by cyclists (*PC1*) and walkers and dogs (*PC3*). The results reported a significant positive relationship between numbers of nesting females and habitat area (Est. = 0.597, $p = 0.005$) but no significant effect of visitation by walkers and dogs (*PC1*) (Est. = -2.416, $p = 0.118$) or cyclists (*PC1*) (Est. = 0.142, $p = 0.134$) (Table 3.3). A GLM with a binomially distributed response variable of nest success at each site indicated that none of the principal components nor habitat area had a significant predictive effect (all $p > 0.05$). A GLM with a binomially distributed response variable of male occupancy included predictor variables of habitat area, *PC1*, *PC3* and *PC4* but likewise reported no significant effects (all $p > 0.05$; Table 3.4). Total site productivity as measured by numbers of nests successful in producing one or more fledglings appeared significantly positively associated with increased habitat area (Est. = 0.521, $p = 0.003$) and visitation by cyclists (*PC1*) (Est. = 0.180, p

= 0.013), with no significant effect of visitation by walkers and dogs (*PC3*) reported (Est. = -1.252, p = 0.210) (Table 3.5).

When visitation levels by cyclists and walkers were each classified as low, medium or high, the best-fitting GLMM with a response variable of nesting females and random effects of year and habitat ID, including data from 2014 and 2015 breeding seasons, incorporated predictor variables of: habitat area, level of visitation by walkers, and level of visitation by cyclists. The results reported a non-significant negative predictive effect of visitation by walkers (Est. = -0.770, p = 0.095), no significant effect of visitation by cyclists (Est. = 0.327, p = 0.247), and a positive effect of habitat area (Est. = 0.444, p = 0.001) (Table 3.6). A GLMM with a binomially distributed response variable of occupancy by a paired male and the same random effects reported a significant negative predictive effect of visitation by walkers (Est. = -90.534, p = 0.008) and a significant positive effect of visitation by cyclists (Est. = 29.801, p = 0.022) (Table 3.7). Figures 3.3 and 3.4 show average numbers of nesting females and rates of paired male occupancy between categories of visitation for walkers and cyclists respectively.

A GLMM with random effects of year and habitat ID and a binomially distributed response variable of nest success indicated no apparent effect of habitat area or categories of visitation upon nest success (all $p > 0.05$), and the same was true of a GLMM for a binomially distributed response variable of male occupancy. Finally, a GLMM with a Poisson-distributed response variable of numbers of nest to produce fledglings reported significant positive effects of increased habitat area (Est. = 0.390, $p < 0.001$) and visitation by cyclists (Est. = 0.546, p = 0.025), and a non-significant negative effect of visitation by walkers (Est. = -0.699, p = 0.067) (Table 3.8).

Two-tailed Mann-Whitney tests demonstrated that average rates of total human visitation were significantly greater in the northern area of the site (W = 67.5, p = 0.016), but that this was largely accounted for by the higher rate of visitation by cyclists (W = 70, p = 0.008), with visitation by walkers not significantly higher than in the south (W = 54, p = 0.216). There was no tendency over breeding seasons from 2010 to 2015 for nests in the more frequently visited northern area of the forest to suffer higher rates of nest failure (W = 1482, p = 0.998), and in fact a two-tailed Mann-Whitney test reported that success rates (classified as success or failure in producing at least one fledgling) in the northern area were significantly higher (North: 0.833 ±

0.063; South: 0.547 ± 0.063; W = 1842, p = 0.004) (Figure 3.5). Of some of the potential non-anthropogenic predators of nightjars and their nests; two-tailed Mann-Whitney tests showed numbers birds of prey observed were significantly greater in the southern area (W = 13, p = 0.018), numbers of corvids observed were greater in the south but non-significantly so (w = 20.5, p = 0.091), and numbers of deer were greater in the south, with the difference approaching significance (W = 24, p = 0.058).

3.2.3 Distribution of nests

A two-tailed paired t-test incorporating nests from all publicly accessible forests (n = 47) over 2014 and 2015 revealed that the distance of located nests from paths was greater than that of randomly generated points within the same areas of habitat (t = 1.863, df = 46, p = 0.068), though a two-tailed t-test reported no significant difference with regard to the distance from habitat edges (t = 1.208, df = 46, p = 0.233). Nonetheless, it was clear from inspection of the data that the difference between distances from paths was relatively small (Figure 3.6); the mean average distance of nightjar nests from the nearest path was 63.898m ± 5.947, while that of randomly generated points was 52.851m ± 5.694. A two-tailed t-test reported no significant tendency for nightjar nests to be located further from paths when compared with randomly generated points in the busier northern site (n = 18) of Clipstone forest than in the southern site (n = 17) (t = 1.282, df = 31.599, p = 0.210).

There did not appear to be any relationship between distances of nests from the nearest path and proportional nest success (W = 175.5, p = 0.159). The mean average distance from the nearest path of successful nests (n = 34) was 65.000m ± 6.291, while failed nests (n = 14) were situated an average of 60.000m ± 14.814 from paths (Figure 3.7).

3.2.4 Survival rates and artificial nests

Mayfield survival estimates for all nests differed markedly between years of 2014 and 2015, with a daily survival rate of 0.983 for 32 nests in 2014, and 0.969 for 19 nests in 2015. When extrapolated over the estimated 36-day duration of the nesting period, this resulted in a 0.325 probability of a nest surviving until fledging in 2015, compared with 0.546 in 2014. It is notable that of the 24 nests recorded as failing, 16 failed at

the egg stage compared with 8 at the chick stage. Causes of predation or abandonment were often unclear, though direct human interference was implicated in the abandonment of two nests in 2015, inferred on each occasion through clear evidence of human activity left at each of the failed nests.

Of the 28 artificial nests, just five were predated in the 18 days for which they were monitored, at just three of the 14 habitats. Predator species were inferred from imprints left on plasticine eggs and sighting of species near the nest on approach. Of the five nests predated, the cause of 'failure' for four was said to be an Avian predator, with a common pheasant *Phasianus colchicus* being positively identified as the cause for two nests at separate sites. For the fifth failed nest, the plasticine egg had been flattened and a deer was considered the most likely cause, with fallow deer *Dama dama* having been sighted at the habitat. A binomial sign test confirmed that there was no significant difference in numbers of nests predated between sets of nests located 10 metres from paths and nests located 50 metres from paths ($n = 2$ and 3 respectively; $p = 1$). Overall, artificial nests had a daily survival probability of 0.989, which extrapolated across the average nightjar nesting period of 36 days produced a nest survival probability of 0.672. This appeared substantially higher than that observed for nightjar nests in both 2014 and 2015 (Figure 3.8).

3.2.5 Female nesting behaviour

Over 2014 and 2015, 14 double broods were located. For 10 of these double broods, the first nest was successful in producing at least one fledgling, while for the other four the first nest was unsuccessful. It was notable that subsequent broods were located less often following a failed brood, which may suggest females were likely to leave the habitat altogether following a failed nest. Average distance between first and second broods where the first brood had failed was 193.263 ± 60.841 metres, while for double broods in which the first brood has fledged the average distance between nests was 93.19 ± 10.60 metres (Figure 3.9). A one-tailed Mann-Whitney test reported a statistically trend for distances between broods to be larger where the first brood had failed ($W = 37$, $p = 0.007$).

FIDs were recorded at 36 nests, for 25 females, and ranged between 0.5 and 6m with an average of 2.167 ± 0.193. A maximal model for a response variable of FID was

constructed with predictor variables of vegetation height, female ID, nest fledging success and distance from the nearest path, but could not be made to fit following stepwise model reduction ($p > 0.05$), seeming to indicate no significant predictive effect of any of the variables included.

A total of 185 days' data were retrieved from thermologgers placed under 23 nests during 2014 and 2015 breeding seasons. ANCOVAs reported that nest ID and times of sunset each had a significant effect upon the timings of nest absences in the evening (sunset: $F_{1,153} = 16.098$, $p < 0.001$; nest ID: $F_{21,153} = 4.652$, $p < 0.001$), and that times of sunrise and nest ID each had similarly significant effects upon nest absences in the morning (sunrise: $F_{1,142} = 32.115$, $p < 0.001$; female ID: $F_{21,142} = 4.829$, $p < 0.001$). Evening nest absences were positively correlated with changes in the timing of sunset ($r = 0.485$, $p < 0.001$), while morning nest absences were negatively correlated with changes in the timing of sunrise ($r = -0.418$, $p < 0.001$). The timings of evening and morning absences were not correlated ($r = -0.051$, $p = 0.524$). Timings of absences appeared highly and significantly repeatable with respect to nest ID in both the evening ($R = 0.597$, $p < 0.001$) and the morning ($R = 0.435$, $p < 0.001$).

3.2.6 Lunar effects upon nesting stages

From 2010 to 2015, hatching dates were recorded for 101 nests in months of June and July. The results of the chi-squared analysis indicated that numbers of clutches hatching was not equally distributed between classes of low, medium and high lunar luminosity ($X^2 = 12.02$, $p < 0.01$) (Table 3.9). Specifically, it was clear that fewer clutches hatched on a full moon than expected by chance, while greater numbers hatched under medium luminosity. Furthermore, it was notable that the deviation from expected values was similar in magnitude for each (high luminosity: expected = 34.11, observed = 20, difference = 14.11; medium luminosity: expected = 31.13, observed = 45, difference = 13.87) (Figure 3.10). When intermediate stages were classified into increasing and decreasing luminosity, there was no apparent difference in the proportion of nests hatching between the two ($X^2 = 0.175$, $p = 0.677$). There was no similar deviation from the distribution among lunar stages expected by chance of inferred laying or fledging dates ($X^2 = 1.702$, $p = 0.427$; $X^2 = 3.302$, $p = 0.192$ respectively).

Of this sample, 10 nests failed to fledge, while 105 fledged successfully. A two-tailed Mann-Whitney test showed no evidence of an effect of lunar luminosity at hatch date upon the likelihood of a nest producing at least one fledgling ($W = 720$, $p = 0.181$).

3.3 Discussion

3.3.1 Habitat selection

Manly selection ratios indicated that habitat selection by nesting nightjars for areas of restock habitat was considerably stronger than for areas of heathland, their traditional habitat (Alexander & Cresswell 1990). From observations during fieldwork, it appeared that restock habitat was generally more homogeneous in composition, with commonly associated species and habitat characteristics such as sparse bracken *Pteridium aquilinum* and patches of bare ground (Dolman 2010) present throughout, while areas of heathland frequently appeared more variable. Consequently, one explanation for this apparent preference for restock habitat may be simply that there is a greater proportion of restock habitat that provides suitable nesting habitat. The results nonetheless demonstrate that forestry restock provides a habitat that is highly suitable, and could even be prioritised over heathland, at least with regard for the preferences of nesting nightjars.

3.3.2 Effects of recreational disturbance

While some the results reported in the current study appear consistent with the hypothesis that visitation by walkers and dogs have a negatively impact upon nesting nightjars, there did not appear to be strong support for the hypothesis that nesting nightjars are strongly negatively impacted by human visitation in general. Visitation by walkers and dogs appeared generally to be negatively associated with numbers of nesting females and fledgling productivity, and there was an indication that female nightjars nested further from paths than predicted by chance. Visitation by cyclists, on the other hand, showed no evidence of a negative impact upon nesting nightjars. Habitat visitation had no apparent effect upon rates of male occupancy, while

occupancy by paired males was negatively associated with visitation by walkers, supporting the conclusions of Lowe et al. (2014), yet positively associated with increased visitation by cyclists. The results of the principal component analysis demonstrated that rates of visitation by walkers and dogs and by cyclists were often dissimilar at individual areas of habitat, and did not covary. Consequently, it is sensible at this site to consider separately the effects that cyclists and walkers may have upon breeding nightjars.

There was an indication that female nightjars nested further from paths than predicted by chance, however the difference was small (< 10m) and so may suggest that nesting female nightjars avoid only areas of habitat especially near to paths. This could be due either to increased disturbance rates or a lower availability of suitable nesting habitat within a few metres of paths which was not accounted for in analyses — if for example there might have been a lower level of vegetation cover, or grassy verges. A clear weakness of this analysis was in the inability to account for differing levels of path use at the different areas of habitat, due to many of them not having been surveyed. Nonetheless, a comparison of differences between nests and randomly generated points in northern and southern sites of Clipstone Forest reported no significant evidence that nightjars tended to practice a greater degree of path-avoidance in the busier northern site.

Results from disturbance analyses appeared to suggest that visitation by cyclists was positively associated with both numbers of nesting nightjar females and in particular habitat productivity, measured as the number of nests to produce fledglings. Notably few previous studies into the effects of human recreational disturbance upon wildlife such as ground-nesting birds have distinguished between such forms as walkers and cyclists in considering the impact of site visitation. Both Clipstone and Birklands sites were observed during fieldwork to be subject to high rates of visitation by cyclists, and it is possible that this may explain in part the lack of any substantial or significant impact upon success rates of distances from paths and site accessibility when compared with contrasting results from studies of populations elsewhere (Langston et al. 2007a; Murison 2002).

None of the forms of site visitation recorded were observed to have an effect upon nest success, and there was no clear relationship between the distance of a nest from the nearest path and the chances of at least one chick fledging (Figure 3.8). Previous

studies have suggested that closer proximity of nests to paths is associated with increased rates of flushing and decreased nesting success rates (Feather 2015; Murison 2002). It was notable in the current study that artificial nests were subject to lower predation rates than were nightjar nests from 2014 or 2015, seemingly unrelated to their distance from the nearest path. This was surprising; a relatively high predation rate had been anticipated given the lack of any parental defence or camouflage, the more so following the finding that the majority of nightjar nests failed at the egg stage.

The finding that artificial nests were subject to comparably low rates of predation may indicate a role of increased flushing of females at nests nearer to paths resulting in attracting the attention of potential predators and, or otherwise, resulting in abandonment of a nest by the female. The latter explanation seems particularly well-suited to explaining the discrepancy between predation rates for nightjar and artificial nests, given that abandonment of nests at the egg stage is relatively common among nightjars (Langston et al. 2007a; Dolman 2010).

By analysing footage from nest cameras placed at nests Langston et al. (2007a) observed two nesting nightjar being flushed by dogs, however in neither case were chicks or eggs predated, and both nests fledged successfully. This may suggest that nesting females are likely to be discouraged from a potential nesting site by occasional flushing in the presence of walkers and dogs, and invasive recreational disturbance in general, but that resulting predation rates are relatively low. Indeed, female nightjars are known to be highly likely abandon a nesting site if flushed prior to laying (Lowe et al. 2014), and this may explain why occupancy by males classified as paired was negatively associated with visitation by walkers. Cyclists, on the other hand, are unlikely to leave paths and so present a less invasive form of disturbance. Cyclists were observed to produce high noise levels, which could conceivably have the effect of deterring nest predators, such as carrion crows *Corvus corrone* or even common pheasants *Phasianus colchicus*, perhaps explaining their apparent positive influence upon fledgeling productivity.

Counter-intuitive relationships are often observed in terms of nest associations between birds and potential predators, termed ‘protective nest associations’ (Quinn & Ueta, 2014), theoretically due to the presence of potential predators deterring others, and particularly nest predators. It seems plausible that a similar dynamic could result

from noisy or otherwise ostentatious forms of human recreational disturbance that are unlikely to be invasive or threaten the nest of ground-nesting birds. If this were the case, a trade-off might be expected between the positive effects that disturbance may have in discouraging potential predators, and the cost of an increased rate of disturbance potentially directly resulting in the failure of a nest. Virzi (2008) observed that although nearby human activity and mammalian presence each appeared to have a negative effect upon American oystercatcher *Haemotopus palliatus palliatus* brood success rates, human presence seemed to have the effect of limiting the negative effects of mammalian nest-predation. There may have been some indication of a similar effect in the current study, as nest success rates were significantly higher in the northern area of Clipstone Forest that was subject to higher rates of site visitation. Moreover, habitats in the northern area were observed to have fewer potential predators such as corvids, deer and birds of prey. While there is no direct evidence of this trend resulting from greater levels of human visitation, the FC have identified a trend for large numbers of deer moving to the southern area of the forest following the growth in human visitation in the north (Lowe et al. 2014).

Beale and Monaghan (2004) placed emphasis on the role of perceived predation risk in determining the effects of human disturbance upon nesting kittiwakes *Rissa tridactyla* and guillemots *Uria aalge.* Another explanation for the apparent positive effects of site use by cyclists may therefore be that nesting females become habituated to frequent visitation, and are as a result less likely to be flushed by disturbance events than an individual nesting at a less frequently visited site. In the current study, sites relatively frequently visited by cyclists were subject to higher rates of visitation in terms of numbers of visitors than were sites visited relatively frequently by walkers, and so this could perhaps provide an alternative or additional explanation for the apparent discrepancy between observed effects of visitation by walkers and cyclists.

It is important when interpreting these results to consider that human recreational disturbance could have impacts upon female behaviours such as foraging and incubation that may not strongly influence fledging success but nonetheless impact offspring fitness (Verhulst et al. 2011). Indeed, if nightjars do exhibit a degree of diurnal torpor this may render them especially vulnerable to behavioural perturbation through daytime flushing.

3.3.3 Nesting behaviour

Recorded FIDs ranging between 0.5 and 6m appear when compared with those of other bird species to indicate that female nightjars practice evasive behaviour only at close proximity (Ruddock & Whitfield 2007). Combined with their effective camouflage and low spatial density (Gill et al. 2001), this may suggest that nightjars should be relatively well suited to areas of habitat with high levels of urban disturbance. Indeed, it seems plausible that allowing potential predators to reach such a proximity before flushing may account for the relatively small difference in distances from nearest paths between nightjar nests and randomly generated points, if females are only likely to be affected by recreational path use within up to six metres either side of the path. No effect of vegetation height was apparent, though it seems likely this will have been due in part to the noise made by fieldworkers brushing past vegetation on approach.

Where first broods had failed, females appeared to lay subsequent broods further from that first nest than in cases where first broods had been successful, which may indicate that females choose nest sites based on previous experience. However, it was notable that for many females second broods were not located following the abandonment or predation of what was thought to be the first brood, and this may suggest that females might occasionally abandon a territory altogether following the failure of a first brood.

It appeared from the data recorded by thermologgers at nests that the timing of absences in the evening and morning were highly repeatable. Field observations suggested that these absences were likely related to foraging activity. While timings of evening absences were significantly and positively correlated with changes in the time of sunset, timings of morning absences were negatively correlated with changes in the timing of sunrise. The latter trend was not anticipated, and it appears likely that the result may have been skewed by the repeatability of timings of absences with respect to female ID.

3.3.4 Effects of lunar intensity

It was anticipated that hatch dates may not be distributed evenly between lunar stages (Mills 1986; Perrins & Crick 1996), and the results of this study found that the distribution of nest hatch dates among lunar stages was significantly different from that expected by chance. Specifically, it was clear that fewer than expected nests hatched on nights of high lunar luminosity, while greater numbers than expected hatched on nights of medium lunar luminosity. This finding may suggest that nesting females avoid laying eggs that will hatch during periods of high lunar luminosity, perhaps due to an increased risk of predation by nocturnal visual predators, combined with pressures to spend more time away from the nest foraging in order to provision chicks. Contrary to previously reported findings (Mills 1986), the disproportionate hatching of nests during periods of medium lunar luminosity did not appear related to whether luminosity was increasing or decreasing. This may indicate that the trend is not related to any importance of relatively high or low luminosity during later stages of provisioning of chicks.

It may appear unlikely that the lunar cycle would strongly influence mating interactions, given the relatively short duration of the breeding season, and the pressure upon both males and females to reproduce. Alternative explanations for the distribution of nests hatching between lunar stages may be that the lunar stage could affect female incubation behaviour, perhaps relating to foraging activity, or the behaviour of chicks prior to hatching. In either case, this could suggest that the commonly used method of back-calculating laying dates from hatch dates using standard incubation periods may not be wholly reliable.

With regard to nightjar conservation, it would appear likely that levels of light pollution could impact nightjar populations for a number of reasons, particularly relating to foraging (see: Feather 2015; Sharps 2014), and any indication that individuals avoid laying broods that will hatch during periods of high lunar intensity may support suggestions that light pollution should be kept to a minimum near to suitable habitats.

3.4 Tables

Table 3.1: The results of habitat selection analysis by nesting female nightjars, showing for each of the habitat classifications: the proportion used, the proportion available, Manly selection statistic (Wi) with corresponding P-values (with a Bonferroni-adjusted significance threshold of 0.005) and standard errors.

	Proportion used	Proportion available	Wi	P-value	St. error
Agricultural	0.000	0.603	0.000	0.000	0.000
Broadleaf	0.000	0.105	0.000	0.000	0.000
Heathland	0.381	0.036	10.639	0.001	2.959
Improved grassland	0.000	0.005	0.000	0.000	0.000
Mature conifer	0.048	0.098	0.488	0.283	0.477
Pole	0.000	0.049	0.000	0.000	0.000
Pre-thicket	0.000	0.008	0.000	0.000	0.000
Restock	0.571	0.011	54.167	0.000	10.237
Thicket	0.000	0.018	0.000	0.000	0.000
Urban	0.000	0.066	0.000	0.000	0.000
Water	0.000	0.001	0.000	0.000	0.000

Table 3.2: Results of a principal component analysis using the variables of interest recorded during 30-minute disturbance surveys. Loadings of < |0.05| are not displayed.

Survey average	PC1	PC2	PC3	PC4
Corvids	0.109	-0.983	0.108	-
Birds of prey	-	-	-	-0.058
Deer	-	-	-	-0.898
Small mammals	-	-	-	-0.43
Walkers	-	-0.103	0.956	-
Dogs	-	-	0.250	-
Cyclists	0.993	0.112	-	-
Vehicles	-	-	-	-
Standard deviation	3.495	2.378	1.138	0.192
Proportional Variance	0.635	0.294	0.068	0.002
Cumulative Variance	0.635	0.929	0.997	0.998

Table 3.3: Results of a GLM with a Poisson-distributed response variable of numbers of nesting females, with predictor variables of habitat area and principal components from disturbance survey data. Eighteen areas of habitat are included, with eight occupied by one or more nesting females, and a total of 12 nesting females identified.

AIC: 37.3

	Estimate	St. Error	Z-Value	P-Value
(Intercept)	-5.106	2.114	-2.416	0.026
Habitat.area	0.597	0.213	2.811	0.005
PC1	0.142	0.095	1.497	0.134
PC3	-2.416	1.545	-1.564	0.118

Table 3.4: Results of a GLM with a binomially-distributed response variable of male occupancy, with predictor variables of principal components from disturbance survey data. Eighteen areas of habitat are included, nine of which were occupied by males.

AIC: 26.4

	Estimate	St. Error	Z-Value	P-Value
(Intercept)	-0.832	0.985	-0.845	0.398
PC1	-0.359	0.288	-1.247	0.212
PC3	1.352	1.108	1.221	0.222
PC4	-17.407	15.665	-1.111	0.266

Table 3.5: Results of a GLM with a Poisson-distributed response variable of numbers of nests from which at least one chick fledged, with predictor variables of habitat area and principal components from disturbance survey data. Eighteen areas of habitat are included, with 12 nests from seven sites successfully producing at least one fledgling.

AIC: 34.5

	Estimate	St. Error	Z-Value	P-Value
(Intercept)	-4.165	1.539	-2.706	0.007
Habitat.area	0.521	0.175	2.969	0.003
PC1	0.180	0.072	2.483	0.013
PC3	-1.252	0.999	-1.253	0.210

Table 3.6: Results of a GLMM with a Poisson-distributed response variable of numbers of nesting females, including random effects of habitat ID and year. Eighteen areas of habitat are included, along with numbers of nesting females in 2014 and 2015.

AIC: 70.1

	Estimate	Std. error	Z-value	P-value
(Intercept)	-3.051	1.010	-3.020	0.002
Habitat.Area	0.445	0.131	3.408	0.001
Walkers	-0.770	0.461	-1.671	0.095
Cyclists	0.327	0.282	1.158	0.247

Random Effects

	Variance	Std. Dev
Habitat ID	0.130	0.361
Year	0.000	0.000

Table 3.7: Results of a GLMM with habitat ID and year included as random effects, and a binomially-distributed response variable for habitat occupancy by paired males at eighteen areas of habitat, including data from 2014 and 2015.

AIC: 12.1

	Estimate	Std. Error	Z-value	P-value
(Intercept)	16.038	9.053	1.772	0.076
Walkers	-90.534	34.070	-2.657	0.008
Cyclists	29.801	13.040	2.285	0.022

Random Effects

	Variance	Std. Dev
Habitat ID	33516	183.1
Year	0	0

Table 3.8: Results of a GLMM with a Poisson-distributed response variable of numbers of fledgling productivity in years of 2014 and 2015 at eighteen habitats, incorporating habitat ID and year as random effects.

AIC: 70.1

	Estimate	Std. error	Z-value	P-value
(Intercept)	-3.003	0.834	-3.600	0.0003
Habitat.Area	0.390	0.106	3.678	0.0002
Walkers	-0.699	0.382	-1.831	0.0672
Cyclists	0.546	0.243	2.247	0.0247

Random Effects

	Variance	Std. Dev
Habitat ID	0.000	0.000
Year	0.000	0.000

Table 3.9: Numbers of nightjar nests located with inferred hatching dates during periods of low (0 - 24.9%), medium (25 - 74.9%) and high (75 - 100%) lunar luminosity between 2010 and 2015, compared with expected numbers in each category.

Luminosity	Observed	Expected	Difference
Low	36	35.76	0.24
Medium	45	31.13	13.87
High	20	34.11	-14.11

3.5 Figures

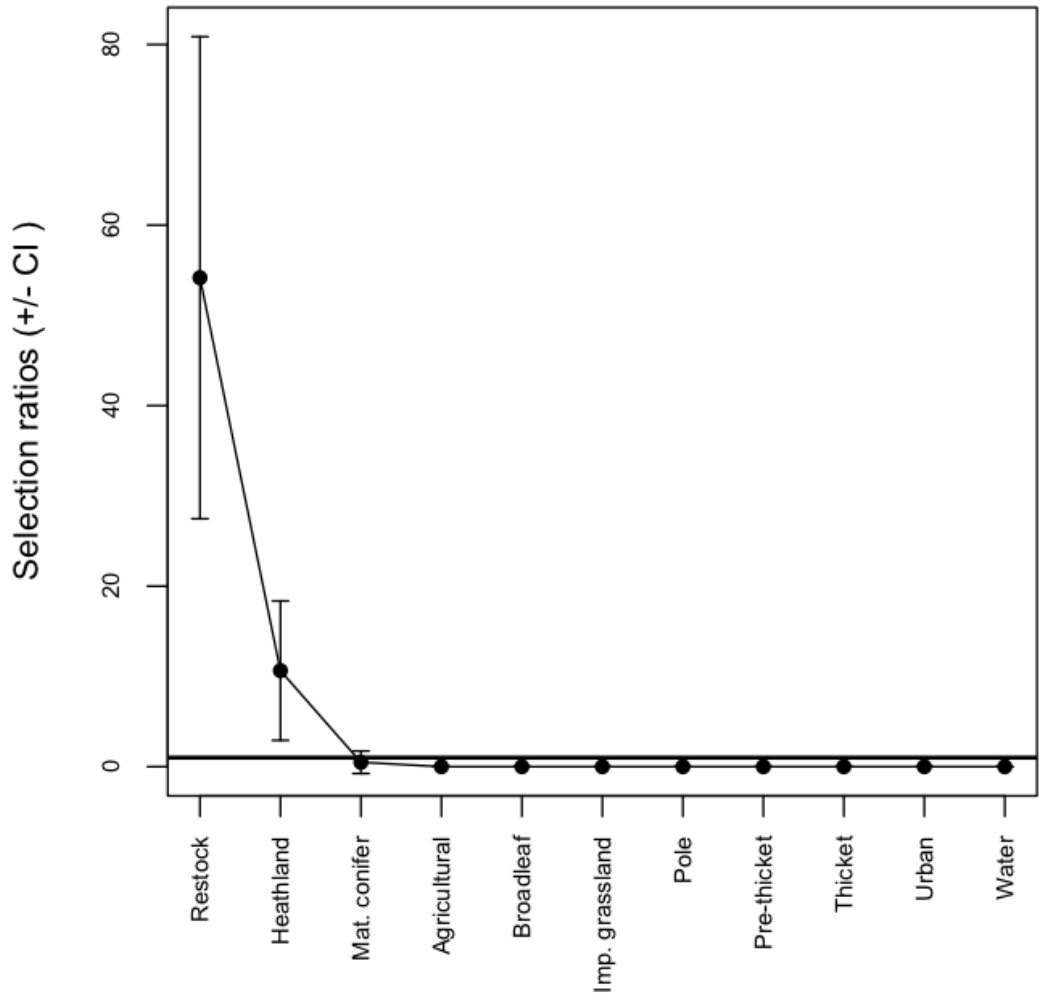


Figure 3.1: Manly selection ratios of nightjar nests for each of the habitat classifications available. Where values are greater than one, selectivity for the corresponding habitat is greater than its availability in the environment. Error bars show ± 95% confidence intervals.

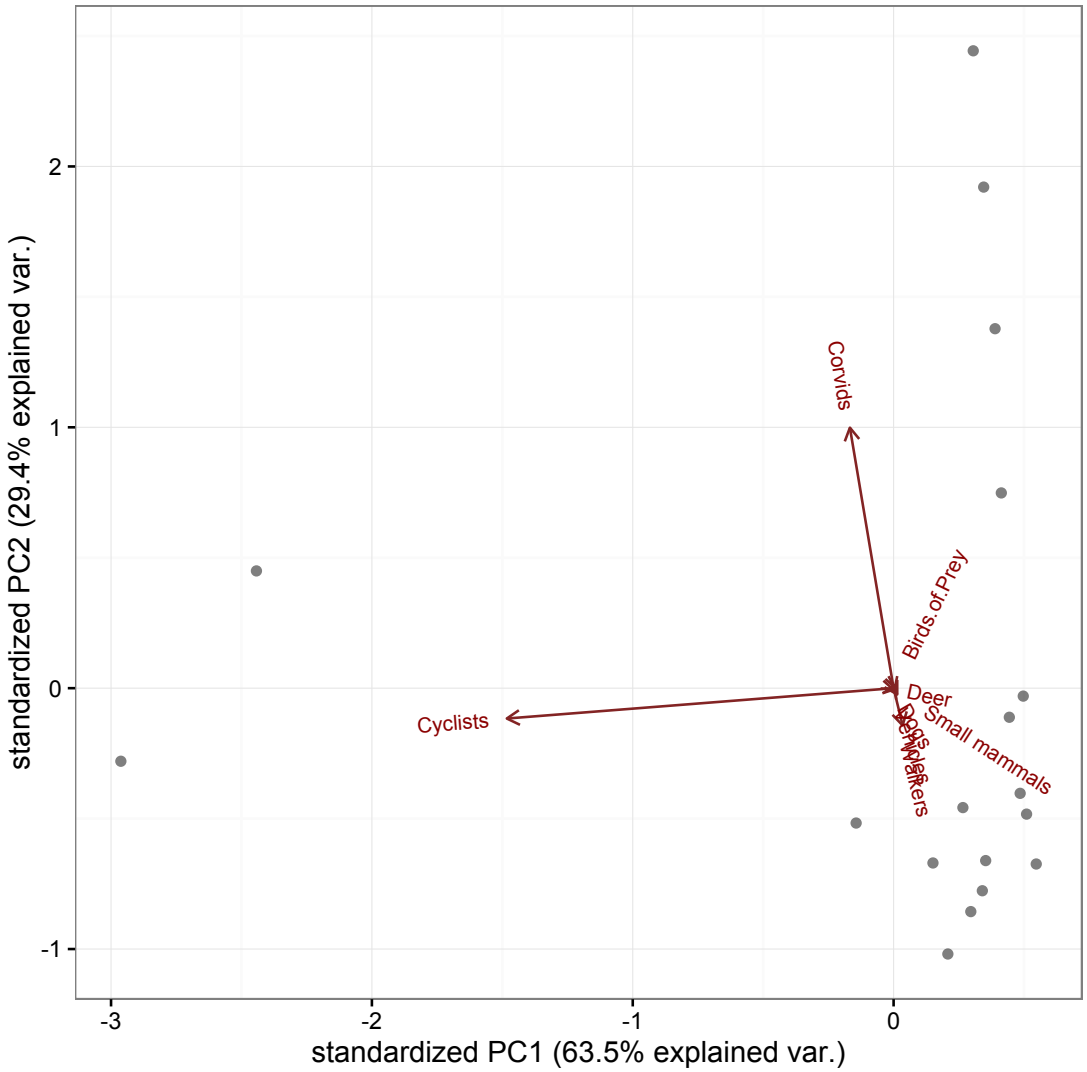


Figure 3.2: Plot of the first two principal components PC1 and PC2 following a principal component analysis of the relationship between variables observed during disturbance surveys, averaged for each area of habitat.

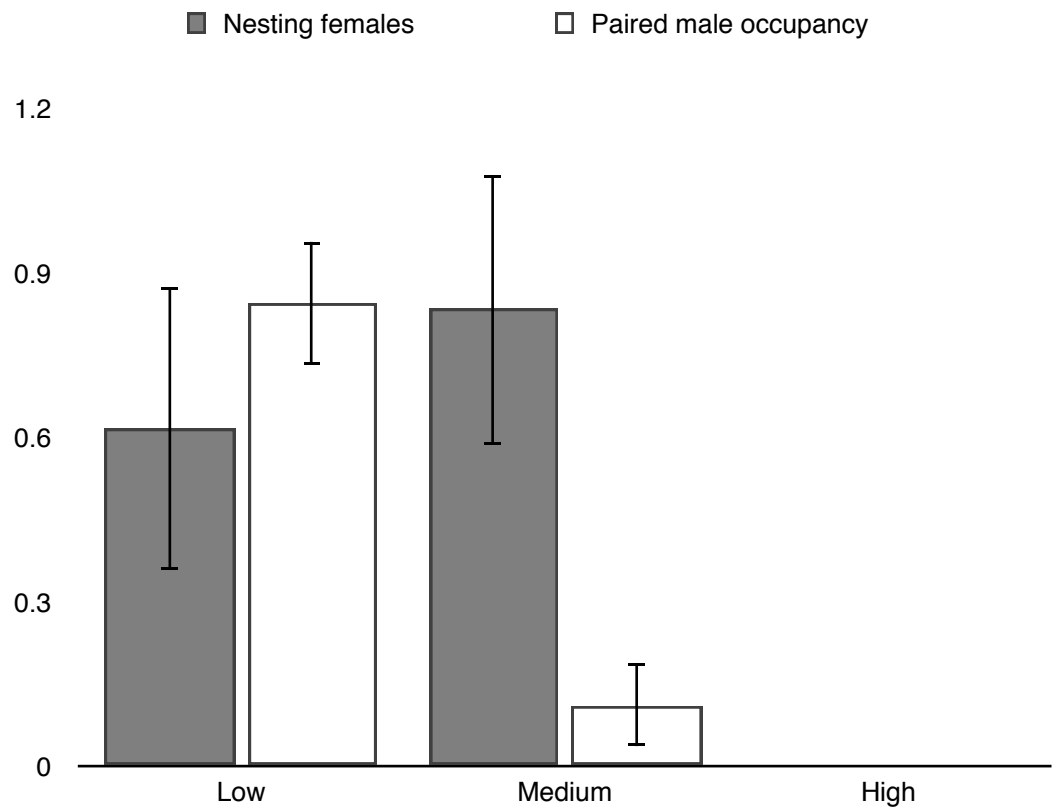


Figure 3.3: Average numbers of nesting females and paired males between 2015 and 2016 at sites of low (seven sites), medium (nine sites) and high (two sites) rates of visitation by walkers. Error bars show ± 1 standard error.

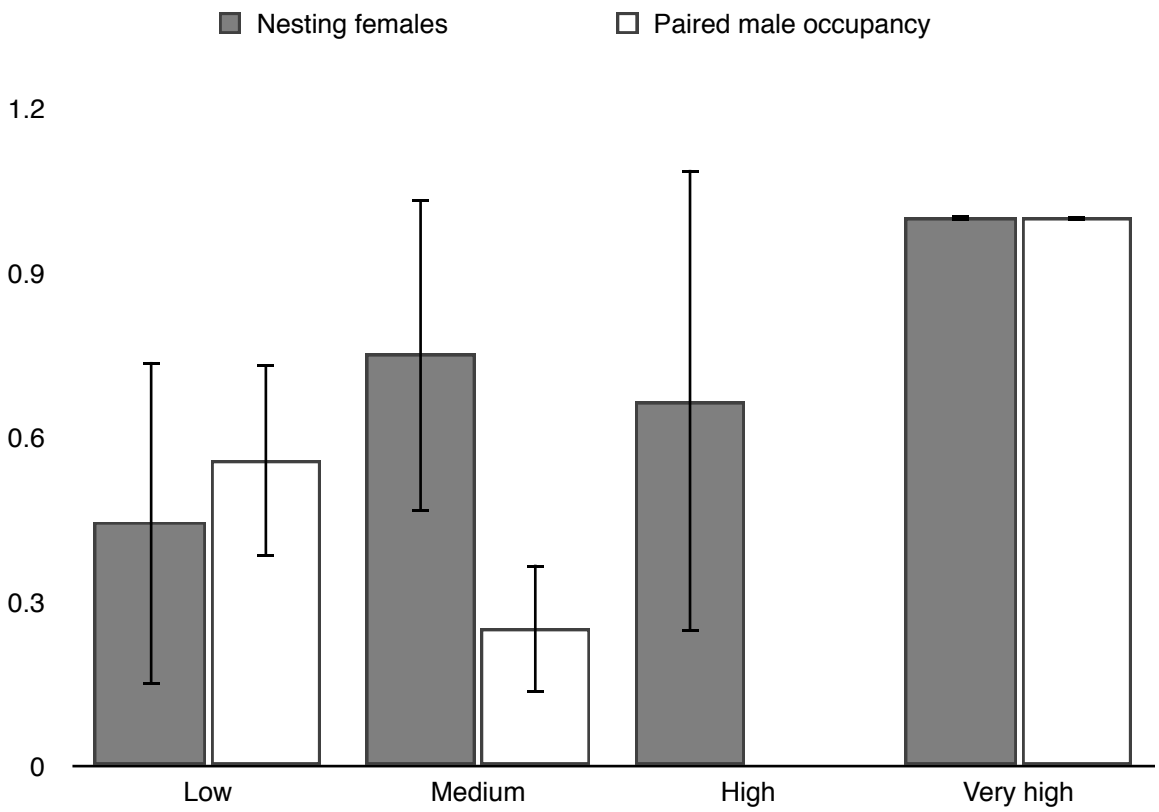


Figure 3.4: Average numbers of nesting females and paired males at sites of low (five sites), medium (eight sites) and high (three sites) and very high (two sites) rates of visitation by cyclists. Error bars show ± 1 standard error.

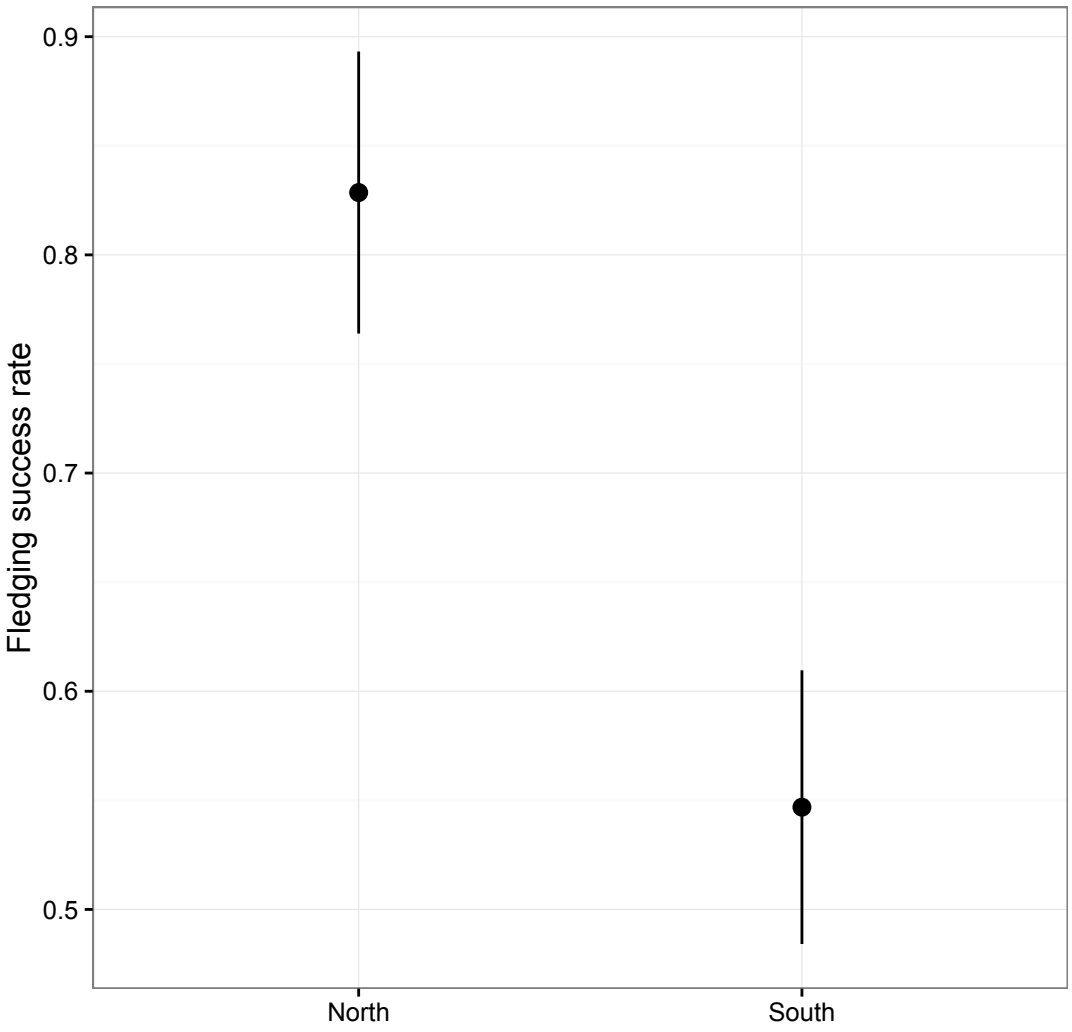


Figure 3.5: Mean average nest success rates in the north (n = 36) and south (n = 64) areas of Clipstone forest between 2010 and 2015. Tails show ± one standard error.

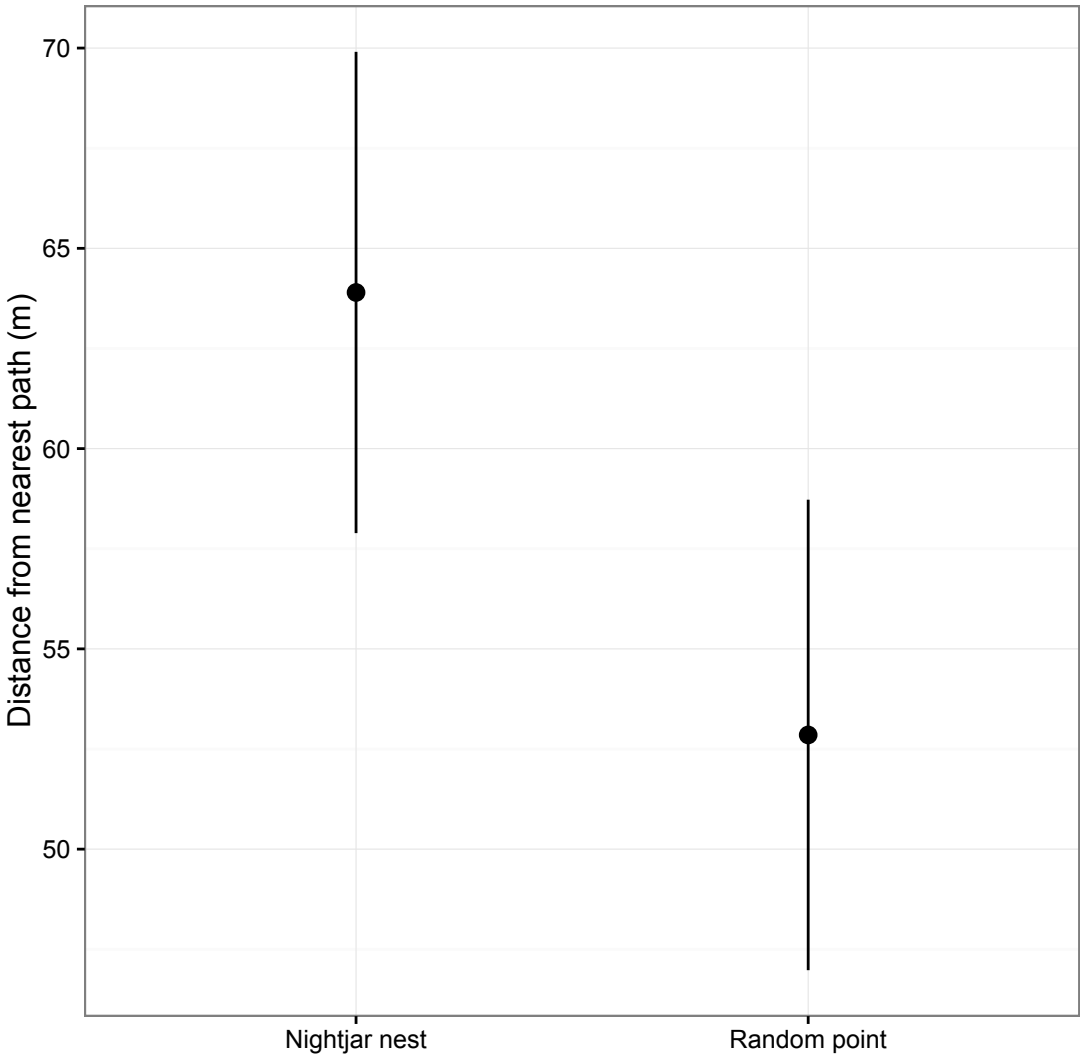


Figure 3.6: Distance in metres from the nearest path of nightjar nests (n = 49) and randomly generated points (n = 47), illustrating the small but statistically significant tendency for nightjar nests to be further from the nearest active path than predicted by chance. Points show mean averages, while tails show ± one standard error.

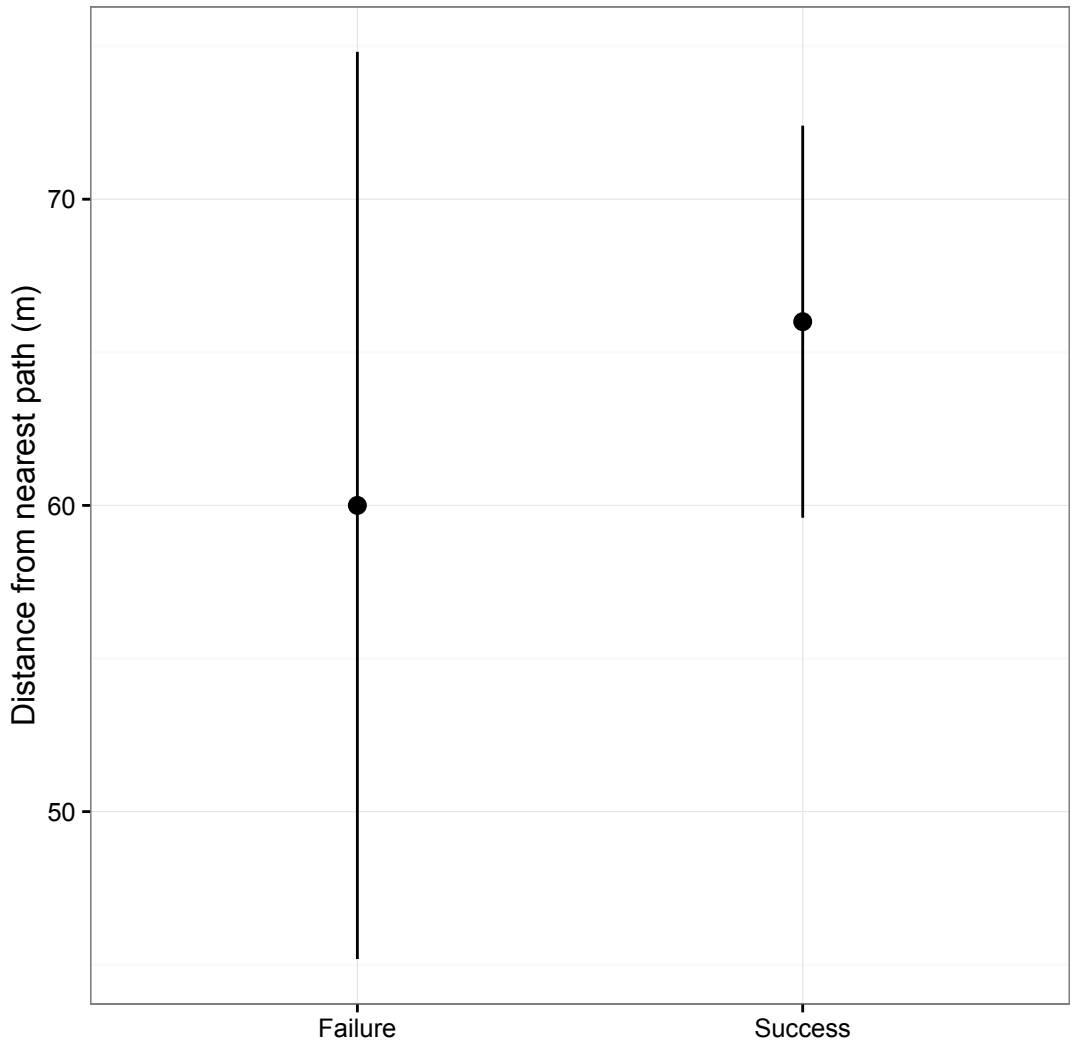


Figure 3.7: The distance from the nearest path for nests that failed (n = 14) and nests that fledged successfully (n = 34), appearing to show no direct relationship. Points show the mean average, tails show ± one standard error.

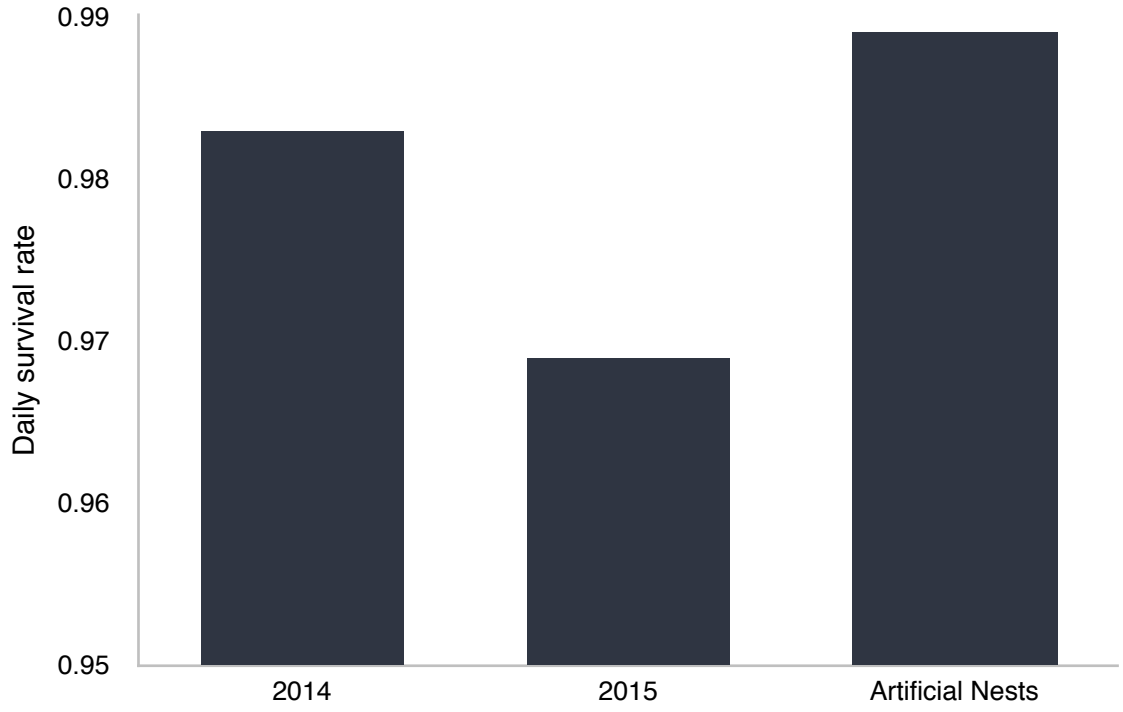


Figure 3.8: Mayfield survival estimates per day for nightjar nests from 2014, 2015 and artificial nests from 2015.

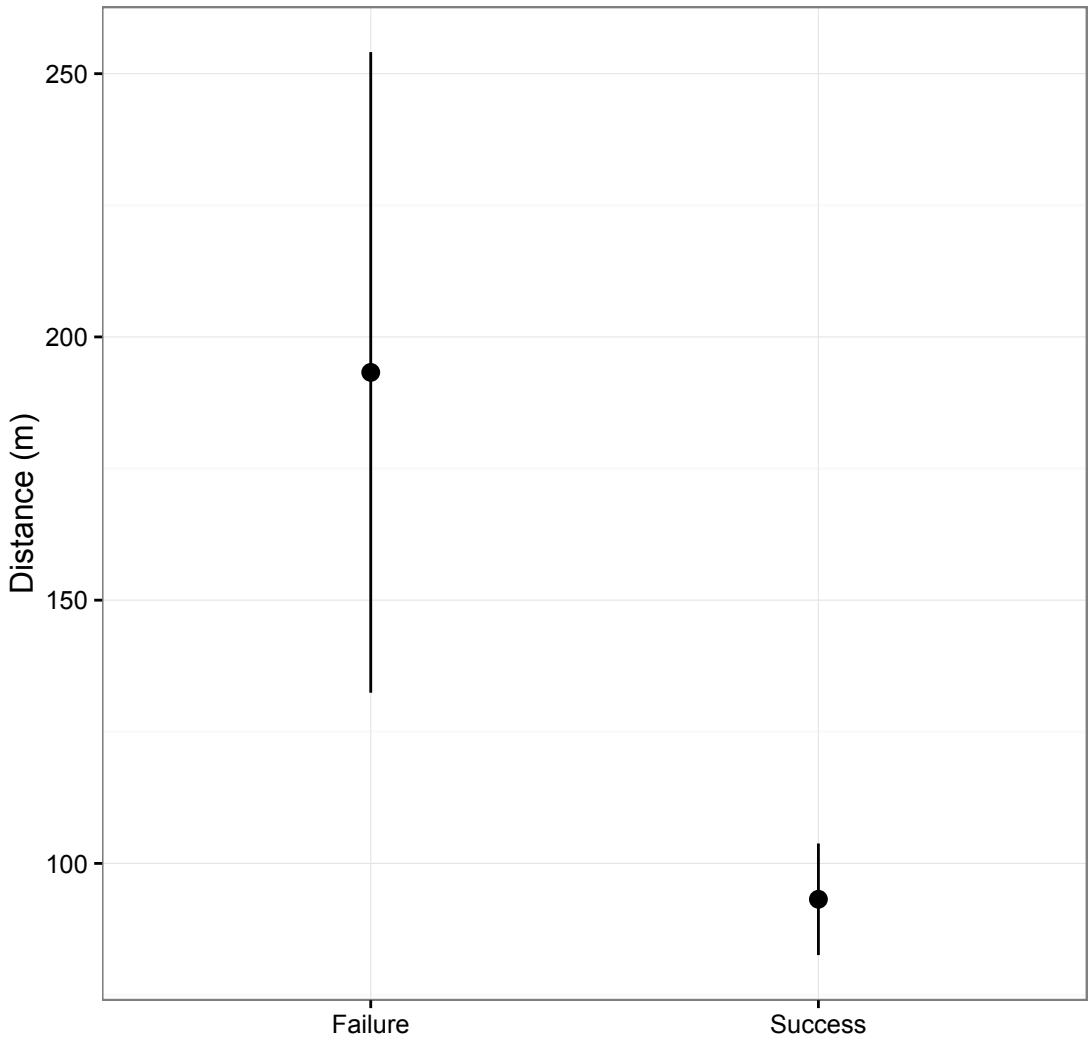


Figure 3.9: Distance in metres between consecutive broods for nests for which the first nest failed (n = 4) and nests for which the first nest was successful in producing at least one fledgling (n = 10). Points show the mean average, and tails show ± one standard error.

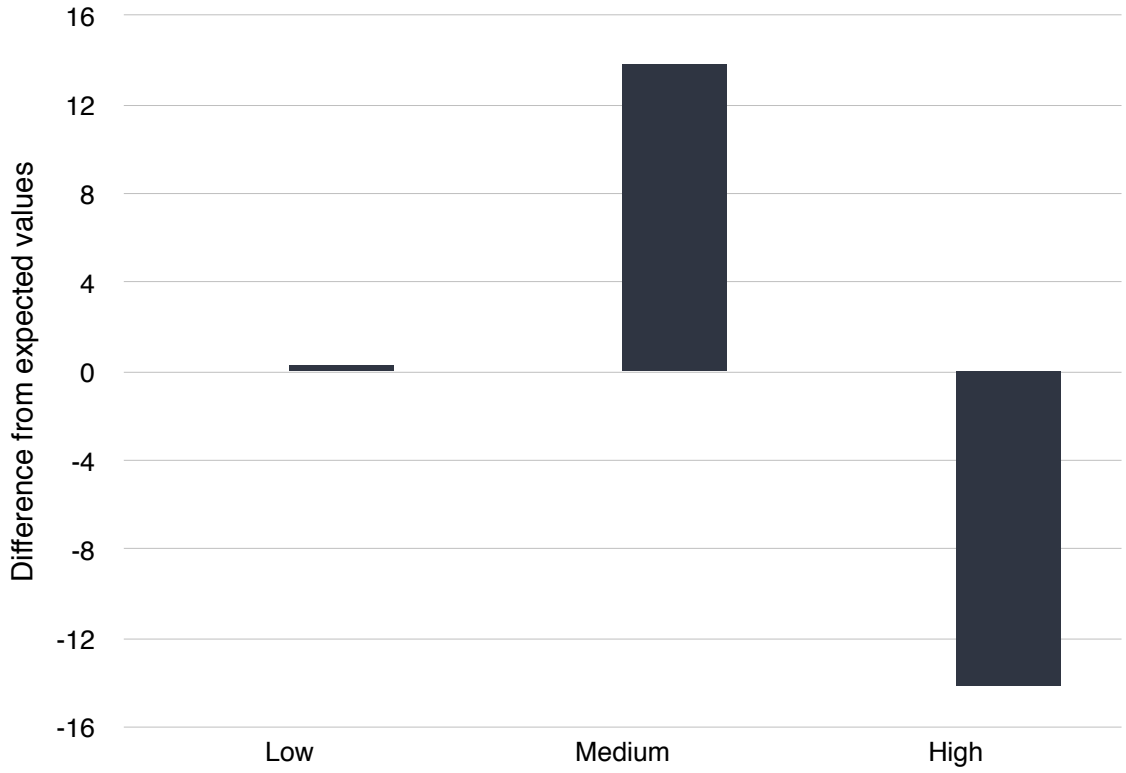


Figure 3.10: Differences between observed and expected numbers of nests in classes of low (0 - 24.9%), medium (25 - 74.9%) and high (75 - 100%) lunar luminosity.

Chapter 4

4. Male breeding ecology

It is common among animals such as birds in which offspring receive parental care for this to be contributed disproportionately by the maternal parent (Knowlton 1982). This trend is generally attributed to the relatively greater gametic investment of females; or 'anisogamy'. The greater energetic input of the female parent with respect to the male as a combined result of increased gametic investment and parental care is widely considered the cause of standard sex-biased mating systems in which males compete for access to females, and are subject to mate choice on the part of the female (Kokko & Jennions 2008). This leads often to a greater variability in breeding success between males (Bateman 1948), as better quality males are able to mate with a larger number of females, while females will tend to devote their energy to raising and provisioning offspring. In such systems, male ornamentation is common and can provide a signal of male genetic quality that is often found to influence female mate choice (Houde 2001). On the other hand, in breeding systems in which male and female parents contribute equally to the production and provisioning of offspring it is expected that intrasexual male competition for females as well as female mate choice will be relaxed (Kokko & Monaghan 2001), and that this could lead to a degree of breeding fidelity between pairs.

While female nightjars lay and are responsible for the diurnal incubation of eggs, it is widely regarded that male parents engage not just in the provisioning of chicks but also often 'relieve' (take the place of) females at the nest while the females forage (Coward 1928; Holyoak 2001). These behaviours as well as field observations have led observers to suggest that breeding pairs are likely to practice a degree of breeding fidelity, or to be monogamous, over the course of the breeding season (Berry & Bibby 1981). Consecutive broods laid by a single female have often been considered as sired by a single male — although field observations have occasionally suggested otherwise (Cresswell & Alexander 1990). If this were the case, it might be expected that nightly movements would differ between 'paired' — those with a female breeding partner — and unpaired males, with unpaired males attempting to elicit matings from females in other males' territories, while paired males may defend their

existing territories and females within them. Such differences in movements are common among paired and unpaired male birds in monogamous breeding systems, although it is often unclear whether unpaired males are simply males that have been unable to obtain a mate, or actually adopt an alternative 'floater male' mating strategy (Penteriani et al. 2010).

It is common among species of birds for males to compete for access to females, and high levels of competition between males are often observed to lead to exaggerated secondary sexual male characteristics (Houle 2001). Aside from the larger bodily mass of females, the most striking sexual dimorphism between male and female nightjars is in the characteristic 'white spots' of the male wing and tail feathers (Holyoak 2001). These white spots appear to differ in size between males, and have been observed to be involved in courting display (Cramp 1985). Enthusiasts and casual observers of nightjars have been known to wave pieces of white material in order to coerce the birds - whether male or female is not clear - to fly near to them, and it has been suggested that this is a result of its emulating the male white spot phenotype (Martin 2010). It is suspected therefore, but not known, that these feather patterns may be important in female mate choice, or in male dominance interactions. It seems plausible that larger white spot area may render males more conspicuous to nocturnal predators, and perhaps also subject to higher levels of parasitism by feather mites (Rothschild & Clay 1953), thereby presenting a handicap (Zahavi 1975) that could provide females with an honest indicator of male quality.

The primary aim of this chapter is to work towards a better understanding of the factors which may influence mating success and behaviour of nightjars. Relatively little is known about interactions between individuals of the two sexes, due to the difficulty in identifying individuals and reliably observing nocturnal behaviours. In recent years, the increasing availability and memory capacity of technologies such as radio- and GPS- tracking tags has allowed researchers to track individuals' movements over single or multiple nights, and test hypotheses regarding habitat selection, range and distances of movements and the timing of activities.

The chapter focuses on two traits, primarily with regard to males, each of which is of speculated importance in nightjar breeding ecology. The first is that of nightly range sizes and movements, which are likely to be intrinsically related to courting effort. Previous studies have suggested male nightly range sizes and movements differ

between paired and unpaired males (Feather 2015; Sharps 2014). This reported trend is investigated using the available sample, and the reliability of studies using observations from individuals on just a single night is explored. A second focus is upon the distinctive male morphology, specifically the presence of conspicuous sexually dimorphic white ‘spots’ on primary wing and tail feathers, and whether it may appear to provide a reliable signal of male quality.

4.1 Methods

4.1.1 Analyses of range and movement

Nightjars were captured using mist nets and GPS-tagged following the procedures outlined in Chapter 2. In the 2015 breeding season ‘nanofix’ (FastTrack, UK) GPS tags were set to record individuals’ positions every 4 minutes, between 8pm and 4am. To retrieve the data, tagged individuals had to be recaptured and the tags removed. In practice, just four tagged individuals were successfully recaptured despite 15 having been tagged — this appeared to be attributable to male cautiousness following an initial capture. Of the retrieved tags, two contained no data and were confirmed by the manufacturer to be faulty, while a third contained data for just two nights rather than the four expected. Because data for two individuals were considered insufficient for the broad-scale analyses, data from these two tags were pooled with GPS data from 12 ‘PinPoint’ (BioTrack, UK) tags with relocation data for a single night for individuals from the same Nottinghamshire population in 2013 and 2014 breeding seasons. The effect of male breeding status upon nightly range sizes was investigated using this expanded sample, and range sizes over multiple nights for the two individuals from 2015 were used as an indication of the validity of observed patterns and analyses that had previously relied upon tracking data from just a single night.

The two tags from 2015 recorded individuals’ locations between hours of 20:00 and 04:00 every 4 minutes over multiple nights. Tags from 2013 and 2014 had recorded locations every 23 minutes over the same period, over a single night. In order to account for potential effects of the increased frequency of relocations for the two individuals from 2015, autocorrelation of fixes was controlled for following the

methods of Schaming (2015). The minimum convex polygon (MCP) is a measure of the area encompassed by an individual's movements, calculated by creating a simple polygon including all of the recorded locations, and calculating the area of the resulting polygon (Nilsen et al. 2008). In this study, 100% minimum convex polygons were calculated for each individual, and considered to provide a measure of individuals' total range nightly movements. The distance of the farthest boundaries for 100% MCPs of each of the individuals were measured in QGIS, and the median of these distances taken. The resulting median distance of 2741m was compared with the maximum recorded distance travelled per minute of all individuals, which was found to be 291m (17.46km/h). Consequently, it would likely take this individual a minimum of 9.42 minutes to travel the median distance from one boundary of movement to another, and only relocations at least 12 minutes apart were included in pooled analyses.

Breeding status of each tracked individual was inferred from field observations and measurements, and always prior to investigation of tracking data. Paired and unpaired males were identified through a number of criteria informed by expert advice (pers. comm., A. Lowe) as well as the methods of Sharps (2014), as described in Chapter 2. Persistent churring bouts while present at a habitat were considered indicative of a male being unpaired, while paired males were often distinguishable by their intermittent churring bouts, frequent interactions with a female and visiting of a known or apparent nest site, being inferred in latter scenario by the movements of the female. Nesting females were identified by the presence of a brood patch. Minimum convex polygons produced in accounting for autocorrelation demonstrated that one of the females had a much larger total range of movement than any other birds tracked (2502 ha, compared with the mean average for all other birds of 40 ha). Similarly large ranges were observed for some nightjar females by Sharps (2014), and were classified as 'prospecting females' without a male breeding partner or nesting territory, a methodology which is followed in this study.

Analyses of range size were focussed around three commonly used measures. Minimum convex polygons have been used historically due to the relative ease with which they can be constructed and calculated, and remain widely used in analyses of home range sizes (Nilson et al. 2008). However, technological advances with regard to computing power have led many researchers to prefer analyses which account for the number, or density, of fixes in a given area using kernel density estimates (KDEs)

(Walter 2011; Kie et al. 2010). These measures factor in not just the total range of movement, but also the proportion of time spent in different areas, in calculating range sizes. Total range sizes are frequently used as measures of total range sizes, while accounting for extreme or outlying points (Worton 1989). Home range estimates - that is, the area in which an individual spends the majority of its time and is primarily active - are commonly ascertained by calculating 50% kernel density estimates, describing movements at closer proximity to the nest or roost (Sharps 2014).

In this study both 95% KDEs and 100% MCPs are used in analyses of nightly movements as they provide slightly different measures, with MCPs describing total range of movement while KDEs account for the geographic density of relocation fixes. Furthermore, calculating both allows greater opportunity for comparison between species and studies which may have used a single measure, particularly as MCPs remain widely used in range analyses (Nilson et al. 2008; Kie et al. 2010). Home range estimates are calculated using 50% KDEs— MCPs were not considered appropriate for home range estimates, as in failing to account for the density of fixes they are likely to include intermediate fixes recorded when moving between areas.

In using kernel density estimates to measure range size, it is important to select a smoothing parameter that fits the data. Over-smoothing can result in bimodal or multimodal ranges in which an individual has two or more centres of activity being interpreted as large, unrepresentative unimodal ranges, while under-smoothing can result in several small kernels where a larger kernel encompassing more points would be more appropriate (Calenge 2015b). A common automated method of selecting the smoothing parameter is to use fitted reference bandwidth (href) values (Calenge 2015b). In this study, href smoothing parameters were calculated for each individual and a mean average of these values (href = 115.94) was used as the smoothing parameter for all kernel density estimates, following the methods of Sharps (2014). The resulting kernels appeared when referenced with relocation data to fit the data sufficiently well. Kernel density estimates were not calculated for the prospecting female, as it appeared that this individual had no defined range.

Distance from the roost over the course of the night was investigated by classifying times into three categories: evening (20:00 - 22:59); mid-night (23:00 - 00:59); early morning (01:00 - 03:59). These classifications were based on field observations, which suggested the majority of foraging activity occurred within the categories of

evening and early morning. For comparative analysis, distances from the roost during each of these categories were mean averaged for each individual.

Habitat selection ratios are used to investigate significant differences between the availability of habitat to an individual or a population, and the proportion of habitat that is 'used' (i.e. the proportion of time spent by an individual or population in that habitat). In this study, type III habitat selection ratios (i.e. habitat selection for individuals with respect to their individual habitat availability; Calenge 2007) were calculated using Manly selection statistics (Manly 2002), to investigate habitat selection by nightjars, and specifically to check the assumption that nightly ranges are influenced primarily by selection for suitable nesting sites. Available habitat is considered as the total area of each of the habitat classifications within individuals' 100% MCP, in other words their total range of movement, while used proportions were calculated using relocation fixes from GPS tags.

4.1.2 White spot analyses

Males were captured and morphometric measures of muscle, weight and the length of outermost tail and wing primary feathers recorded following the methods outlined in Chapter 2. Once caught, males' left wing and left side of the tail were photographed using a digital camera, with white spots clearly visible and a ruler placed by them, as close to equidistant from and perpendicular to the camera as possible in order to provide an accurate and usable scale reference. All 29 individual males captured had their left wing and left side of the tail photographed, while 17 were also photographed for the right wing and side of the tail. While photographing both left and right wings and sides of the tail for all individuals could have provided useful further data, this was found in practice to substantially increase handling time, and so was avoided in order to avoid making capture a stressful event — a particular concern where individuals were GPS tagged with the intention of subsequent recapture. Henceforth, wing and tail spot areas refer to measurements taken for just the left side unless otherwise stated.

To avoid effects of potential bias, measuring of white spot areas was performed blindly with respect to individual ID and morphometric data, and photos of feathers that appeared highly frayed were removed from the sample for analysis due to

difficulty in measuring spot size. White spot areas were measured for males that had been photographed using ImageJ 1.50e (Schneider et al. 2012), with a calibrated scale set using a 1 mm gradated ruler, and image contrast lowered in order to render the white spots easily distinguishable from the dark surrounding feathers. White areas of feather were selected using the polygon selection tool (Figure 4.1) and their area measured in mm2 by reference with the pixel-to-mm2 ratio produced by the scaling tool.



Figure 4.1: White spots on the two leftmost primary tail feathers of a male nightjar, with the area of the white spot selected on the second feather in. Photograph credit: Ann Ward.

4.1.3 Statistical analysis

For individuals tracked over multiple nights, only the first night of tracking's data was included in analyses. Potential effects of breeding status upon each measure of range size were investigated using Kruskal-Wallis tests. Post-hoc comparison of paired and unpaired males' range sizes were conducted using one-tailed t-tests, with the hypothesis that unpaired males would have larger range sizes for each of the dependent variables of range size. One-tailed Mann-Whitney tests were used to investigate whether unpaired males visited a greater number of nesting habitats than did paired males. Analysis of variance (ANOVA) was used to investigate the effects of breeding status and time categories upon average distances from the starting roost. Distances from evening roosts were normalised by log-transformation. Repeatabilities of range size estimates and distances from roosts were calculated using restricted maximum likelihood (REML) Linear Mixed Models (LMMs) following the advice of Nakagawa and Schielzeth (2010).

The predictive significance of morphometric measures of muscle score, weight, wing feather length and tail feather length with regard to tail feather spot area, wing feather spot area and total feather spot area was investigated using multiple linear regression. Maximal models were constructed for each response variable, after which stepwise model simplification was performed until models produced fits of $p < 0.05$.

Normality of all response variables was investigated using Shapiro-Wilks tests and diagnostic histogram and Q-Q norm plots, while assumptions of equality of variances between groups were satisfied through inspection of fitted vs residual plots. Post-hoc examinations of relationships were conducted using t-tests, Tukey's HSD tests and Pearson's product-moment correlations.

MCPs and kernel density estimates were computed using the R package *AdehabitatHR* (Calenge 2016b). Habitat selection ratios were produced using the R package *AdehabitatHS* (Calenge 2016a) and rasters produced in QGIS from shapefiles constructed following the procedures outlined in Chapter 2. REML LMMs investigating the repeatability of range sizes were performed in the R package *rptR* (Nakagawa & Schielzith, 2010).

4.2 Results

4.2.1 Nightly range analyses

Habitat selection ratios were significant for all individuals ($p < 0.001$), with significant positive selection ratios reported for areas of restock and heath (Table 4.1; Figure 4.2), which were known by field observations to be frequent nesting sites. From observations during fieldwork, numerous male individuals were commonly observed to be active at the same habitat and it was apparent that much of their activity involved courting displays such as wing-clapping and interacting with females. Inspection of the movements of the paired male tracked over four nights in 2015 showed regular movements centred around locations within two separate suitable nesting habitats (Figure 4.3), both of which were known from field observations of female activity to be very near to nightjar nests, although GPS co-ordinates for the nests were not available.

Among the pooled sample five males were classified as paired and seven as unpaired, while two females were classified as nesting and one as prospecting. Each of the male individuals tracked over multiple nights in 2015 were classified as paired during fieldwork, and inspection of nightly movements appeared to corroborate this, as roosts were near to active nests and the nests frequently visited by the male over the course of the night. Although multiple nights' data were available for just two individuals, REML LMM-based repeatability analyses indicated that nightly range sizes appeared highly repeatable, and approached significance despite the small sample size ($R = 0.755$, $p = 0.068$). Visual inspection of individuals' ranges over multiple nights suggested there was a degree of repeatability in the movements and placement of kernel densities with respect to areas of heath and restock habitat over multiple nights (Figures 4.3 and 4.4), though time spent at each site appeared to differ as indicated by the placement of 50% kernel densities and comparison of nightly distances from the roost (Figure 4.5). Inspection of nightly movements showed that nesting females, paired males and unpaired males all returned to the habitat area of their evening roost at the cessation of nightly activity.

Whether an individual was a nesting female, paired male or unpaired male had a significant effect upon nightly ranges measured by 95% KDE ($X^2 = 8.776$, df = 2, p = 0.012), home range size measured by 50% KDE ($X^2 = 6$, df = 2, p = 0.049) and total range of movement measured by 100% MCP ($X^2 = 6.678$, df = 2, p = 0.035) (Figure 4.6). Unpaired males had significantly larger nightly range sizes as measured by 95% KDEs compared with paired males (t = -3.036, df = 6.760, p = 0.001), and significantly larger total nightly ranges of movement as measured by 100% MCPs (t = -2.612, df = 9.623, p = 0.013), but there was no significant difference in home range sizes as measured by 50% KDEs (t = -1.662, df = 8.828, p = 0.067). There was no significant tendency for unpaired males to visit a greater number of nesting habitats over the course of the night (W = 10, p = 0.101).

Time classes of early night, mid-night, and early morning did not appear to have any main effect upon the average distance from the roost during each category (F2,42 = 0.716, p = 494), while breeding status did appear to have a strong and significant effect (F3,42 = 16.738, $p < 0.001$). There was no significant interaction between the time of night and breeding status upon the average distance from the roost (F6,42 = 1.749, p = 0.133). Individuals' average distances from the roost were significantly repeatable between time factors, indicating a degree of consistency in differences between individuals over the course of the night (R = 0.389, p = 0.008). Post-hoc Tukey HSD tests demonstrated that average distances from the roost were significantly greater for unpaired compared with paired males ($p < 0.001$), and significantly greater for unpaired males compared with nesting females (p = 0.003), but that there was no apparent difference between paired males and nesting females (p = 0.890). (Figure 4.7).

Because the majority of the nests that paired males appeared to focus activity around had not been located by the date for which tracking data was available, it was not possible to investigate the effects of nest stage upon paired males' movements. However, among the five males classified as paired, it appeared that nightly range sizes as measured by 95% kernel density estimates became larger over the course of the breeding season (Figure 4.8), with a strongly positive rank correlation between relative date of tracking and 95% kernel densities that approached significance despite the small sample (r = 0.9, p = 0.083).

4.2.2 White spot analyses

For each of the 29 males caught and photographed there were white spots on each of the outermost three primary feathers of each wing, and two on the two outermost primary feathers on either side of the tail. Of the photos taken, wing and tail spots were deemed suitably clear for 21 of the male birds, and only these 21 were included in analyses. Mean total wing spot area was 483.459mm^2 ± 25.160, while mean tail spot area was 848.869mm^2 ± 32.457. Mean total spot area including both wing and tail feathers was 1334.572mm^2 ± 53.922. Individual's wing and tail spot areas were not significantly correlated ($r = 0.186$, $df = 19$, $p = 0.420$) (Figure 4.9). Among the males for which both wings and both sides of the tail were photographed, there appeared to be some indication that spot areas on each side were correlated, though the correlation did not reach statistical significance (wing spots: $r = 0.556$, $df = 11$, $p = 0.060$; tail spots: $r = 0.509$, $df = 10$, $p = 0.075$).

Multiple regression analysis with a response variable of total tail feather spot area reported significant predictive effects of the length of the tail feather (Est. = 22.093, $p = 0.008$) and muscle score (Est. = 236.829, $p < 0.001$), with a model fit of $F_{2,19} = 15.130$, $p < 0.001$; $R^2 = 0.574$. No model with a response variable of total wing spot area could be made to fit ($p > 0.05$). The best-fitting model for the sum of white spot areas on wing and tail feathers reported a significant predictive effect of muscle score (Est. = 396.189, $p < 0.001$) and a non-significant effect of weight (Est. = 14.484, $p = 0.0611$) with an overall model fit of $F_{2,18} = 12.750$, $p < 0.001$; $R^2 = 0.540$. A one-tailed t-test demonstrated that individuals with the larger muscle score of 2 tended to have significantly larger total white spot areas than did individuals with a relatively low muscle score of 1 ($t = - 4.4024$, $df = 18.509$, $p < 0.001$) (Figure 4.10).

4.3 Discussion

The results of the range analysis demonstrate clearly that unpaired males tend to have larger nightly ranges than paired males and nesting females. It was notable that although unpaired males had larger total range sizes than did paired males, there was no significant difference in the number of nest sites visited or home range size, and visual inspection of movements (Figure 4.1) indicated that even paired males may regularly visit and spend substantial time at additional nest sites. Results from

analyses of white spot area demonstrated a significant link between overall spot area and individual muscle score in particular, suggesting that white spot area offers an honest indication of male quality that may be likely to influence female mate choice and/or male dominance interactions.

The reported effect of male breeding status upon total nightly range size supports the findings and conclusions of previous studies (Sharps 2014); namely, that males without a breeding partner frequently move between suitable nesting habitats. Given the lack of any significant differences in home range sizes between paired and unpaired males, and the morning return of each to their evening roost, it appears unlikely that unpaired males represent 'floaters' (Penteriani et al. 2010) adopting an alternative mating strategy and are instead similarly territorial males that have been unable to attract a mate.

It was clear from the results of this study that even paired males tended to travel frequently between suitable nest habitats, and that considerable time was spent by males away from their roost despite apparently contributing to parental provisioning. It had previously been suggested that while unpaired males appear to often have somewhat bimodal nightly ranges, the ranges of paired males are more unimodal (Feather 2015), however monitoring of the movement of paired males over consecutive nights appeared to suggest that this is not necessarily the case, and that paired males' ranges may too appear bimodal. Inspection of the range of the paired male tracked over multiple nights strongly suggested that this individual moved frequently between two areas of habitat that were known to be nearby nightjar nests (Figure 4.2). While this male's paired status was inferred by frequent interaction with a female at the habitat closer to his roost, these movements may indicate that he was also regularly interacting with a female at a second nest site.

The most obvious explanation for the disparity in range sizes and time spent further from the evening roost between unpaired males and paired males or nesting females is that nightly range sizes are strongly influenced by courting effort. Kernel density estimates and analyses of distances from evening roosts showed unpaired males tended to spend more time further from their home territory each night, likely due both to the importance of courting females in order to achieve copulations and a lack of parental provisioning responsibility. The larger nightly total range sizes of unpaired males compared with paired males and nesting females appear to demonstrate that

nightly ranges, particularly for males, are influenced not only by foraging activity but also, and perhaps primarily, by courting effort and interactions between sexes. Conclusions of studies of nightjar habitat use that have presumed movements are related primarily to foraging (see: Alexander & Cresswell 1990) and draw conclusions of foraging suitability of various habitats from analysis of these movements may not be wholly reliable, therefore, particularly where males constitute a large portion of the sample.

The finding that paired males also appear frequently to visit other nesting sites may suggest a tendency to seek extra-pair copulations in order to maximise their reproductive output. Nesting females on the other hand appeared less likely to leave their nests for extended periods, likely due to parental responsibilities of incubation and/or provisioning. Because males are often observed to assist in parental provisioning, and particularly to 'relieve' females of their nest by incubating in their absence, it has been commonly held that paired males and females will practice a degree of breeding fidelity (Holyoak 2001). However, males frequently visiting nesting females at territories separate from their own suggests a likelihood of extra-pair copulations, potentially suggesting in turn that the perception of nightjars as largely monogamous is unlikely to be wholly accurate, and that the multiple nests often laid by females may be sired by different fathers, as has been suggested previously as a result of field observations (Cresswell & Alexander 1990). Moreover, comparatively high courting effort of males along with lower rates of parental care and provisioning are likely to lead to competition among males for access to females, and potentially strong sexual selection pressures. This may indicate a role of the secondary sexual characteristic white spots displayed by males, in providing an indicator of male quality that influences female mate choice.

Total white spot area on males' feathers was found to differ substantially and significantly between assigned muscle scores, with individuals with a great level of muscle tending to exhibit larger white spot area. Of the morphometric and physiological measures available for the male nightjars photographed, muscle seemed the most likely to provide a reliable indicator of male quality. A greater level of muscle mass may be likely to facilitate early arrival at breeding grounds, which is widely expected to have a positive impact upon individual reproductive success (Dickerson et al. 2005; Janiszewski et al. 2014; Velmala et al. 2015), and has been observed to be of selective importance in migratory pied flycatchers *Ficedula*

hypoleuca (Velmala et al. 2015). Furthermore, considerations of genetic quality impacting offspring fitness, thought to play a role in sexual selection (Kokko & Monaghan 2001), may provide a further incentive for females to choose males that appear to be of better quality.

In addition to considerations of sexual selection enforced through female mate choice, plumage patterns may play a role in male dominance interactions and therefore in accessing receptive females. Male plumage patterns are often reported to play a role in intrasexual competition, for example between males of the white-throated sparrow (Watt 1984), which can often involve aggressive and costly interactions between individuals. Being able to assess the quality and strength of a rival male may allow an individual not only to avoid potentially life threatening injury but also to save valuable energy by not engaging in a conflict he is unlikely to win. Male nightjars are often observed to compete for access to females, and to perform aggressive displays such as holding their wings in a 'V' shape during flight and wing-clapping (Coward, 1928) which are likely to be highly energetically costly. Muscle mass is likely to have a direct impact upon a male's likelihood of success in aggressive intrasexual conflict, and white spot plumage may therefore provide important signals to other males of male physical condition without engaging in aggressive energetically costly displays. Alternatively, it is possible that white spots could accentuate such displays, which may be important in influencing female receptivity.

In addition to providing honest and direct indicators of male genetic and/or physical quality, the presence of larger areas of white spots on feathers may provide an indication of male quality in terms of their ability to survive with a handicap. Zahavi (1975) popularised the 'handicap hypothesis', which suggests female mate choice may operate upon phenotypes that are costly to male fitness as the ability to survive in spite of the costs therefore provides an honest indication of male quality. Areas of white spots on the male nightjar plumage have been observed to be host to feather mites not found on other feathers (Rothschild & Clay 1957). It is possible furthermore that larger white spots render males more conspicuous to potential predators. During fieldwork a large number of tawny owls *Strix aluco* were captured in nets, seemingly due to their attraction to male calls played on the tape lure. Moreover, fieldworkers could easily distinguish males from females in flight following the visual conspicuousness of the white wing and tail spots. It is possible therefore that the

ability to survive with larger white spots despite the increased conspicuousness to nocturnal predators such as owls would represent a handicap that may influence female mate choice.

The results of the habitat selection analysis demonstrate a significant overall preference of nightjar for areas of heath and restock habitat. It is clear from inspection of nightly movements that this preference relates not only to home and nesting territories, but also to breeding ecology; particularly for males. The finding that males frequently spend considerable time in habitats distant from or non-contiguous with their roosting territory may suggest that the geospatial patterns of habitat mosaics will have important effects upon breeding population interactions. For example, paired males may be unwilling to travel long distances to additional nesting habitats due to energetic cost, or the likelihood of other males visiting their own territory, potentially affecting the reproductive output of females. Behavioural barriers to movements of birds have often been disregarded given their range of movement, but are becoming increasingly recognised (Harris & Reed 2002). With regard to nightjar conservation and censusing efforts the results of this study also support and reiterate the concerns of Sharps (2014) and Feather (2015) in suggesting that non-simultaneous site surveys are highly likely to lead to repeated sampling of single male individuals.

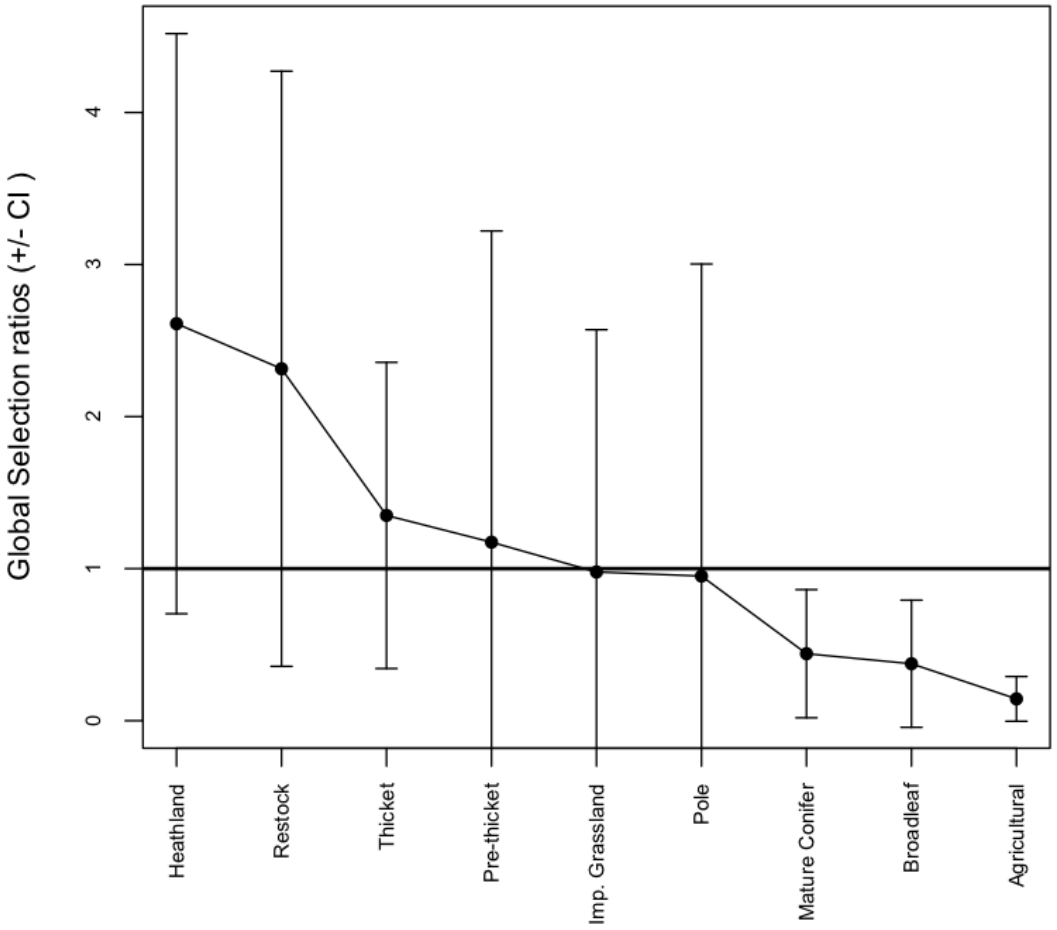
4.3 Tables

Table 4.1: Manly selection statistics (Wi) and their standard error and 95% confidence intervals for each habitat classification calculated using nightly relocations of males and nesting females within 100% MCPs. Urban and water habitats are not included, as they were not represented within the 100% MCPs that were used to define individual habitat availability.

Habitat	Wi	SE	95% Confidence Interval	
			Lower	Upper
Agricultural	0.143	0.058	-0.017	0.304
Broadleaf	0.375	0.165	-0.082	0.831
Heathland	2.611	0.751	0.528	4.694
Improved grassland	0.978	0.628	-0.762	2.718
Mature conifer	0.441	0.166	-0.020	0.901
Pole	0.951	0.808	-1.290	3.193
Pre-thicket	1.174	0.806	-1.062	3.409
Restock	2.315	0.771	0.177	4.452
Thicket	1.349	0.397	0.250	2.450

4.4 Figures

Figure 4.2: Manly selection statistics for each of the different habitat classifications over the course of nightly movements for all males and nesting females. Where values are greater than one, selectivity for the corresponding habitat is greater than its availability. Error bars show 95% confidence intervals.



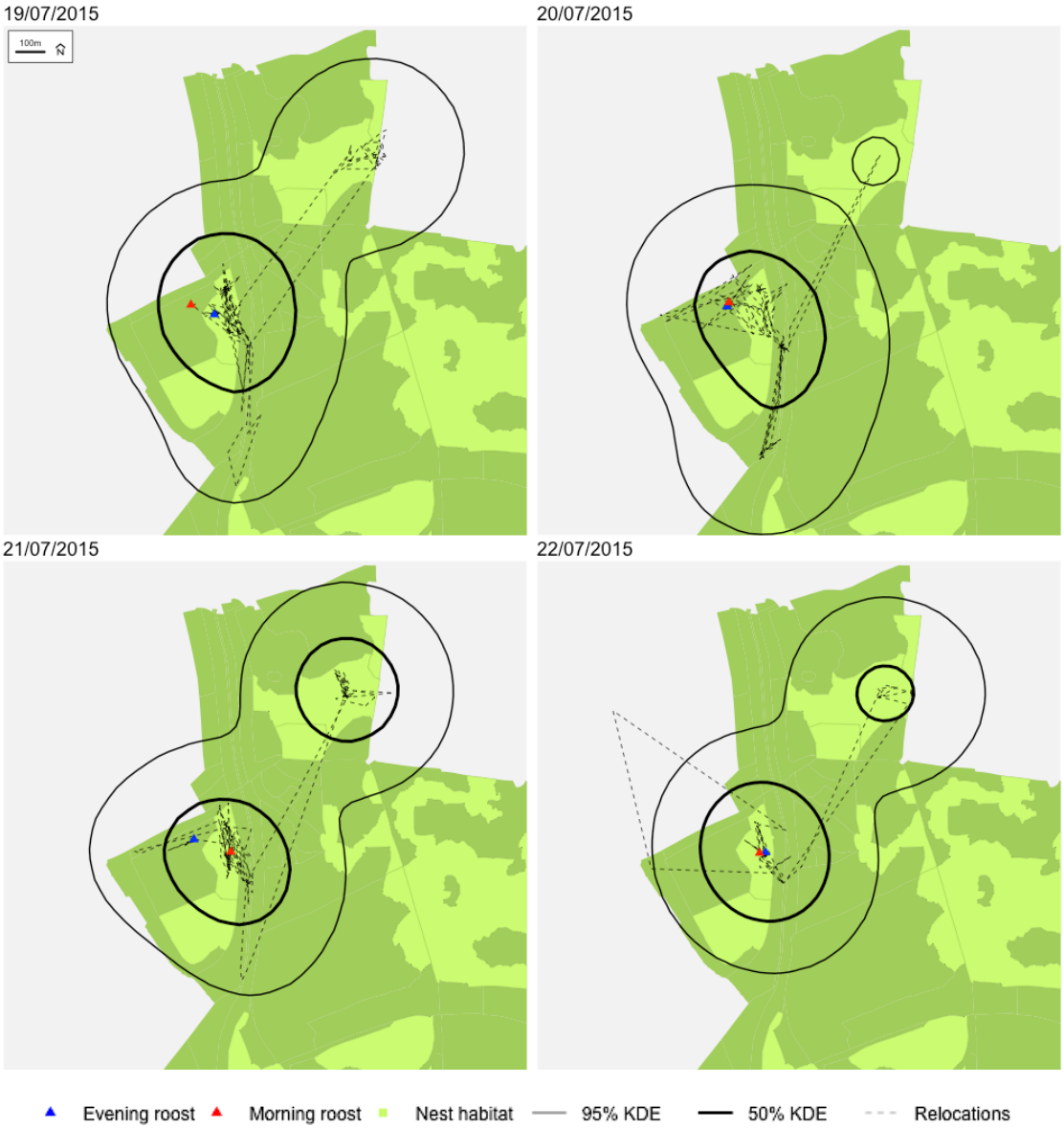


Figure 4.3: 50% and 95% kernel density estimate range sizes and track of individual movements over four consecutive nights between 20:00 and 04:00 for a paired male. Grey areas represent primarily agricultural habitat outside of the forest.

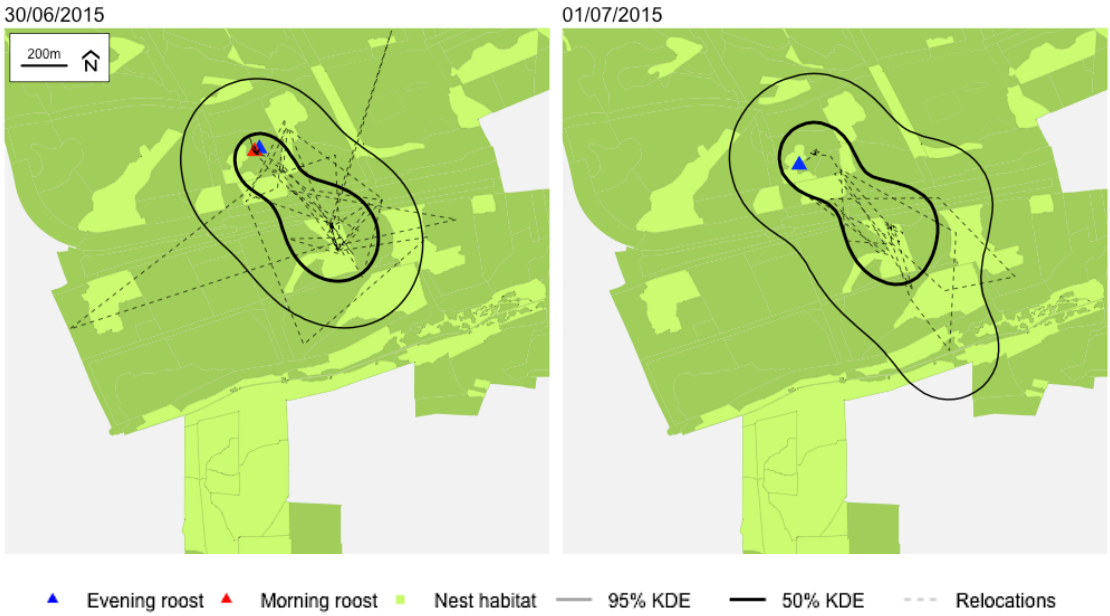


Figure 4.4: 50% and 95% kernel density estimate range sizes and track of individual movements over two consecutive nights, between 20:00 and 04:00 on 30/06/2015 and 20:00 and 00:00 on 01/07/2015 for a paired male. Grey areas represent primarily agricultural habitat outside of the forest.

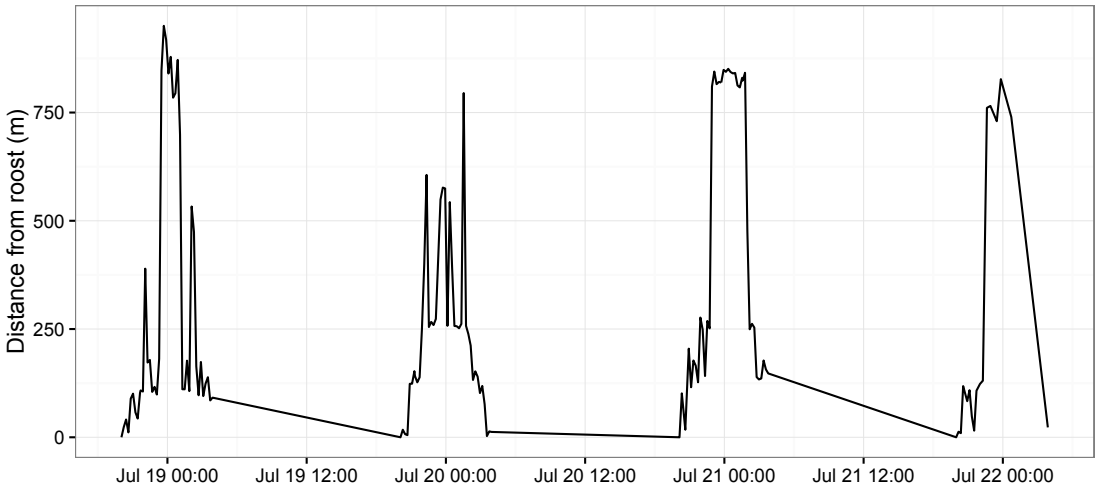


Figure 4.5: Distance from starting roost over four consecutive nights between hours of 20:00 and 04:00 for the paired male in Figure 4.3.

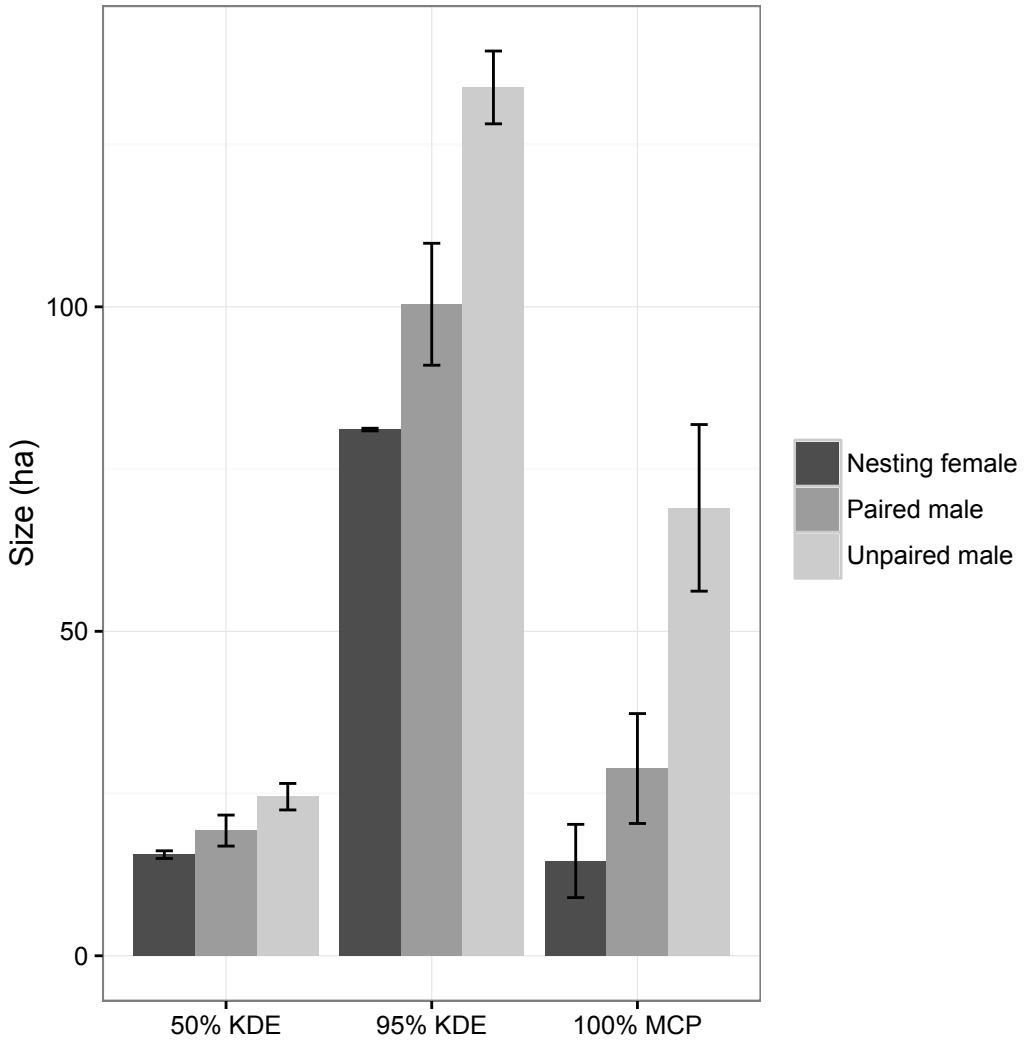


Figure 4.6: All home and range size estimates for each of nesting females (n = 2), paired males (n = 5) and unpaired males (n = 7). Error bars show ± one standard error.

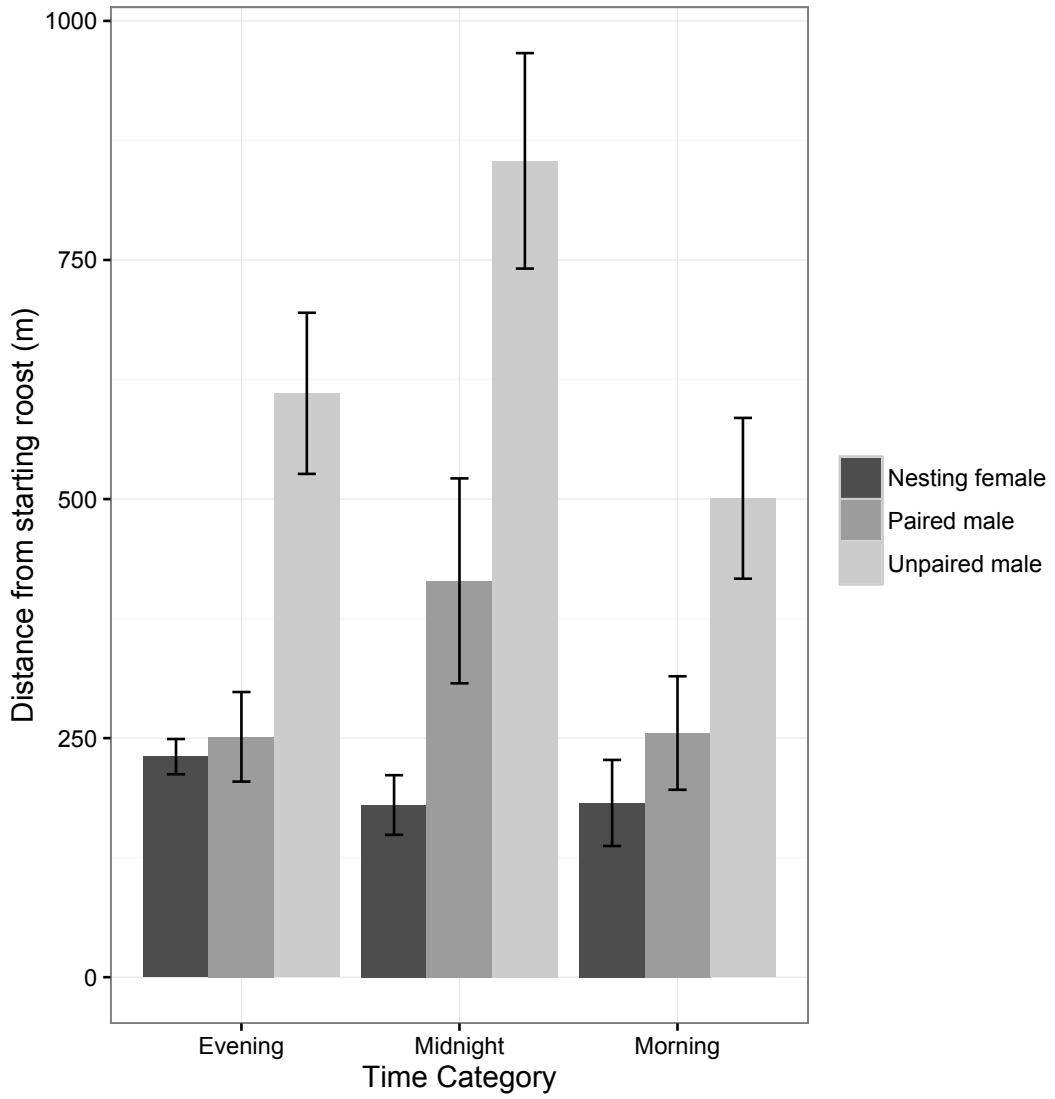


Figure 4.7: Mean average distances from the evening roost for nesting females (n = 2), paired males (n = 5) and unpaired males (n = 7) during classes of evening (21:00 to 22:59), mid-night (23:00 to 00:59) and morning (01:00 to 02:59). Error bars show ± one standard error.

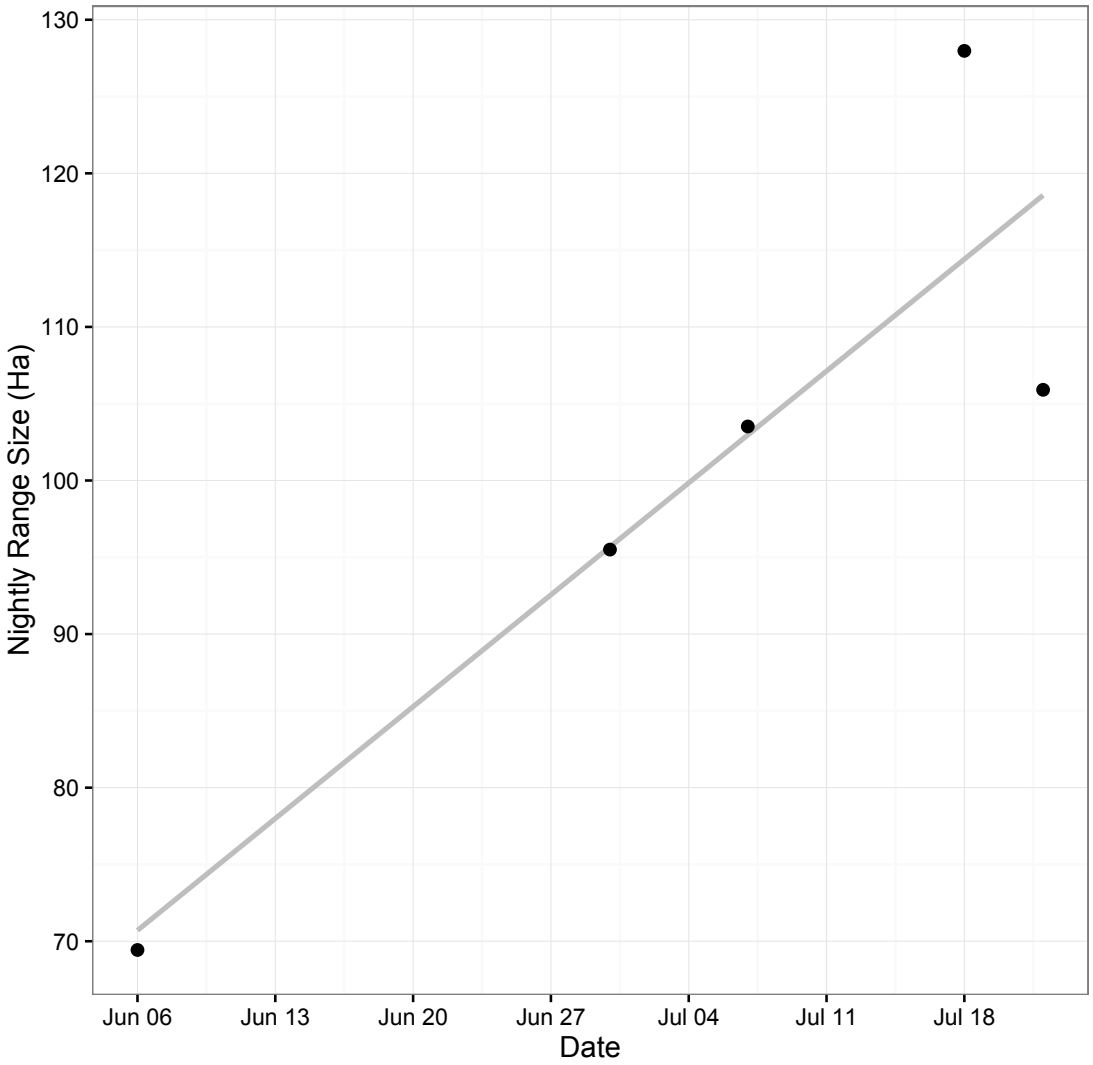


Figure 4.8: Nightly range size as estimated by 95% kernel densities for paired males over the course of the breeding season.

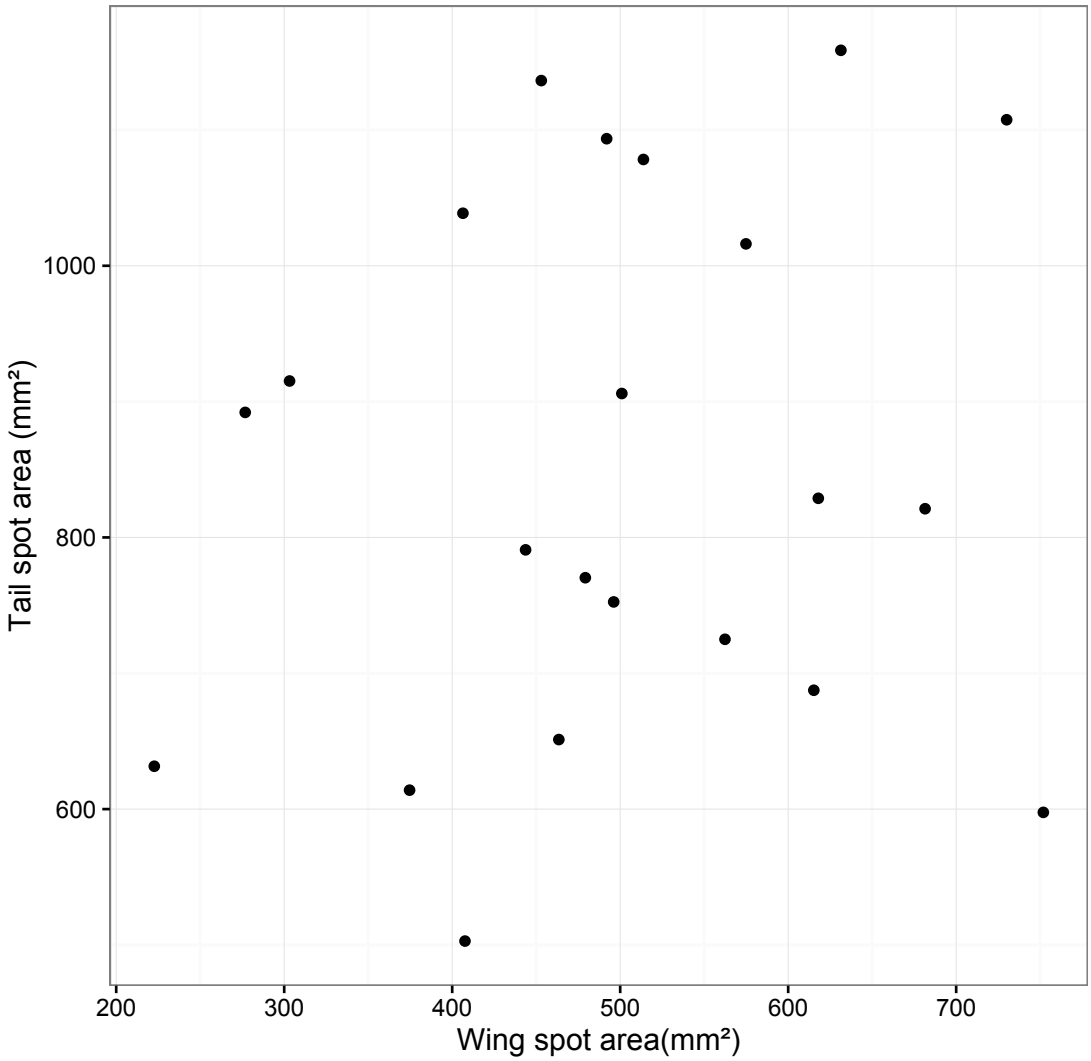


Figure 4.9: Total area of white spots on wing feathers plotted against the area of white spots on tail feathers of male nightjars, illustrating the lack of any significant or apparent correlation between the two.

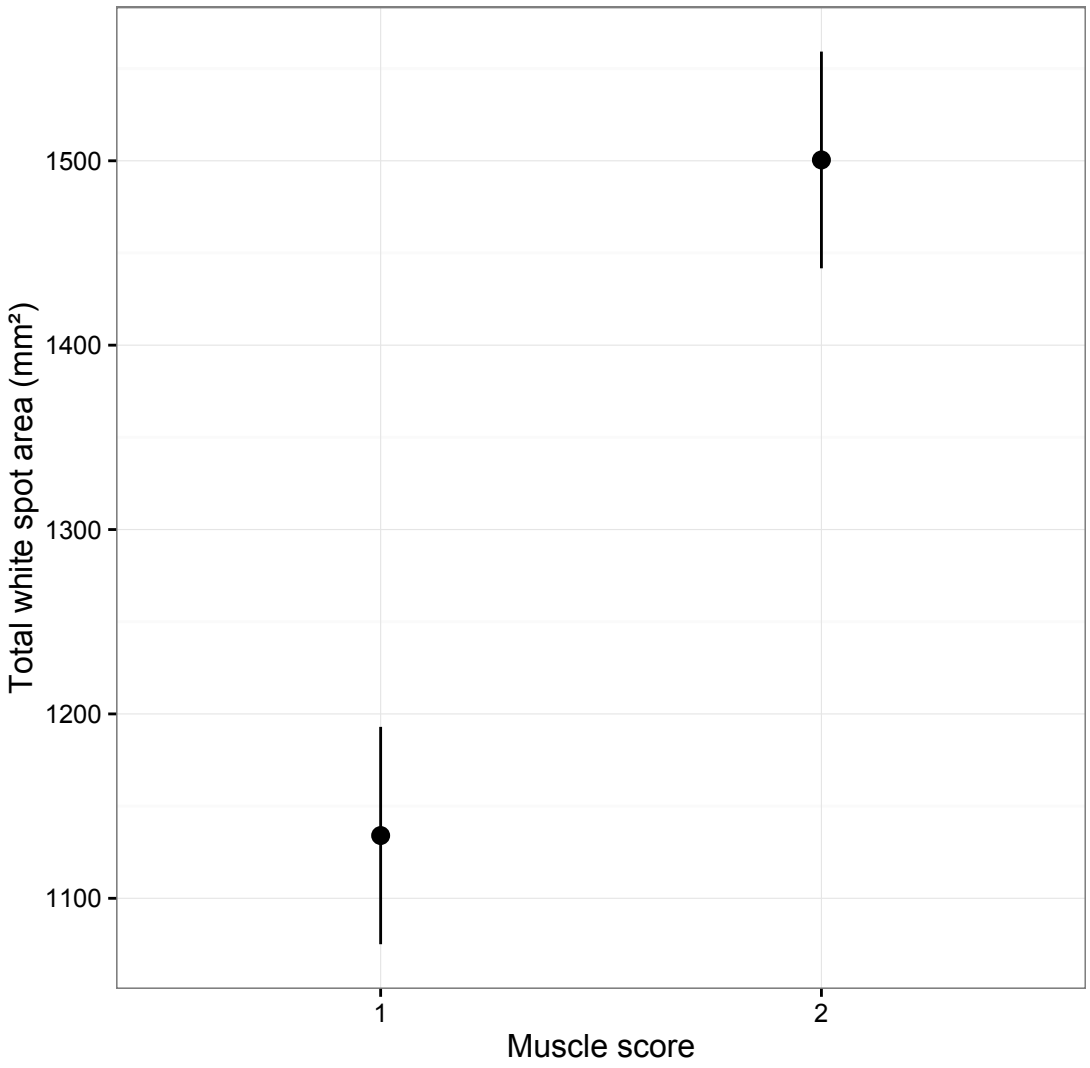


Figure 4.10: Differences in total white spot area in mm^2 between males with pectoral muscle scores of 1 (n = 9) and 2 (n = 12). Points show mean averages, while tails show ± one standard error.

Chapter 5

5. Molecular ecology

Understanding how populations interact is of great importance in ecology, and particularly in the conservation of threatened species. A key tool in addressing questions of population ecology is in molecular analyses utilising genetic markers to assess issues of genetic diversity and the degree of genetic differentiation between populations (Paetkau & Strobeck 1994). Utilised in such a way, molecular ecology can offer extremely valuable insights into the broader scale population ecology and habits of a species, as well as its resilience to further declines. Indeed, one of the major developments in conservation biology during in the late twentieth century involved the integration of genetic data into models assessing the threats faced by species, with substantial implications for conservation policy (Lande 1988; Frankham 2003).

Although the nightjar suffered rapid declines in overall numbers throughout the 20th century, particularly in Western Europe and the UK, the species has not generally been perceived as threatened by extinction due to its large range (Eaton et al. 2014). However, suitable habitat is often - and increasingly - highly fragmented, with large distances between sites suitable for sustaining breeding populations, and it is not clear whether or to what degree populations themselves mix. Addressing whether or not individuals appear frequently to move between populations, or whether populations are to some degree genetically isolated, is therefore extremely important in understanding the habits of the species, and in understanding whether its conservation status is justified as well as the threats posed by widespread habitat loss and fragmentation.

The life history of the nightjar makes it an intriguing species for the study of population genetics. While breeding site habitats tend to be sparse and fragmented, such that within the UK large breeding populations are often hundreds of miles apart (Conway et al. 2007), it appears likely that discrete and largely geographically separated breeding populations could ‘overlap’ or share nearby habitat in African wintering grounds, and/or the long-distance migration. Recent research using mark-recapture methodology has suggested overwintering neotropical migratory birds often move between wintering habitats (Ruiz-Guiterrez et al. 2016), suggesting migratory

birds from different breeding populations will often come into contact with one another. In general, migratory birds tend to exhibit a lower degree of population structure when compared with non-migratory birds (Haig et al. 2011), perhaps indicating that movement between breeding populations is relatively common.

Studies of limited numbers of birds using long-distance 'light logger' trackers have found that nightjars tagged in geographically separate populations within the United Kingdom migrate to habitat in Central Africa at close proximity to one another (Cresswell & Edwards 2013). Ringing records have demonstrated that nightjars frequently return to the same breeding populations (Silvano & Boano 2012), however in the Nottinghamshire population a number of unringed adult birds are captured every year. Occasional movement between breeding populations could indicate they in fact represent metapopulations - spatially segregated but interacting populations (Haig et al. 2011) - and would be likely to strongly influence molecular ecology both at the scale of individual populations and species as a whole.

Nightjar populations throughout the UK and western Europe suffered rapid declines throughout the early half of the 20th century. Although a large number of populations as well as the species overall has since partially recovered, it is possible that this decline could have resulted in a genetic bottleneck, and a reduction in genetic variance thereby that can reduce fitness at the level of the population and have strong deleterious effects upon morphology (Durrant et al. 2010). As of the 2004 census, the Nottinghamshire nightjar population was estimated to include approximately 66 males and to be in decline (Conway et al. 2007). It is unclear however how much movement there may be between sites within northern Nottinghamshire, and whether this constitutes a single effective population. Outbreeding organisms are suggested to suffer a chronic lack of fitness at effective population sizes of fewer than 50 to 100 individuals (Franklin 1980, Soulé 1980). Consequently, if individuals exhibit a high degree of breeding site fidelity, and do not appear to move between breeding sites within or outside of Nottinghamshire, this would be likely to have negative impacts upon the fitness of the population(s), and their robustness to future declines.

Genetic drift in populations of less than approximately 500 individuals (Soulé 1985) is suggested to lead to a progressive loss of genetic variation, with concomitant reductions in fitness (Beardmore 1983) and evolutionary potential (Franklin & Frankham 1998). Given that the Nottinghamshire nightjar population seems to fall

within this bracket, it is important therefore to ascertain a picture of the population genetic structure of the species, and determine whether individuals may move between distantly separated breeding grounds. The alternative scenario, in which individuals do not regularly move between breeding grounds, would have negative implications for the health and persistence of both the Nottinghamshire population(s) and the species at a broader scale. At present, there is little indication from ringing records that population mixing regularly occurs, with no records of the capture of individuals in Nottinghamshire that had been originally ringed elsewhere (A. Lowe, pers. comm.). Provided the extremely large breeding range of the species (Eaton et al. 2015) however, it may be relatively unlikely that any individuals arriving from another breeding site would have been ringed, and so this does not preclude low rates of movement between populations.

In addition to population genetic analyses, genetic markers can be utilised in order to address questions of individual relatedness, and gain insight into breeding ecology. Nightjar females are generally found to double-brood, and while it has often been suggested that males and females 'pair' early in the season and subsequently exhibit a degree of mating fidelity, previous studies and observations (Cresswell & Alexander 1990) as well as personal observations during fieldwork undertaken have suggested that females may also change male partners between broods. Extra-pair copulations and mate-switching are frequently observed among a wide range of apparently monogamous bird species (Westneat et al. 1990). Indeed, such a tendency may appear to be theoretically beneficial to both males and females in the context of genetic 'bet hedging' strategies (Botero & Rubenstein 2012), or to encourage males to engage in the provisioning of 'neighbourhoods' rather than single broods owing to the likelihood of nests containing their own offspring (Eliassen & Jørgensen 2014). Nonetheless, evidence both for and against mating fidelity in either male or female Nightjars is lacking notably, and studies of inferred relatedness of offspring from first and second broods could help elucidate upon the nature of the nightjar mating system.

Due to the lack of detailed genomic data for the majority of species, a commonly utilised approach in molecular ecology is to focus on microsatellites; areas of selectively neutral, non-coding DNA that nonetheless develop mutations and exhibit allelic polymorphism (Ellegren 2004). Genetic mutations at a given locus are extremely rare (Ellegren 2004), and the frequency at which they arise provides an

indication thereby as to the effective population size and its health. Relatively high levels of allelic variance may indicate that a population does not suffer from appreciably low levels of genetic diversity, while the contrary scenario may be a cause for concern, particularly if the locus is found to be variable at the level of species or between populations.

In this chapter, DNA samples taken from birds are analysed using cross-species utility Avian microsatellites. Tests for population differentiation based on allelic frequencies in the genetic data are used to investigate whether populations between sites and years appear significantly different, potentially indicative of geographic isolation. Samples from the Nottinghamshire population are compared with a small number of samples from birds in Yorkshire, and analyses then focus on comparing allelic frequencies between two closely situated habitats to assess whether there is a degree of population differentiation at finer scales (i.e. between nearby forests in Nottinghamshire, that individuals might feasibly move between during a single breeding season), which could suggest a high degree of fine-scale site fidelity. Potential differences in allelic frequencies within the population between years are also investigated, in order to gain an understanding of whether there is a difference in allelic frequencies between seasons, which may be indicative of a large amount of annual movement between genetically distinct breeding populations. Finally, an attempt is made to compare the apparent degree of relatedness between first and second broods from the same female, in order to provide insight into whether mate-switching or extra-pair copulations seem to regularly occur.

5.1 Methods

Adult individuals were captured using the mist-netting procedure outlined in Chapter 2, while chicks were located at known nests. Samples were obtained by taking buccal skin cell samples using Sterilin swabs (ThermoFisher, USA) which were then stored in a -20°C freezer until extraction with repeated thawing cycles avoided (Shao et al. 2011). Extracts from previous years obtained through the same methods and that had been stored at -80°C were thawed and re-used. A small number of samples (n = 4) were also obtained from a geographically separate nightjar population located in the Humberhead peatlands (53°37′48″N, 0°54′36″W) of Yorkshire, approximately 45 kilometres from the Nottinghamshire population, for genetic comparison. Cheek-

swabbing is a relatively non-invasive procedure for obtaining DNA samples from birds that minimises stress. Although overall extraction yields are likely to be of a lower quality than blood or tissue samples, previous studies had found the process to be effective for the purposes of amplification of microsatellites (Feather 2015; Wellbrock et al. 2012).

Microsatellite primers and loci were obtained using the guidelines of Dawson et al. (2010), who, through cross-referencing genomes of distantly related species of zebra finch *Taeniopygia guttata* and domestic chicken *Gallus gallus domesticus,* provided details of 35 microsatellites for use in studies of bird species for which species-specific primers were not available. In the original study the microsatellites were tested on a wide range of species, however neither nightjars nor any Caprimulgiformes were included. Following the recently improved phylogeny for Aves published by Prum et al. (2015), the closest known relative included in the sample, rufous hummingbird *Selasphorus rufus,* an Apodiforme, was used as an indication of which micro-satellites would be most likely to amplify reliably and exhibit variance.

Sample DNA was extracted using DNEasy blood and tissue extraction kits (Qiagen, Germany), removing the swab at at the appropriate stage, placing it end-first in a 1.5ml eppendorf and removing the handle using a pair of pliers that were sterilised between uses with 99% ethanol. DNA extracts were subsequently amplified using the Polymerase Chain Reaction (PCR) in a Techne cycler (Techne, UK) using a 10µl mixture for each sample of 5µl Reddy mix (ThermoFisher, USA) containing Taq polymerase, 0.4µl MgCl2, 0.5 µl of each primer (or 0.25µl of each when multiplexed), 2.6µl ddH2O and 1µl of DNA. The lid was heated at 105°C for 4 minutes, after which initial denaturation at 94°C for 1 minute was followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 60 seconds. A negative sample that had undergone the DNA extraction process without a swab was included in order to identify possible cross-contamination, and where loci had previously been amplified using the same methods a positive control was also included. PCR was performed in singleplex (i.e. with a single set of primers) where time allowed, but was occasionally run in multiplex with two sets of primers in a single reaction where the binding of the two sets of primers was considered unlikely.

PCR products were visualised by electrophoresis, run on a 1% agarose gel alongside 100 bp DNA ladder in order to ascertain whether or not amplification had been

successful. Because all microsatellite loci amplified were longer than 100bp, and tended to vary in size only by up to 10bp, it was not often feasible to differentiate between alleles on the gel itself. Subsequently, all PCR products were diluted to 1:100 or 1:000 (based on the relative brightness of gel bands) in distilled H_2O and fragment analysis carried out using an ABI 3700 automated sequencer (Applied Biosystems, USA). The results were analysed using Peak Scanner version 2.0 (Applied Biosystems, USA).

Population genetic tests of Hardy-Weinberg equilibrium and population genic and genotypic differentiation were conducted in GENEPOP 4.2 (Raymond & Rousset 1995; Rousset 2008) using exact G tests and with P-values estimated through Markov chain algorithms. In carrying out population genetic tests, assumptions of random and independent sampling were accounted for as best as possible by including only the genotype of one chick from each nest — either by including the individual that had amplified at more loci or, if equally amplified, selecting via random number generation. Samples from the same individual identified in the process of analysis were removed from samples during population genetic analyses, but were included in analyses of genetic differentiation between seasons where samples were from different years. It is possible that the likely inclusion of related parent and offspring genotypes may mean that individuals are likely to be more genetically similar than expected by chance. However, it was not possible to control for this possibility due to the lack of confident assignment of parentage from field observations. Moreover, individuals were suspected to move frequently and independently between nearby sites, and to often return to the same breeding population in subsequent years, and so it was considered unlikely that this would substantially bias tests for population differentiation.

Hardy-Weinberg exact tests were used to identify loci at which there were potentially ‘null alleles’ which do not amplify reliably, and thereby lead to significant differences between observed and expected genotype frequencies, and in particular excess homozygosity, with respect to the frequencies of the alleles themselves (Brookfield 1996). In order to attempt to account for the presence of null alleles, only loci which were not significantly departed from Hardy-Weinberg equilibrium at the level of $p < 0.05$ were used in population differentiation and relatedness analyses. Given that a number of loci were variable and amplified reliably, increased probability of type I errors was accounted for using Holm or ‘sequential Bonferroni’ correction, following

the advice of Waples (2015). Sequential rather than standard Bonferroni correction was preferred as it tends to produce less conservative thresholds for significance, and was in turn more conservative in terms of avoiding type II errors, i.e. loci containing null alleles appearing non-significantly departed from Hardy-Weinberg equilibrium.

Allelic richness was calculated for each locus at which null alleles did not appear to be present using Fstat 2.9.3.2 (Goudet 2002), using rarefaction in order to account for the effects of sample size (Kalinowski 2004). Allelic richness statistics at loci were compared between nightjar samples and samples from outbred populations of chaffinch *Fringilla coelebs* (n = 19) and bullfinch *Pyrrhula pyrrhula* (n = 24), calculated using data from Dawson et al. (2010), in order to evaluate whether nightjars appeared to exhibit appreciably low levels of allelic richness. For comparison, F_{is} P-values were also produced in Fstat for each of the loci for each species, with significant ($p < 0.05$) Bonferroni-adjusted P-values indicative of the presence of null alleles (see: Durrant et al. 2010).

Only loci that appeared not to exhibit null alleles and scored reliably were included in population differentiation and relatedness analyses in GENEPOP. Following population genic differentiation analyses, post-hoc pairwise genic differentiation comparisons were performed, with P-values Bonferroni-corrected to account for numerous comparisons. Because relatively few samples were available for some of the populations of interest, such as the Yorkshire population (n = 4), no numerical cutoff was used for investigating genetic differences between populations.

Likelihood ratio tests for full-sibship of samples from consecutive broods of the same female were calculated in ML-Relate (Kalinowski et al. 2006), by producing a P-value for the putative relationship of full-sibship with respect to the alternative relationship of half-sibship. This test evaluates the maximum likelihood of the putative relationship against the alternative relationship using the equation:

$$\Lambda = \mathrm{Ln}\left[\frac{L(\mathbf{K}_{Putative})}{L(\mathbf{K}_{Alternative})}\right]. \quad (1)$$

Where Λ represents the likelihood of the putative as opposed to alternative relationship, $L(K_{putative})$ represents the calculated likelihood of the putative

relationship, and $L(K_{alternative})$ the likelihood of the alternative relationship. The likelihood of each hypothetical relationship is defined by the probability of sharing no alleles (half-sibs: P = 0.5; full-sibs: P = 0.25), one allele (each P = 1) and of sharing two alleles (half-sibs: P = 0; full-sibs: P = 0.25) for any given locus. One-hundred iterations were used for each simulation. Likelihoods were calculated using the allelic frequencies observed within the population as a whole, including all samples from Nottinghamshire in all years. Full details of the methodology for calculating likelihood ratios in ML-Relate are given in Kalinowski et al. (2006).

In carrying out likelihood ratio tests, the probability of full- as opposed to half-sibship was calculated for individuals from the same brood, and for individuals from first and second broods. It was considered highly likely that individuals from the same brood would be full-siblings, and so for comparison likelihood ratios for inter- and intra-brood relatedness were mean averaged for each female.

5.2 Results

A total of 112 DNA samples were extracted, with samples from breeding seasons of 2013 (n = 18), 2014 (n = 44) and 2015 (n = 48). Once siblings and duplicate samples were removed 85 remained, with 17 from 2013, 36 in 2014, and 32 in 2015. Samples were generally found to show moderate DNA extraction success, with sufficient quantities of DNA for loci to amplify somewhat reliably. Nonetheless, a number of DNA extractions failed in this respect, though this was often thought to be due to human error at the extraction stage. Moreover, it appeared from sequencing results that multiplexed PCR often had lower amplification rates for the respective loci than did singleplex PCRs.

Of the 21 primer combinations tested, 20 amplified reliably (amplification in > 0.5 of the total samples tested). Of these 21 primer combinations tested, 11 were amplified for all or the majority of samples, summaries of which are shown in Table 5.1. Six of the samples for which the loci were amplified were unintentional duplicates, with the same individual having been sampled and amplified and analysed for each of the loci twice. These duplicates were only identified from ring numbers after fragment analysis and scoring of peaks, and had been scored identically for each of the loci for which

they had amplified successfully, except for one discrepancy at the locus *TG04-061*, which had already been identified as a locus for which scoring was problematic.

Hardy-Weinberg exact tests revealed that of the 15 loci tested for the majority of samples, 10 exhibited variance and showed no significant deviation from Hardy-Weinberg equilibrium (Table 5.2). The two loci that did appear to be significantly deviated from equilibrium were considered likely to have null alleles which failed to amplify, and were therefore omitted from all subsequent analyses along with the three loci that did not exhibit variance in alleles. Of the 10 loci remaining, one locus (*TG04-061*) was removed from analyses due to difficulty in objectively classifying peaks, as a number of alleles appeared to be just 1 bp apart, and peaks themselves were 'mountainous' — i.e. exhibited slippage. Subsequently it was not possible at this loci to reliably distinguish between homozygote samples and samples heterozygous for alleles 1 bp apart. Exact tests using only the remaining 9 loci reported a statistically significant overall departure from Hardy-Weinberg equilibrium (X^2 = 29.499, df = 18, p = 0.043). A comparison of numbers of alleles at loci between the Nottinghamshire population of nightjar and an outbred sample of chaffinch *Fringilla coelebs* using data from Durrant et al. (2010) appeared to suggest that the Nottinghamshire nightjar population does not exhibit appreciably low levels of allelic richness (Table 5.3).

There was no apparent genic differentiation between geographically discontiguous forests of Birklands, Clipstone, Rainworth and Park Forests (X^2 = 20.288, df = 18, p = 0.317), nor significant genotypic differentiation (X^2 = 18.2445, df = 18, p = 0.439). There appeared likewise to be no significant genic differentiation between the Nottinghamshire population as a whole and a small sample (n = 4) of individuals from a population in Yorkshire (X^2 = 22.575, df = 24, p = 0.545).

Tests for genic differentiation between samples collected from populations in different years throughout the forests of northern Nottinghamshire reported significant differences (X^2 = 32.9, df = 18, p = 0.017). Once Bonferroni-adjusted for multiple comparisons, statistically significant genetic differentiation was not reported for any single locus (Table 5.4). Subsequent pairwise tests for genic differentiation between samples collected from populations of 2013, 2014 and 2015 did not report a significant difference between samples from 2013 and 2015 (X^2 = 31.538, df = 18, P = 0.025), 2014 and 2015 (X^2 = 22.823, df = 18, P = 0.197), or 2013 and 2014 (X^2 =

19.607, df = 18, P = 0.355), with a Bonferroni-adjusted P significance threshold of 0.0167. When Hardy-Weinberg exact tests were performed upon samples from each of the three seasons separately, each season's population produced X_2 statistics considerably further from statistical significance than when samples had been pooled between seasons (2013: $X^2 = 9.052$, df = 16, P = 0.911; 2014: $X^2 = 16.246$, df = 16, P = 0.436; 2015: $X^2 = 16.806$, df = 16, P = 0.398; all seasons: $X^2 = 42.165$, df = 48, P = 0.710).

Likelihood analyses of full versus half-sibship tended to report values some way from statistical significance, and it would not have been possible to reliably classify which individuals were or were not full sibs without *a priori* field observations. Nonetheless, there appeared to be an indication that comparisons between broods produced P-values further from significance than those between individuals from the same brood ($\bar{x} = 0.515 \pm 0.111$; $\bar{x} = 0.314 \pm 0.088$, respectively). It was notable however that the comparison which reported a probability of full sibship as opposed to half-sibship closest to significance (p = 0.06) was between subsequent broods. Figure 5.1 shows the distribution of averaged relatedness scores between the two sets of comparisons, while Figure 5.2 shows the distribution of relatedness between the two sets including all pairwise comparisons between first and second brood chicks. Among the latter set, there was no significant tendency for between-brood comparisons to produce P-values further from significance when compared with within-brood comparisons (W = 33, P = 0.186).

5.3 Discussion

Population genetic analyses appeared to confirm that nearby but geographically discontiguous populations of nightjar in Nottinghamshire do not differ genetically, but suggest that populations between years were significantly genetically differentiated. The finding that nearby but geographically separate populations do not appear to differ genetically is as anticipated, and would appear to suggest that there may be relatively high rates of movement between these habitats by individuals, either within or between breeding seasons — or indeed both. Furthermore, there did not appear to be a significant genetic difference between the Nottinghamshire population and a more geographically distant breeding population from Yorkshire, however this result appears somewhat inconclusive given the small number of individuals from Yorkshire

for which swabs were available. Additionally, the Yorkshire population is relatively nearby with regard to the full breeding range of the species across Europe and Asia (Birdlife International 2012), and comparison with more geographically distant populations would be likely to provide a greater degree of insight into the population structure of the species as a whole, as genetic differentiation of populations is often closely related to geographic distance (Rousset 1997).

The finding that populations between years are genetically differentiated is relatively difficult to interpret, and was not anticipated. One suggestion was that the result may have been influenced by sampling bias between years, with a greater proportion chicks in particular swabbed in the 2015 season. This is possible, however it appears unlikely that this would strongly skew allelic frequencies given that Hardy-Weinberg analyses provided no indication that populations within each breeding season were inbred. It was considered possible moreover that analyses may have been skewed by an aberrant locus (Raymond & Rousset, 2008), however there didn't appear to be a strong indication that a single locus was responsible for the statistically significant result (Table 5.4). Lastly, scoring of peaks and therefore the assignment of alleles was often subjective, and despite the removal of loci that were especially difficult to score or showed significant deviation from expected frequencies heterozygosity, the potential for scoring errors should be considered in interpretation of results. Nonetheless, any errors or subjectivity in assigning alleles should have been consistent throughout the sample, and so it appears unlikely that this should be the cause of this unexpected result.

The suggestion that populations differ significantly in their genetic composition between breeding seasons could indicate that there is an amount of mixing or movement of individuals between genetically distinct populations. If this were the case, it would violate the assumption of Hardy-Weinberg equilibrium that there is no movement into or out of the population, and may therefore explain why the test for Hardy-Weinberg equilibrium over multiple seasons suggested that the population was not at equilibrium ($p = 0.051$). Previous studies have interpreted the lack of a genetic structure in bird populations as evidence of gene flow between them (Barnett et al. 2008). On the other hand, it has been observed from ringing records that nightjars show a high degree of philopatry and are often recaptured in subsequent years at the same or nearby sites (Silvano and Boano 2012), and this was observed in the current study, with one male having been captured at different locations in Nottinghamshire

for each of the past three years. Nonetheless, large numbers of un-ringed adult individuals are captured each year, despite ongoing and near-comprehensive ringing efforts at field sites throughout northern Nottinghamshire. Moreover, previous studies of migratory bird species have reported that despite an overall tendency for migratory connectivity, discrete breeding populations overlapped at wintering grounds and degrees of migratory connectivity differed between populations (Finch et al. 2015).

It is possible that individuals or populations could differ in their degree of migratory connectivity (Finch et al. 2015), or from another perspective in their tendency to move between breeding populations. One possibility may be that the likelihood of returning to the same breeding or wintering grounds is influenced by individual age (Cresswell 2014). Another possible and intuitive explanation for such a phenomenon may be if the breeding site fidelity or movement of individuals, particularly of males, is influenced by breeding success or mating strategy (Penteriani et al. 2010). Speculatively, a male may be more likely to return to the same breeding grounds if he has previously experienced a high level of breeding success, while a less successful male may be more likely to migrate to a different breeding ground location in order to maximise his chances of breeding and therefore his fitness (Gauthier 1990). Data for the current study appeared insufficient to investigate a difference between sexes in genic differentiation, as sex was not easily classifiable for most chicks or juvenile individuals, but this could provide a focus for future research.

In relatedness analyses between broods, likelihood ratio scores for full-sibs were generally quite weak and in most cases would have been insufficient to confidently assign full-sibship without field observations *a priori*. The assignment of siblingship would be likely to benefit from the amplification of loci exhibiting a greater degree of allelic polymorphism, possibly obtainable through development of nightjar-specific microsatellite sets, and from more reliable DNA extraction, perhaps through collecting high quality blood samples. As a consequence of the low confidence of sibship assignment, no strong conclusions can be drawn from these results. Nonetheless, it appeared that comparisons between subsequent broods of the same female produced lower likelihoods of full-sibship than within-brood comparisons. Consequently, the reported findings do appear to provide some support, albeit weak, for the hypothesis that females may often breed with different males in producing subsequent clutches. The validity of comparisons between relatedness of chicks from first and second broods may have been skewed by the genotype of parents.

Unfortunately, it was difficult to control for this possibility by means other than averaging relatedness for single female parents, as confident parental assignment through field observation was extremely difficult.

It is generally regarded that there is a degree of subjectivity in interpreting the results of fragment analysis of microsatellites (Renteria & Krohn 2013; Waples 2015). Indeed, this was found to be an issue with two loci, *TG04-061* and *TG08-024 set 2* with each seeming to exhibit large degrees of variation in alleles separated by just 1bp, meaning individual alleles were often difficult to distinguish from the slippage that is often characteristic of fragment analysis of PCR product (Dieringer and Schloterrer 2003). These loci were subsequently not used in any of the population genetic analyses after the initial tests for departure from Hardy-Weinberg equilibrium, although *TG04-061* was not reported as being significantly departed from Hardy-Weinberg equilibrium. Aside from these two loci, there was no substantial difficulty in classifying peaks, and this is indicated by the consistency of classification between duplicate samples. It was noted that singleplex PCR cycles amplified DNA more reliably than did multiplex cycles, in which multiple pairs of primers were included. It would be sensible therefore to prioritise singleplex PCR in future studies, despite its comparatively time-consuming process, to ensure the greatest possible utilisation of resources.

Overall, few strong conclusions can be drawn from the results of this chapter, but may provide useful directions for future research. The suggestion that breeding populations differ in genetic structure between seasons is interesting and would have positive implications with regard to the species conservation status, but requires further investigation.

5.4 Tables

Table 5.1: Details of alleles and amplification rates for each of the loci tested for all or the majority samples, including N (number of samples tested), Amp. (number of samples that successfully amplified), and k (number of alleles observed).

Locus	N	Amp.	Prop.	k	Alleles
TG01-000	112	77	0.688	7	205, 207, 209, 211, 213, 215, 220
TG01-147	112	77	0.688	2	268, 269
TG02-120	89	61	0.685	6	232, 233, 234, 235, 236, 238
TG03-002	112	61	0.545	3	123, 125, 127
TG03-031	112	76	0.679	3	187, 190, 192
TG03-035	89	69	0.775	1	210
TG04-004	112	91	0.813	3	167, 169, 171
TG04-12A	112	86	0.768	2	240, 242
TG04-061	89	69	0.775	8	200, 202, 203, 204, 205, 206, 207, 208
TG05-053	89	69	0.775	10	215, 217, 218, 219, 220, 221, 222, 223, 224, 225
TG06-009	89	64	0.719	1	117
TG08-024 set 2	89	56	0.629	7	244, 245, 247, 249, 250, 251, 252
TG12-015	112	82	0.732	4	275, 277, 279, 287
TG13-016	112	99	0.884	2	138, 142
TG13-017	89	70	0.787	1	213

Table 5.2: The number of alleles observed (k) and results of a Hardy-Weinberg exact test for each of the loci that were tested for all or the majority of samples. P-Values are adjusted using Holm correction, with $p < 0.05$ used as an indication that the locus contained non-amplifying null alleles.

Locus	k	P-Value	St. Error
TG01-000	7	1.000	0.005
TG01-147	3	1.000	0.000
TG02-120	6	0.069	0.001
TG03-002	3	1.000	0.001
TG03-031	3	0.210	0.001
TG03-035	1	-	-
TG04-004	3	1.000	0.000
TG04-061	8	0.210	0.002
TG05-053	10	0.040	0.001
TG06-009	1	-	-
TG04-012A	2	1.000	0.000
TG08-024 set 2	7	0.006	0.000
TG12-015	4	1.000	0.003
TG13-016	2	1.000	0.001
TG13-017	1	-	-

Table 5.3: A comparison of number of alleles observed (k) and allelic richness (Estimated k) at loci for nightjar, and outbreeding populations *Fringilla coelebs and* bullfinch *Pyrrhula pyrrhula.* Non-significant F_{is} P-values suggest null alleles are not present.

Locus	Species	k	Estimated k	F_{is}	P-value (F_{is})
TG01-000	Nightjar	7	6.82	0.114	1.000
	Chaffinch	1	1.00	-	-
	Bullfinch	6	5.55	0.063	1.000
TG01-147	Nightjar	2	2.00	-0.058	1.000
	Chaffinch	4	3.43	-0.088	1.000
	Bullfinch	1	1.00	-	-
TG03-002	Nightjar	3	3.00	0.009	1.000
	Chaffinch	3	2.32	-0.167	1.000
	Bullfinch	3	2.69	-0.004	1.000
TG03-031	Nightjar	3	2.79	0.298	0.198
	Chaffinch	1	1.00	-	-
	Bullfinch	1	1.00	–	-
TG04-004	Nightjar	3	2.66	-0.063	1.000
	Chaffinch	3	2.17	-0.056	1.000
	Bullfinch	3	1.77	-0.029	1.000
TG04-12A	Nightjar	2	2.98	-0.043	1.000
	Chaffinch	1	1.00	-	-
	Bullfinch	1	1.00	-	-
TG12-015	Nightjar	4	3.93	-0.085	1.000
	Chaffinch	4	3.36	0.086	1.000
	Bullfinch	2	2.00	0.217	1.000
TG13-016	Nightjar	2	2.00	-0.159	1.000
	Chaffinch	4	3.34	0.500	1.000
	Bullfinch	6	4.27	0.061	1.000

Table 5.4: The results of an exact G test for genic differentiation between seasons of 2013, 2014 and 2015 at each of the 9 loci, with a Bonferroni-adjusted significance threshold of p = 0.006. The resulting P-value across all loci was 0.016.

Locus	P-value
TG01-000	0.821
TG01-147	0.628
TG02-120	0.206
TG03-002	0.123
TG03-031	0.114
TG04-004	0.470
TG04-12A	0.022
TG12-015	0.028
TG13-016	0.166

5.5 Figures

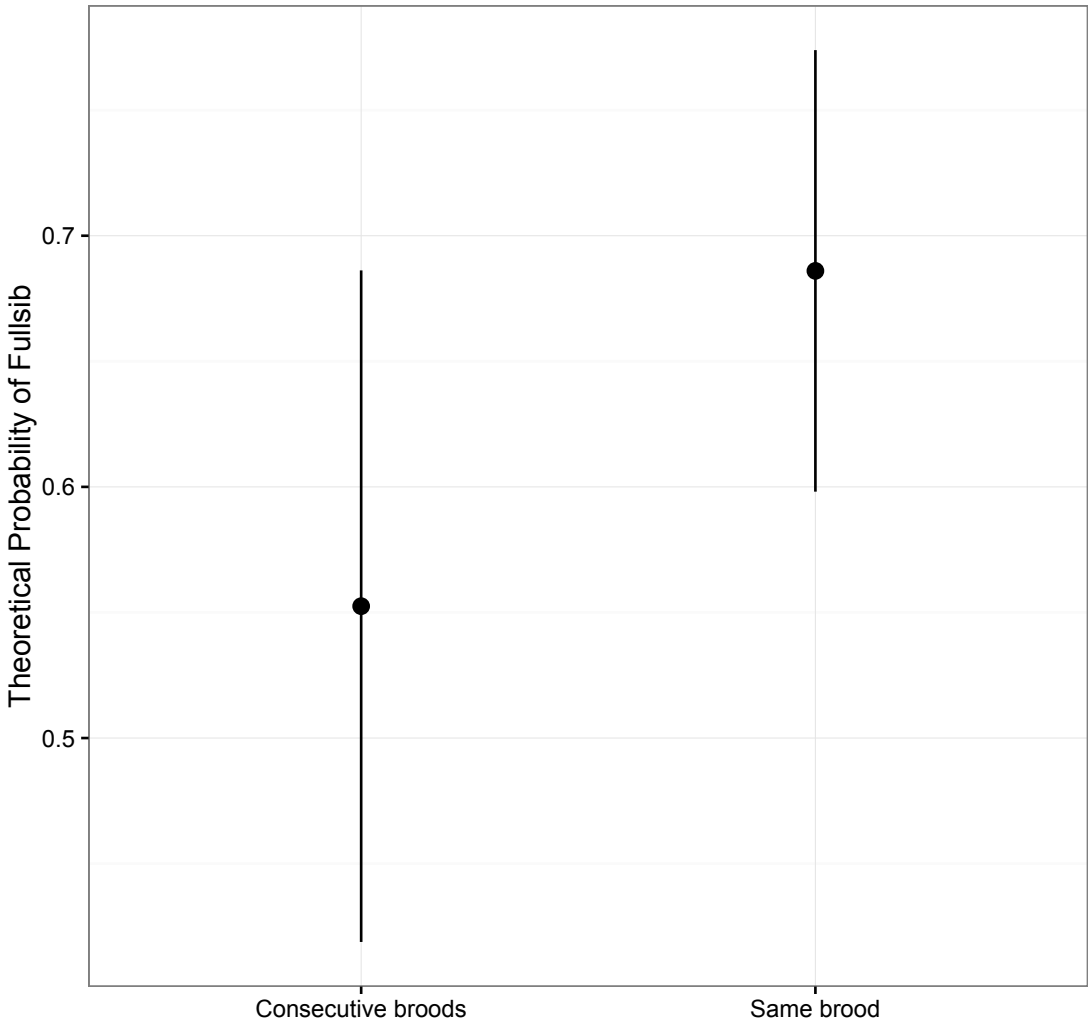


Figure 5.1: A comparison between average theoretical likelihood of full-sibship with respect to half-sibship (1 - p) of chicks sampled from consecutive broods (n = 4) and within the same brood (n = 5) of the same female. Tails show ± one standard error.

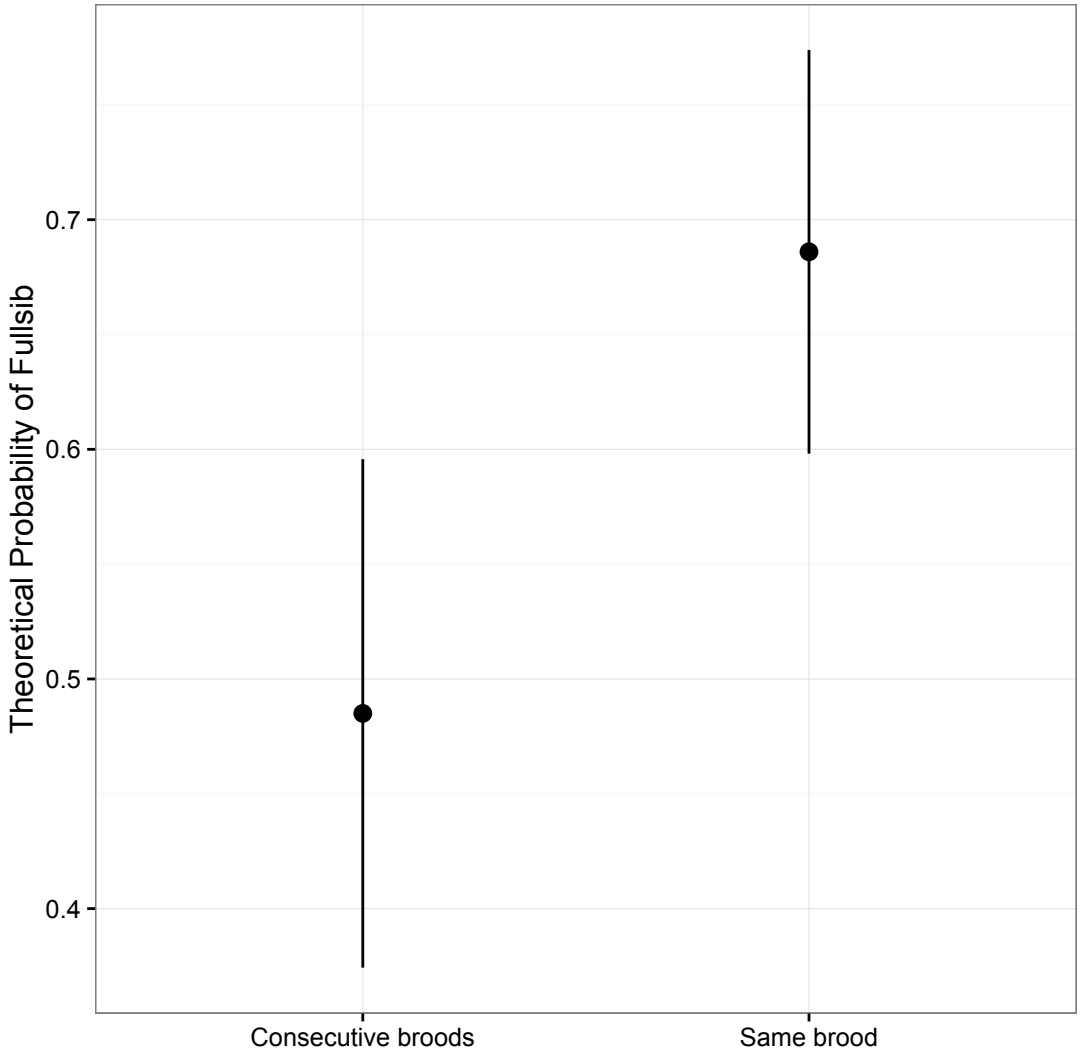


Figure 5.2: Theoretical probability of full as opposed to half-sibship (1 - p) of offspring from consecutive broods (n = 10) and from the same brood (n = 5), including all pairwise comparisons between subsequent broods. Tails show ± one standard error.

Chapter 6

6. General conclusions

Each of the three sections of this thesis sought to investigate a particular aspect of the ecology of nightjar breeding populations, with applications in conservation and species understanding. Together, the results of this study into nightjar breeding and behavioural ecology are likely to be of interest to land managers and organisations such as the Forestry Commission, for whom the conservation of nightjar is a stated priority (Lowe et al. 2014), while also elucidating upon behavioural and ecological habits of the species more generally, with potential applications in the undertaking of fieldwork. The main findings of the study can be broadly categorised into areas of: effects of human recreational disturbance, nightjar breeding ecology more generally, and nightjar molecular ecology.

6.1 Considerations of land management

The results of an investigation into the effects of recreational disturbance suggested that different forms of recreational disturbance have differing impacts upon rates of habitat occupation by nesting nightjars, and that the results of recreational disturbance as a whole need not be negative. Indeed, it appeared that increased site visitation by cyclists was positively associated with total site productivity in terms of numbers of nests fledging. The most immediate conclusion to be drawn from the results reported is that cyclists, and by extension similarly non-invasive forms of human recreational visitation, do not have an appreciably negative impact upon breeding nightjar populations. The apparent disparity between forms of disturbance may have important implications for land management in sites subject to high levels of human visitation.

There was some indication that recreational disturbance has effects not just upon nesting ecology, with nesting females and fledgling productivity, but also upon male breeding ecology. While site visitation appeared not to have any effect upon rates of male habitat occupancy, a negative effect of visitation by walkers was observed upon the likelihood that a habitat would be occupied by a paired male. There was again no evidence of a similarly negative impact of numbers of cyclists, and visitation by

cyclists was in fact positively associated with the likelihood that a habitat would be occupied by a paired male. These results seem to support the conclusions of Lowe et al. (2014) in suggesting that males occupying habitats subject to higher rates of disturbance by walkers and dogs may have difficulty attracting mates.

There was no evidence of any substantial impact of site visitation by walkers or cyclists upon nest success. This may have been due in part to possible compensatory behaviours or avoidance of busy areas, however none of the results appeared to support the commonly held notion that moderate rates of human recreational site use have strongly negative impacts upon the survival of nightjar nests (Langston et al. 2007a; Showler et al. 2010). Occasionally, however, direct and deliberate human interference was identified as the cause of nest failure, and this is an important caveat to bear in mind when considering the effects of recreational site use. Restricting human access to nest sites is unlikely to be feasible at a number of sites which are open to the public, however the practice of fieldworkers in not marking nests so as to avoid drawing attention to active nests (Feather 2015) is clearly advisable.

Altogether, results from the study into the effects of recreational disturbance suggest that preventing recreational site visitation need not be necessary so long as certain invasive forms of disturbance are kept to a minimum, or paths and habitats are planned so that more invasive forms of disturbance are minimised at areas of suitable habitat. Such an approach is likely to be both more sustainable and have greater application in land management. It is also likely to be more popular with members of public, the engagement of whom is highly beneficial in the practice of implementing effective conservation measures (Gill 2001). There is at current a large degree of inconsistency in the results of studies into the effects of human recreational disturbance upon ground-nesting birds (Showler et al. 2001), and the results of this study may suggest that this could be due in part to the failure of the majority of studies to distinguish between forms of disturbance that are likely to have differing impacts.

Aside from the effects of recreational disturbance, nesting nightjars showed a significant preference for areas of heath and restock. It was notable that habitat selection for areas of restock was considerably stronger than for heathland, the habitat generally and traditionally considered best-suited for nightjar occupancy. One explanation offered for this was simply that restock habitat tended to be more

consistently suitable for nesting females, while heathland tended to be more variable throughout its extent. Regardless of the reasons for this apparent preference, the results support suggestions that forestry restock provides excellent nesting habitat for nesting nightjars (Sharps 2014; Wichmann 2004), and with regard for nesting nightjar could even be prioritised over the maintenance of heath habitat.

Among nightjars for which GPS tracking data was obtained, analysis of nightly movements reported significant and positive selection for habitats of restock and heathland. This was likely to be explained largely by foraging suitability of these habitats (Sharps 2014), however it was also clear that unpaired males in particular, and to a varying degree paired males too, frequently visited and spent considerable time in areas of heath and restock habitat distant from their own roost. This finding seems to suggest that forests with discontiguous areas of heath and restock habitat are suitable for sustaining nightjar populations. On the other hand, if areas of heath and restock habitats within sites are largely geographically separated this may impact upon the nightly movements and breeding habits of nightjars by restricting movement between nesting sites or, for example, reducing the ability of males to defend multiple territories. This is a concern that could be taken into consideration in allocating the spatiotemporal availability of suitable habitat, and patterns of clearfelling. Factors that create behavioural barriers to movement between habitats have often been neglected in the study of birds, however their importance in conservation is increasingly recognised (Harris & Reed 2002).

Overall, it seems highly advisable given the results of this and previous studies (Langston et al. 2007a), to limit wherever possible forms of invasive disturbance by, for example, advising pedestrians to keep dogs on leads. Though investigation of habitat selection ratios was not a primary aim of the current study, having been utilised mainly in order to facilitate the interpretation of results from other analyses, it is clear from the results that areas of forestry restock in addition to semi-natural heathland provide females with suitable nesting habitat. Preserving these habitats, or at least continuing to ensure that similar habitats are always available, should be a priority of forestry planning with regard to nightjar conservation. This may be achievable through advising commercially managed forestry sites, and organisations such as the FC, to maintain a proportion of suitable low-lying habitat at all times, in order to maintain heterogeneous landscapes and avoid possible scarcity of viable nesting and foraging habitat as plantations mature simultaneously.

6.2 Nightjar breeding ecology

Results from analyses of breeding status and male plumage provided some insight into the breeding ecology and interactions of the nightjar, which can be difficult to observe interacting in the field. Both total nightly range sizes and distances from roosts indicated that unpaired males spend significantly more time away from their roost and inferred home territory, when compared with paired males or nesting females. The most parsimonious explanation for this trend is that unpaired males devote more time and effort to courting females at habitat that does not constitute their home territory. Because home range sizes did not appear to differ between paired and unpaired males, it was considered that unpaired males did not represent floater males with an alternative mating strategy as described by Penteriani et al. (2010), but were in fact males that had been unable to attract a mating partner. It was clear nonetheless that all males, paired or not, regularly visited habitat that was not part of their own territory, and the habitats visited were frequently known to host nesting females. Moreover, it appeared from tracking data over multiple nights that paired males' movements between sites showed a degree of repeatability, but that time spent away from roosts differed quite substantially between nights.

The area of distinctive and sexual dimorphic white spots on primary feathers of males was significantly correlated with muscle score, indicating that these plumage patterns may provide a useful and honest indication of male quality. Subsequently, it seems likely that these white spots could be of importance in female mate choice and male dominance interactions. Given the results of movement analyses, showing that males and in particular unpaired males often spend a substantial amount of time at nesting habitats outside of their own territory, it seems plausible that they must compete for access to females and also to coerce females to mate with them. White spot area seems as though it may play a role in such interactions, by potentially influencing their ability to access females and, or otherwise, the receptivity of females.

In general the results of the current study would appear to suggest that males compete for access to and matings with females, and that unpaired males seek to coerce nesting females into mating while paired males may seek extra-pair copulations (see: Alexander & Cresswell 1990). These inferences appear partially

corroborated by relatedness analyses of DNA samples from offspring from the same and subsequent broods from a single female, in that subsequent broods appeared to often have lower likelihoods of full sibship when compared with offspring from the same brood — potentially indicative of differing paternity.

6.3 Nightjar population genetics

The results of population genetic analyses indicated no genetic difference between individuals captured at geographically nearby forests, seeming to justify the perception of a single Nottinghamshire breeding population, at least within a single breeding season. An unexpected finding was that allelic frequencies appeared to differ significantly between years, and that the Nottinghamshire populations did not appear to be at Hardy-Weinberg equilibrium. One possible interpretation for this result was that migration may occur between genetically distinct nightjar populations between breeding seasons, which would certainly be of interest in approaching their conservation. This result appears to warrant further investigation, with reviewers of the application of genetic data in population ecology having stressed that interpretations of genetic population differentiation analyses between bird populations should be corroborated by observation or ringing records (Bossart & Prowell 1998; Lowe & Allendorf 2010).

Chapter 7

7. Limitations and future research

7.1 Effects of recreational disturbance

In the current study, detailed site visitation data was collected for just 18 areas of habitat within a single forest. Given that a number of factors besides human recreational disturbance will influence the suitability and occupancy of nesting habitat, similar data from a number of other similar sites would be beneficial in order to draw reliable conclusions regarding the effects of recreational disturbance, particularly with regard to possible effects upon nest success rates. This is one area in which further data collection would be highly valuable.

A minimum of 11 surveys each lasting 30 minutes were performed for each site, and with surveys randomly scheduled within a large site, which often meant travelling long distances between surveys, rendering data collection somewhat arduous. Performing so many surveys for each area of habitat was considered necessary, as it was not known how variable site visitation may be over the course of the day. In fact, it was clear from observations during fieldwork that variability in visitation at each site was relatively low, and that differences between sites tended to be consistent throughout time categories. Consequently, although repeat-surveying of sites is recommended, it would appear that performing so many repeat surveys may have been overly conservative in this study, and so should not discourage researchers from conducting similar studies.

Flight initiation distances were investigated with regard to vegetation cover, distance from paths, and individual repeatability. No significant effects of vegetation cover or distance from paths were reported. Given that previous studies have found among a large number of species a general trend for populations to have reduced FIDs along a rural-urban gradient (i.e. to practice evasive behaviour at lower distances in areas presumed to have more recreational disturbance) (Lin et al. 2012) another approach in investigating whether recreational disturbance affects the behaviour of nesting females may be to compare FIDs between sites with contrasting levels of human recreational disturbance. However, a study conducted by by Beale and Monaghan

(2004) using recorded FIDs of turnstones *Arenaria interpres* suggested that these measurements may reveal more about individual condition than behavioural responses to human disturbance, and so may not be a valid means of investigating impacts of human disturbance.

7.2 Breeding ecology

It was evident from tracking data that breeding status played a significant role in influencing male behaviour and movement. However, it was noted from results and the visualisation of data that there was considerable variance in range sizes and nightly movements between males assigned the same breeding status. It seems clear that factors additional to whether or not a male is paired with a female will influence their movements and courting effort. For example, a male that is paired may be more likely to expend greater effort in attempting to achieve extra-pair copulations if a nest he has sired has hatched. Conversely, a paired male may exhibit a greater willingness to spend more time away from the nest during the incubation period, prior to the onset of duties in parental provisioning of chicks. Another possibility may be that paired males guard females and territories more strictly during key intervals in order to maximise their chances of paternity. It has been frequently suggested that lunar stages will affect individual movements, particularly at stages of high luminosity (Cramp 1985; Mills 1986), though a recent study reported no differences in churring vocalisation relating to moonlight, which may indicate that it does not strongly affect male behaviour (Reino et al. 2015). Data available in this study were insufficient to address these questions, due to the small sample size and, or otherwise, failure to locate and subsequently monitor corresponding nests during the appropriate period.

Future research into male movements and breeding ecology is likely to benefit from continued data collection and larger samples, and from detailed surveying for nests in areas surrounding roosts. In this study, GPS-enabled tags where used for tracking of movements, without radio-tracking capabilities. Radio-tracking capabilities have frequently been useful in tracking movements of individuals in real-time, and in locating roosts and nests (Cresswell & Alexander 1990; Sharps 2014). While GPS-enabled tags without radio-tracking capabilities may have greater memory capacity, and therefore record data over more nights or at more frequent intervals, it appears there could be a trade-off between the quantity of data recorded and the ease with

which complementary fieldwork can be conducted, that should be of consideration in planning research and choosing between technologies. Regardless, the data gathered in this study for individuals over multiple nights did provide some insight into repeatability of movements between sites, and the collection of similar data for a greater number individuals would facilitate further analysis into patterns of nightly movements over consecutive nights.

Results from white spot analyses seemed somewhat conclusive in demonstrating a significant predictive effect of muscle score, and a relationship therefore with male quality. Nonetheless, the sample used in this study included only muscle scores of one and two, and the photographing of wing and tail spots for males with the highest muscle score of three could strengthen this analysis. Two interesting additional focuses may be to investigate a link between male breeding status and white spots, and to investigate differences in male spot plumage between populations; anecdotal evidence has suggested that males captured in Yorkshire often exhibit different patterns of white spots on the primary feathers of each wing (A. Lowe, pers. comm.), compared with those observed for males in the Nottinghamshire population.

7.3 Genetic analyses

Genetic relatedness was analysed in the current study using cross-species utility Avian microsatellites (see: Dawson et al. 2010). While the results of population genetic analyses using these genetic markers seemed to suggest they were suitable and sufficiently variable to carry out analyses for genetic differentiation, attempts to investigate questions of individual relatedness, such as maternity and paternity, were largely unsuccessful. Future research into the relatedness of individuals and the structure of the nightjar mating system may therefore benefit from the design and development of bespoke microsatellite primers that may amplify and score more reliably. Indeed, this is an avenue that is currently being pursued (K. Durrant pers. comm.).

An attempt was made to investigate whether populations from separate breeding grounds in the UK appeared genetically distinct. While there was no indication of any genetic differentiation between the two populations in Nottinghamshire and Yorkshire, analyses were likely to be weak due to small number of samples available from the

Yorkshire population. The collection and extraction of additional DNA samples from geographically separate breeding populations may provide further insight into whether populations may be genetically distinct. Collection of samples from areas such as mainland Europe may be particularly useful in this respect, given the frequently observed relationship between distance between populations and genetic differences (Rousset 1997).

7.4 Population ecology and migratory connectivity

With regard to both conservation and understanding of the species in general, it seems likely that many of the most fruitful avenues for future research into nightjar ecology appear will involve a focus upon locations of wintering grounds and migratory routes (Morrison et al. 2013; Sanderson et al. 2006). While it has been suggested that nightjars in the UK are beginning to recover following declines over the course of the 20th century (Langston et al. 2007b), a number of Afro-palearctic migrants continue to suffer declines (BirdLife International 2005). It seems likely therefore that the greatest threat to nightjar populations may be posed by habitat loss and/or anthropogenic change thought to be occurring as a result of agricultural intensification throughout the African continent (Tilman et al. 2002). Moreover, effects of climate change are likely to be particularly pronounced among long-distance migratory species, given the increased likelihood of habitat loss and general perturbation of ecosystems within their migratory range (Finch et al. 2015). Knowledge of the locations of important wintering grounds and stopover sites would be useful in predicting whether or how severely the species as a whole may be impacted by regional changes of climate.

There would appear to be three approaches suited to investigation into the migratory routes and population ecology of the nightjar. The first involves the use of long-distance loggers, such as ‘light’ loggers and long-distance GPS tags. Studies involving light loggers attached to small numbers of nightjars at breeding grounds in the UK have already reported some success in revealing the location of wintering grounds in Africa (Cresswell & Edwards 2013), however greater sample size as well as tracking of birds from more geographically distant breeding populations are likely to provide much greater insight. The utilisation of long-distance tracking technologies has often provided valuable insight into long-distance movements of similarly migratory species. For example, data collected from long-distance tracking of

European rollers *Coracias garrulus* indicated a moderate degree of migratory connectivity overall, but suggested that connectivity varied between geographical populations (Finch et al. 2015).

A second approach may involve approaches of population genetics and the collection of DNA samples from geographically distant populations across nightjars' breeding range. Evidence for or against genetic differentiation between distantly geographically separated breeding populations is likely to be of use in establishing an understanding of the population structure and ecology of the species. Evidence of any such population structure may indicate that individuals practice a high degree of breeding site fidelity, and that there is a limited degree of population mixing. Conversely, evidence to suggest that populations appear relatively genetically homogeneous may indicate that population mixing is common. (Haig et al. 2011)

A third approach, commonly utilised in studies concerning the movement of migratory bird populations, may be to conduct isotope analysis of feathers collected from individuals at breeding grounds. Nightjars are thought to moult and regrow primary feathers at wintering grounds in Africa, and so analysis of the isotopic constitution of feathers may provide useful clues as to the location of wintering grounds. One potential concern in adopting this approach may be the relative lack of isotopic variation in Africa when compared with that of North America and Europe (Bowen et al. 2005), where utilisation of stable isotope analyses has often proved fruitful (Inger & Bearhhop 2008). Results from isotopic analyses may nonetheless provide useful insight into the migratory movements of nightjar, particularly when combined with tracking or genetic data (Chabot et al. 2012; Macdonald et al. 2010).

Chapter 8

8. Concluding remarks

The results from each of the main sections of this thesis have together provided insight into the ecology of nightjar breeding populations, and of the Nottinghamshire nightjar population in particular. Many of the most pervasive questions regarding nightjar ecology are discussed, and the findings should be of use in identifying areas for future research, as well as providing guidance for land managers and fieldworkers.

Results from investigation of the effects of recreational disturbance and of the nightjar mating system appear to indicate that females are deterred from nesting in areas with high rates of potentially invasive disturbance, but that non-invasive disturbance has no similarly negative effect, and that this may too impact upon the breeding success of males who hold territories in these areas. While males appear to have the opportunity to mate with more than one female, results from this thesis and prior field observations suggest that females may also practice mate-switching, or seek extra-pair copulations. Together, these results seem to indicate that the nightjar mating system is perhaps less monogamous than had previously been thought, while external and anthropogenic factors such as site visitation have a substantial impact upon the viability of a habitat and the breeding success of its inhabitants. Genetic analyses reported no evidence of differences in the genetic composition of geographically nearby breeding populations, but suggest that genetic composition of these populations may change over time, or between breeding seasons. It is possible that this is a result of movement of individuals between genetically distinct breeding populations, however this surprising result could have been influenced by sampling bias and requires further research.

The nightjar remains an elusive and cryptic species that is difficult to study *in situ*. Recent advances in technology have improved the ability of researchers to study nightjar behaviour and ecology in the field, and provided new avenues for research. Future technological advances and collection of data are likely to have a similarly positive impact, and provide increasingly reliable conclusions regarding the nightjar's behaviour and ecology, with useful applications in their conservation. In particular, the utilisation of technological advances in the study of migratory movements and

potential population mixing will elucidate upon both the behaviour and sustainability of the species and its individual populations.

9. References

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