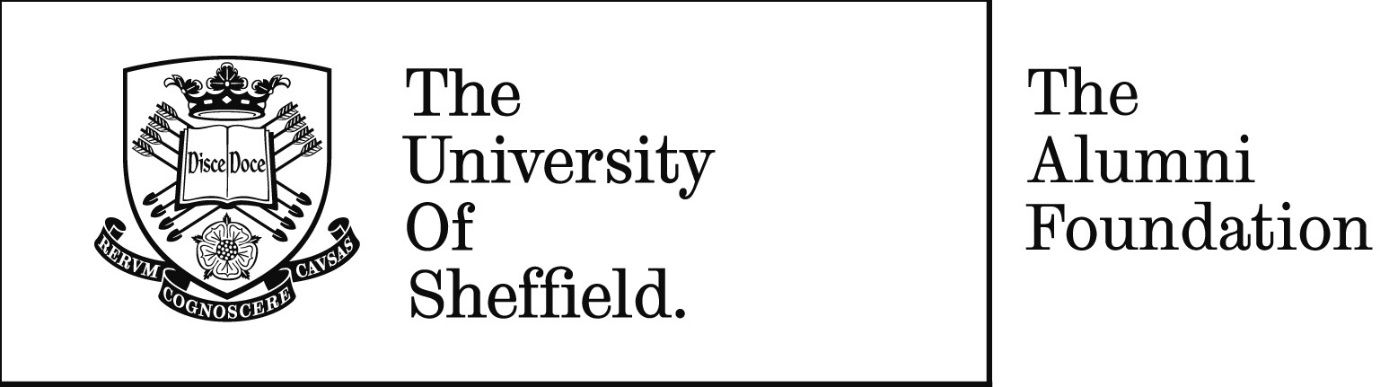
Evolutionary ecology of avian nest design and function in a variable environment



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**General Summary**

Reproduction is the only source of fitness for most organisms, which is why despite the inherent costs to an individual’s own condition, they invest their finite resources into reproductive attempts. However, individuals must weigh investment in any single attempt against any future attempts to breed because investing heavily in one attempt can reduce the likelihood of surviving to breed again. This cost of reproduction is likely to vary in relation to many factors, including environmental conditions. This thesis investigates the effects of variable environmental conditions on nest placement and structure, and incubation behaviour and duration in a passerine bird.

These problems are addressed using the long-tailed tit *Aegithalos caudatus*, which has been studied in the Rivelin Valley, Sheffield since 1994. I used this long-term dataset to answer questions about the effects of predation on nest placement, and the influence of environmental conditions on incubation period duration and hatching success. I also conducted experiments on nests to explore how their thermal properties were affected by ambient temperature and simulated rainfall under controlled conditions. Finally, I determined the influence of environmental conditions on incubation behaviour.

The probability of predation and predator type varied significantly with nest placement, but personal experience of nest predation did not affect nest placement. Nest site choice was neither a heritable trait, nor was it a result of imprinting. Variation in incubation period duration was a function of environmental conditions, and also influenced hatching success. Experiments showed that nests effectively buffered the potential impact of adverse environmental conditions (temperature and rainfall) on nest contents. Finally, females adjust their incubation behaviour in order to buffer variation in ambient temperatures, with consequences for incubation period duration and hatching success. I conclude that variability in nest-building and incubation behaviours allows individuals to adapt to their environmental conditions.

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**Statement of intellectual contributions**

In this thesis, the research chapters (2-5) have benefitted from the collaboration with and advice of several colleagues. My primary supervisor was Ben Hatchwell, who conceived and continues to manage the long-tailed tit study. Karl Evans co-supervised this project. Long-term data collection and population monitoring has been contributed to by numerous field researchers. Contributions made by all co-authors are detailed below, all other work is my own.

Chapter 2: I completed the data analysis and wrote the manuscript. Ben Hatchwell and Karl Evans contributed to the conception and design of the data analysis, interpretation and provided comments that improved the manuscript. DNA extraction and genotyping were conducted by Natalie dos Remedios and Maria-Elena Mannarelli, under the guidance of Deborah Dawson and Terry Burke at the NERC Biomolecular Analysis Facility at the University of Sheffield.

Chapter 3: I conducted the data analysis and wrote the manuscript. Karl Evans and Ben Hatchwell contributed to the conception and design of the data analysis, interpretation and provided comments which improved the manuscript.

Chapter 4: I conceived this study with Ben Hatchwell and Karl Evans. Joel Pick provided the R code for the modelling of cooling rates, which I then adapted for my data. Katherine Assersohn (for 2016 and 2017 nests) and Jacob Peers-Dent (for 2018 nests) ran the nest insulation quality experiments. I completed the data analysis and wrote the manuscript. Ben Hatchwell and Karl Evans contributed to the interpretation of results and provided comments that improved the manuscript.

Chapter 5: I collected incubation behaviour data, analysed this data and wrote the manuscript. Ben Hatchwell and Karl Evans contributed to the conception and design of the data analysis, interpretation and provided comments that improved the manuscript.

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**Chapter 1.**

# **General introduction**

## **1.1 Investment in reproduction**

Producing offspring which then go on to reproduce themselves is the only source of fitness for most organisms (Trivers 1972). For this reason, most species invest energy and resources to advance the evolutionary fitness of their offspring (Smiseth, Kӧlliker & Royle 2012). However, parents have a finite amount of energy therefore they must balance their investment in raising any current offspring with other essential activities, such as foraging, which improves their own body condition and allows for potential future breeding attempts (Trivers 1972; Stearns 1989). The amount of investment an individual makes in any one reproductive attempt is likely to be dependent upon the probability that the reproductive attempt will be successful and the likelihood of future reproductive attempts. For example, those organisms with a low annual mortality rate usually have a lower reproductive output each year, whilst those with high annual mortality usually have a much greater investment in each reproductive attempt and have a higher annual reproductive output (Stearns 1989; Promislow & Harvey 1990).

The first investment which must be made into any reproductive attempt is in finding a mate. This investment and potential cost may be lessened in long-term (more than a single breeding season) socially monogamous species, i.e. white-chinned petrels *Procellaria aequinoctialis* (Bried & Jouventin 1999), oystercatchers *Haematopus ostralegus* (van de Pol *et al.* 2006) and Stellar’s Jay *Cyanocitta stelleri* (Gabriel & Black 2012) (for review see Griffith 2019). However, even within socially monogamous bird species mate replacement is common, either due to mate mortality or divorce, although the levels at which this occurs are likely to be highly variable between bird species (Jeschke & Kokko 2008). Divorce between a pair becomes especially likely following a sub-optimal breeding performance (Culina, Radersma & Sheldon 2015). In addition, likely a species will breed with the same partner through multiple years is dependent on the relative costs and benefits of long-term social monogamy within that species. Some studies have suggested that the behaviours required to maintain the pair bond are potentially costly (Huxley 1938; Butterfield 1970) i.e. contact calling, beak-touching, allopreening and displays which occur after mate choice. However, more recent studies have suggested that these costs may be outweighed by the advantage of not spending time searching for a new mate, as this allows pairs to breed earlier in the year and have higher reproductive success in a number of species, i.e. blue-footed booby *Sula nebouxii* (Sanchez-Macouzet *et al.* 2014), bearded reedling *Panurus biarmicus* (Griggio & Hoi, 2011) and zebra finches *Taeniopygia guttata* (Crino *et al.* 2017).

Individuals must not only decide how much of their resources, such as time and energy, to invest in each reproductive attempt but must also divide their resources between different reproductive stages and tasks. The division of reproductive tasks may also depend upon sex. For example, only females invest in egg production, while males typically invest more in territory defence (Burger, 1981). In birds, the process of raising offspring can be clearly split into several more or less discrete stages: nest building, egg production and laying, incubation and the provisioning of young (Reid, Monaghan & Ruxton 2000a). The provisioning of young has often been thought of as the most costly stage of reproduction, and as such has received more attention (Collias & Collias 1984; Hansell 2000). However, other work has shown that incubation is as energetically costly as provisioning (Vleck 1981; Williams 1996; Monaghan & Nager 1997; Nord & Williams 2015; Marasco & Spencer 2015), and that nest building is also a costly stage of reproduction (Moreno *et al.* 2008; Moreno *et al.* 2010; Lambrechts *et al.* 2012; Mainwaring & Hartley 2013). Both nest function and incubation behaviour are vital for reproduction as they ensure that eggs are maintained within a temperature range that is necessary for optimal embryonic development (Webb 1987; DuRant, Hopkins, Hepp & Walters 2013). This thesis will concentrate on nest building and incubation behaviour.

The amount of resources that individuals invest in a single breeding attempt may also depend on their own quality, as poorer quality individuals in lower body condition may need to spend more time on self-maintenance to ensure their own survival and therefore have less time to spend on activities associated with reproduction (Lens, Wauters & Dhondt 1994). In species with biparental care, an individual’s investment is also dependent on that of their partner (Houston & Davies 1985; Harrison et al. 2009). The differential allocation hypothesis states that an individual’s investment may vary depending on the quality of their mate; in barn swallows *Hirundo rustica*, for example, females invest more (by laying larger clutches) when their males invest more in nest building as this is likely to reflect the amount they will invest in provisioning (Soler, Møller & Soler 1998). Great reed warbler *Acrocephalus arundinaceus* (Mahr *et al.* 2012)andblue tit *Cyanistes caeruleus* (Jelínek *et al.* 2016) males also invest more in provisioning when paired with a higher quality female.

## **1.2 The effect of environmental factors**

Variation in environmental factors influences the costs of reproduction by affecting the energetic requirements for breeding and altering breeding success (Erikstad, Fauchald, Tveraa, & Steen 1998). Factors such as the likelihood of nest predation (Cresswell 2008), parasite abundance (Clayton & Moore 1997), weather conditions (Siikamiiki 1995; Nord & Nilsson 2012) and food availability (Hails & Bryant 1979) have all been found to change the costs of reproduction. In this thesis I will concentrate on two main environmental factors - risk of predation and weather - as these factors are key to the survival of eggs to hatching.

### **1.2.1 Predation**

Predation is the main cause of nest failure in most passerines (Westmoreland & Best 1987; Sanders & Maloney 2002), with predation rates as high as 93% in some species (Lloyd *et al.* 2000). It is expected that a high risk of nest predation will cause individuals to invest less in an individual breeding attempt (Cresswell 2008). Predation can also have non-lethal effects on breeding behaviour, such as influencing the size of clutches (Skutch 1949; Westmoreland & Best 1987), incubation behaviour (Conway & Martin 2000a), incubation periods (Cresswell *et al.* 2003), parental provisioning (Fontaine & Martin 2006; Ghalambor *et al.* 2013; Hua *et al.* 2014), time of fledging (Harfenist & Ydenberg, 1995) and the colour of eggs (Hanley, Cassey & Doucet 2013) (for review see Cresswell 2008). These changes in behaviour and the predicted reduction in reproductive investment due to high predation risk, are likely to affect the quality of the offspring if they do survive.

### **1.2.2 Weather**

The microclimate of the nest is extremely important in providing the optimal incubation temperature for the eggs and aiding with thermoregulation of the chicks (Webb 1987; DuRant, Hopkins, Hepp and Walters 2013). These optimal temperatures are typically substantially higher than the ambient environmental conditions in temperate environments. Therefore incubation behaviour, in terms of time spent on the nest and the temperature of the brood patch, will be the predominant drivers of the microclimate of the nest (Haftorn 1988). However, poor environmental conditions, such as low ambient temperature and strong wind, will decrease the temperature in the nest and influence the amount of energy required to reach and maintain optimal egg temperature (Reid *et al.* 2002). Rainfall may also dampen nesting material, increasing nest conductance and increasing energetic requirements through increased heat loss from eggs (Reid *et al.* 2002; Hilton *et al.* 2004; Heenan 2013). It is notable that the impact of rainfall on energy budgets during incubation has received much less attention than other aspects of ambient conditions, such as temperature. Changes in ambient conditions may also influence the amount of energy that individuals require to maintain their own body condition (Conway & Martin 2000b), leading them to neglect their reproductive attempt (MacDonald *et al.* 2013; Fu *et al.* 2017). However, birds are not necessarily passive responders to these changes in environmental conditions and have been shown to alter their behaviour in order to buffer their potentially adverse effects (Deeming & Reynolds 2015).

## **1.3 Buffering of environmental conditions**

The bird-nest incubation unit theory states that the incubating bird and the nest have evolved together to ensure that eggs are incubated effectively, especially in small birds (Deeming 2015). As such there are two main aspects of behaviour that they can alter to compensate for suboptimal environmental conditions, their nest placement and structure, and incubation behaviour.

### **1.3.1 Nest placement and structure**

Production of an external structure that is heritable and impacts the fitness of an individual, can be characterised as an extended phenotype (Dawkins 1982). Niche construction theory suggests that animals may also use these structures to manipulate their environment to better meet their needs, so extends the extended phenotype theory to include the feedback between the organism and the environment (Laland & Sterelny 2006). Nests fit well within both of these definitions as they are external structures built by animals which affect fitness, with nest size shown to be heritable (Jarvinen *et al.* 2017). They also allow animals to better respond to ambient conditions. Nests, like any other phenotypic trait, are likely to exhibit variation between individuals with low quality nests having repercussions for fitness. For example, in birds an inability to create an adequate microclimate and incubate eggs at the optimal temperature can have consequences for the survival of embryos (DuRant, Hopkins, Hepp & Romero 2013) or extend the incubation time and thus increase the risk of nest predation (Lombardo *et al.* 1995). It would therefore follow that those individuals that build better nests are likely to be able to raise more offspring and have more copies of their genes represented in future generations.

There may also be variation in nest characteristics within individuals that may be caused by a plastic response to environmental factors (Wilson *et al.* 2009). Phenotypic plasticity is the ability of a genotype to express different phenotypes under different environmental conditions, and it plays an important role in adaptation in many species (West-Eberhard 1989; Scheiner 1993). Birds have been shown to demonstrate flexibility in nest building under different environmental conditions, such as investing more in nest lining under cooler environmental conditions (Mainwaring *et al.* 2012).

Before building the nest, a nest site must first be selected. Nest site selection would be expected to minimise the effect of inclement conditions that may cause physiological stress to the incubating bird and offspring (Quirici *et al.* 2014). Sheltered nest sites with extensive vegetation cover are generally favoured in high precipitation areas, and in areas subject to strong winds, birds gain shelter from trees by nesting on the opposite side to the prevailing wind (Collias & Collias 1984). In addition to influencing nest microclimate, nest placement can be influenced by nest predation. Nest predation risk is often affected by nest conspicuousness, which may be a function of concealment in thick vegetation and nest height (Hatchwell, Russell, *et al.* 1999; Colombelli-Négrel and Kleindorfer 2009; Latif *et al.* 2012). However, the evidence for adaptive changes in nest site is mixed, perhaps due to the choice of sites being restricted by trade-offs between different environmental pressures. Such trade-offs occur when the enhancement of one function causes the degradation of another, with attempts to balance conflicting pressures causing neither to be at an optimal level (Götmark et al. 1995). Thus, nest placement can reflect the trade-off between microclimate and predation risk; for example, shading affects microclimate (Lima 2009), but birds may shift to safer, but cooler nest sites when predation risk is high (Eggers *et al*. 2006).

If nest placement influences the likelihood of a breeding attempt succeeding, evolutionary selection pressure would be expected for choice of a ‘good’ nesting site. For this to be the case nest site would have to be, at least partially, genetically determined but few studies have investigated whether nest site selection is a heritable trait in birds (but see Yeh *et al.* 2007). In addition, it would be adaptive to exhibit a degree of behavioural plasticity in nest site selection to allow individuals to respond to changing conditions and predation pressures (Roff 1992). For this to be possible the birds must be capable of judging the level of predation (Forstmeier & Weiss, 2004), and adjusting their reproductive investment accordingly (Eggers *et al.* 2006; Zanette *et al.* 2011). These studies indicate that birds are able to gauge predation probability from the levels of corvid calls (Eggers *et al.* 2006; Zanette *et al.* 2011). Similarly, passerines did not establish territories in habitats that were sprayed with American mink *Neovison vison* excrement (Forsman *et al.* 2013) and when playbacks of predator vocalisations were played (Hua *et al.* 2013). In general, it would be adaptive for individuals to use their own personal experience of both nesting success and failure to influence their choice of site location (Komdeur 1996; Chalfoun & Martin 2010).

Like nest placement, nest building has also been shown to be altered by individual experience, with zebra finches changing their material preferences following successful breeding attempts with those materials (Muth & Healy 2011). In addition, birds are unlikely to select materials simply because they are the most abundant in the environment, but instead, should select materials according to their different properties (Aguilar *et al.* 2008; Wesołowski & Wierzcholska 2018). Bailey et al. (2015) also showed that zebra finches select the nesting material that most closely matches the background colour to camouflage their nests. When bird species across Australia were compared, the materials used depended upon temperature and precipitation of the area in which the nest was built (Heenan, Goodman & White, 2015). At low temperatures, materials with high thermal insulation were used, independent of precipitation levels. However, in areas with warm temperatures and high precipitation levels, materials that dried quickly were preferred. Surprisingly, the selection of materials for their ability to waterproof nests has received little attention.

Building a well-insulated nest can be important for reducing the costs of incubation (Deeming & Gray 2016). When the incubating parent is away from the eggs they are exposed to abiotic factors, such as ambient temperature, wind, precipitation and solar radiation, each of which can affect egg temperature (Webb & King, 1983). Experimental removal of nest lining increases the cooling rate of eggs (Reid *et al.* 2000b; Windsor *et al.* 2013) and produced significantly smaller nestlings (Lombardo *et al.* 1995). The insulating properties of nests have been correlated to the amount of lining and the size of the nest, although the two variables are often correlated (Windsor *et al.* 2013). However, nest-building is costly in terms of time because it may delay the start of egg laying and hatching, which can impact food availability for offspring. Nest-building also requires energy to collect materials (Moreno *et al.* 2008; Moreno *et al.* 2010; Lambrechts *et al.* 2012; Mainwaring and Hartley 2013), so birds would not be expected to over-invest in nest construction. In addition to energetic costs associated with nest building, larger nests have also been found to suffer from higher predation rates due to increased visibility (Møller 1990; Rohwer *et al.* 2015). Therefore, birds are predicted to adjust their investment in nest building according to prevailing environmental conditions and predation risk (McGowan *et al.* 2004; Mainwaring *et al.* 2012; Rohwer *et al.* 2015).

Nest design is also thought to influence predation risk (Nice 1957; Møller 1989; Hall *et al.* 2015), enclosed nests being regarded as less vulnerable than open cup nests because the contents of the nest would be concealed. Part of the rationale for this is that nest predation is believed to be higher in the tropics where many more species build enclosed nests, compared to temperate zones. However, a recent meta-analysis by Martin *et al.* (2017) revealed that nest predation is not higher in the tropics and that the nest type (enclosed vs. open) did not affect nest predation rates. Instead, enclosed nests may primarily confer benefits by improving nest microclimate; in the tropics roofs may protect eggs and nestlings from intense rain and solar radiation (Collias 1986; Martin *et al.* 2017). Indeed, recent studies demonstrate the importance of nest mass, and specifically the thickness of roofs, in moderating ambient temperatures in the nests of zebra finches (Wilson *et al.* 2017) and sociable weavers *Philetairus socius* (van Dijk *et al.* 2013). Lamprecht and Schmolz (2004) also showed that eggs in enclosed nests cooled at a slower rate than those in open nests. However, the effects of rainfall on enclosed nests have not been directly tested in experimental settings.

### **1.3.2 Incubation behaviour**

Incubation is critical for the optimal development of the embryo because development of the embryo occurs at 34-40˚C and eggs are unable to generate their own heat (Tatner & Bryant, 1993; Williams, 1996; DuRant, Hopkins, Hepp & Romero 2013). Fluctuating embryo temperatures and extended time outside of this optimal temperature range lead to slower embryonic growth (Olson *et al.* 2006), production of fewer offspring (Reid *et al.* 2000a, 2000b; DuRant, Hopkins, Hepp & Romero 2013), and lower quality offspring (Lombardo *et al.* 1995; Tombre & Erikstad 1996; Dawson *et al.* 2005; Nord & Nilsson 2011; DuRant, Hopkins, Hepp & Romero 2013). In addition in zebra finch, the exposure of eggs to sub-optimal temperatures for a prolonged period of time can also reduce long-term survival of those offspring (Berntsen & Bech 2016).

The energetic requirements of incubation are determined by environmental conditions because the further ambient temperatures are from the optimal incubation temperatures, the more energy will be required by the incubating parents to ensure that the optimal egg temperature is reached (Bryan & Bryant 1999). The energy required for incubation was reduced by artificially heating the nests in great tits *Parus major* (Bryan & Bryant 1999). In artificially heated nests, females were also able to invest more in incubation leading to clutches hatching more synchronously in tree swallows *Tachycineta bicolor* (Ardia *et al.* 2009), nestlings having greater mass close to hatching in great tits (Vaugoyeau *et al.* 2017), and greater fledging success in common starlings *Sturnus vulgaris* (Reid *et al.* 2000a). In common starlings, more pairs also went on to attempt to rear a second brood (Reid *et al.* 2000a). These studies suggest that birds adjust their incubation behaviour so that under cooler ambient temperatures the optimal incubation conditions are maintained, even though there may be negative knock-on effects on offspring body condition and future reproductive attempts. Other studies have found that increased rainfall (Coe *et al.* 2015; Fu *et al.* 2017) and wind speed (Klimczuk *et al.* 2015) cause changes in incubation behaviour, although the effect of these changes on adults and offspring body condition have received less attention. However, during nestling provisioning, rainfall has been shown to increase energetic requirements for parents in sand martins *Riparia riparia* (Westerterp & Bryant 1984)*,* perhaps because increases in energy expenditure during flight under higher rainfall.

Under increased predation, birds may also adapt their incubation behaviour in order to reduce the costs of incubation and the chance that their nest is discovered by predators (Rompre & Robinson 2008). Martin and Ghalambor (1999) showed that males reduced their incubation feeding under high nest predation, which was correlated with a reduction in female nest attentiveness. However, contrary to expectations, the experimental reduction of nest predator density also caused females to reduce their nest attentiveness (Fontaine & Martin 2006). These results together highlight a more complex relationship between nest predation risk and incubation behaviour than may have been anticipated, which should be taken into consideration in future studies.

## **1.4 Thesis background and objectives**

Much of the previous research done on the behavioural ecology of long-tailed tits (*Aegithalos caudatus*) has focused on their cooperative breeding behaviour (Hatchwell, 2016). However, long-tailed tits are also remarkable for their complex nests (Hatchwell, Russell, *et al.* 1999), which require substantial investment of time and energy to build (McGowan *et al.* 2004). The reason for this high investment is poorly understood, especially given the high nest predation rates they suffer (Gullett *et al.* 2014). In addition, the population shows considerable variation in their nests, in terms of nest placement (Hatchwell, Russell, *et al.* 1999), size and composition (McGowan *et al.* 2004), and in the duration of their incubation periods (Hatchwell, Fowlie, *et al.* 1999). The overall objective of this thesis was to investigate the effects of variation in environmental conditions (including biotic and abiotic factors) on the placement and structure of nests, and incubation behaviour and duration.

### **1.4.1 Study species**

Long-tailed tits are small (7-8 g) passerines with a broad geographic distribution across much of the Palearctic (Huntley *et al.* 2007). They are a sexually monomorphic, insectivorous species that is common throughout the UK. During the non-breeding season they live in flocks of *c*.10-20 birds, foraging and roosting together (Lack & Lack 1958; Hatchwell 2016). These mixed-sex and mixed-age flocks consist of both related and unrelated individuals. There is some turnover in flock membership through the non-breeding season as birds disperse, and sometimes multiple groups come together for relatively short periods of time (Hatchwell *et al.* 2001; McGowan *et al.* 2006).

Pairs are usually formed with a member of the same flock in February or March, at which point the flock begins to break up (Hatchwell 2016). Pairs usually remain within their wintering home range and are not territorial (Lack & Lack 1958). Pairs are socially monogamous and extra-pair paternity within broods is low (~10%; Green & Hatchwell 2018). However, pairs divorce frequently both within and between season…. Once the pair has formed, nest building begins. Long-tailed tits build enclosed nests, the main structure of which is comprised of moss and spiders’ silk; the outside of the nest is covered in lichen and the inside is lined with up to 2,500 feathers (Fig 1.1; McGowan *et al.*, 2004).



Figure 1.1 Female incubating eggs in the nest. Long-tailed tits construct an enclosed nest made of moss and spiders’ silk covered in lichen and lined with feathers. Photo credit: Jacob Peers-Dent.

McGowan et al. (2004) found that nest building in long-tailed tits represents a relatively large investment of time and energy with their first nest taking approximately 38 days to construct. However, the amount of time taken to build was shortened to ~11 days when a new nest is built following predation of a previous nesting attempt, but these nests are usually smaller and contain fewer feathers. Nests are commonly located 2-3m from the ground, in thorny vegetation such as bramble *Rufus fruticosus*, gorse *Ulex europaeus* and holly *Ilex aquifolium*. However, some pairs build their nests much higher (up to 30m) in a tree fork or the canopy, typically in birches *Betula* spp*.* or conifers.

Long-tailed tits lay large clutches, typically 8-11 eggs, with one egg being laid per day. The clutch is incubated by the female alone but the male will feed the female on the nest (Hatchwell, Russell, *et al.* 1999). The incubation period lasts on average 15. The brood is then provisioned by both parents for 16-17 days whilst chicks remain in the nest, and after fledging the parents continue to provision offspring for approximately three weeks (Cramp & Perrins, 1993).

Long-tailed tits are single-brooded but breeding attempts frequently fail due to predation by both avian and mammalian predators (Gaston 1973; Hatchwell, Russell, *et al.* 1999). Following nest failure, long-tailed tits may go on to re-nest if there is sufficient time left within the breeding season. However, if failure occurs later in the season, long-tailed tits are very unusual among European species by exhibiting redirected helping, failed breeders assisting other pairs in provisioning of nestlings and fledglings (MacColl & Hatchwell 2002; Hatchwell *et al.* 2004). Helping is kin-selected, with helpers gaining indirect fitness benefits by preferentially directing their care towards kin (Hatchwell *et al.* 2014).

### **1.4.2 Study population**

The Rivelin Valley population of long-tailed tits has been studied intensively since 1994. The study site is a 2.5 km2 area of the Rivelin Valley, Sheffield (53˚23′ N, 1˚34′ W); the habitat is a mix of open pasture, deciduous woodland and scrub. Approximately 95% of breeders are individually identifiable, as they are ringed with a British Trust for Ornithology ring and a unique combination of two colour rings. Nestlings are ringed 11 days after hatching and any unringed immigrants to the study site are captured and ringed at the beginning of the breeding season; blood samples (5-30µl) are taken at the time of ringing for genetic analysis. Unringed individuals are assumed to be first-years breeders who have fledged from nests outside of the field site, given the high site fidelity shown by repeat breeders in the population (McGowan, Hatchwell, & Woodburn, 2003).

Each year there are on average 50 breeding pairs in the area (range: 25-72 pairs), whose breeding attempts are monitored (range: 33-114 breeding attempts per year). Nests are located through observation of breeding pairs with most nests discovered during the building phase. However, a small number of nests (*c*. 5%) are not found each year but these undetected nesting attempts usually fail early in breeding (Sharp *et al.* 2008). Predation accounts for 72% of nest failure (Gullett *et al.* 2014), and if there is sufficient time within the breeding season pairs will attempt to re-nest, but if nest failure occurs after the end of April or early May then pairs abandon breeding for the year (MacColl & Hatchwell 2002). These potential re-nests are intensively searched for.

Long-tailed tit nests are generally accessible, although approximately 10% of nests are located above 5m and are therefore inaccessible (Gullett *et al.* 2014). The status of the nest is checked at least every other day until the nest has failed or the nestlings have fledged, with nests being checked every day around the time of hatching. For accessible nests, the contents of the nest are checked, to provide accurate reproductive parameters including: lay date, clutch size, fledging date and failure date (accurate to ± 1 day). For inaccessible nests, we gain the same reproductive measures through observation of the breeding pair’s behaviours (accurate to ± 2 days). Once inaccessible nests have fledged, we endeavour to ring and take blood samples from the fledglings while they remain close to their natal nest. Further methodological details relevant to specific aims are described in the relevant chapters.

### **1.4.3 Aims and thesis structure**

The overall aim of this thesis was to investigate the effects of variation in environmental conditions, including biotic and abiotic factors, on the placement and structure of nests, and on incubation behaviour and the duration of incubation periods. A key objective was to determine whether long-tailed tits were able to buffer these adverse environmental conditions. In chapter two of my thesis, I investigated nest placement, focusing on the height of nests above ground. First, I examined how nest height influenced the probability of predation and predator identity. I then investigated individual repeatability and heritability of nest sites, and finally how the risk of predation and the personal experience of individuals influenced nest site selection. In chapter three, I investigated the effects of environmental conditions and other factors, such as timing of breeding and clutch size, on the duration of incubation and hatching success. I also determined how variation in incubation periods attributable to these factors affected the predation risk to which nests were exposed. Chapter four describes experiments that I conducted under controlled conditions in the lab to investigate the ability of nests to buffer the potentially adverse effects of variation in ambient temperature and rainfall on nest contents. In chapter five, the final data chapter, I examined how female incubation behaviour was affected by changes in environmental conditions. I also examined how behavioural decisions made during nest building and incubation affected breeding success, in terms of the duration of incubation and hatching success. Finally, in chapter six, I synthesise the results of these data chapters to address the question of whether long-tailed tits are able to buffer variation in environmental conditions in order to ameliorate their impact on reproduction.

# **Chapter 2.**

# **Genetic and environmental determinants of nest placement**

**Summary**

The high rates of nest predation experienced by many bird species makes choice of a ‘safe’ nesting site critical to breeding success, and therefore under strong selection pressure. Nest height from the ground influences predation rate in a number of bird species, with individuals being shown to alter their nesting height following predation. However, in short-lived species, individuals have little chance to gain experience of predation events, so nest height selection would be expected to have a heritable component. Here, we investigate the repeatability and heritability of nest sites in long-tailed tits (*Aegithalos caudatus*), along with the effects of breeding density, bird’s individual experience of predation and the density of predators in a given year on nest height. First, we found that although nest height affected breeding success, there was only weak evidence that this was a repeatable or heritable trait. Nest height was not significantly related to the breeder’s age, nor did it differ significantly between years. Nest height was also not significantly associated with breeding density, indicating that nest site availability was not a limiting factor. Within the same breeding season, individuals did not change their nesting height in response to the type of predator that destroyed an earlier nest, and across years there was no indication that the relative predator density influenced nest height. We conclude that nest height in long-tailed tits is a relatively plastic trait, with very low heritability. This is probably due to the high likelihood of predation at all heights and variability in nest predator abundance, which together results in no optimum nest height, and hence no consistent selection in favour of a particular nest height.

## **2.1 Introduction**

Nest site selection by birds is often critical to breeding success (Martin 1995; Weidinger 2004; Frevola & Walters 2016). The quality of a nest site is a function of several environmental factors, including predation risk (Martin 1988; Amat & Masero 2004; Forstmeier & Weiss 2004; Eggers *et al.* 2006), microclimate (Wiebe & Martin 1998) and competition (Deng & Gao 2005; Robertson & Rendell 1990; for reviews see Mainwaring, Hartley, Lambrechts, & Deeming 2014). Choosing a safe nest site is likely to be especially important among small passerines because predation is the primary cause of nest failure (Ricklefs 1969; Martin 1995), even if choice of safer nest sites must be traded off against other considerations, such as a poorer microclimate (Forstmeier & Weiss 2004; Eggers *et al.* 2006). Nests and nest site characteristics have been shown to influence nesting success, including the height, crypsis and structure of nests, use of cavities and protective nesting associations (Collias & Collias 1984). Thus, the variation in nest placement between and within species (Hansell 2000) is likely to be determined, at least in part, by the probability of predation and the type of predators that species experience.

The height at which nests are built influences predation rate in a number of bird species (Nilsson 1984; Hatchwell, Russell, *et al.* 1999; Forstmeier & Weiss 2004; Colombelli-Négrel & Kleindorfer 2009; Beckmann & McDonald 2016) and may also determine the type of predator a nest is most vulnerable to. For example, high nests are particularly vulnerable to avian predators, whilst lower nests are more frequently depredated by mammals (Söderström *et al.* 1998). Therefore, the optimum nest height will vary according to the identity of nest predators (Forstmeier & Weiss 2004; Eggers *et al.* 2006).

Assuming that birds are able to assess predator abundance directly (Forstmeier & Weiss 2004), breeders should alter their nest site characteristics and/or their investment in reproduction according to perceived predation risk (Eggers *et al.* 2006; Fontaine & Martin 2006; Ghalambor *et al.* 2013). If predator abundance is difficult to assess before breeding, individuals may alter their nest site preferences only after they have gained first-hand experience or public information about nest predation risk (Marzluff 1988; Martin & Martin 2001; Fontaine & Martin 2006; Peluc *et al.* 2008). For example, individuals may adopt a ‘win-stay, lose-switch’ strategy, changing nest placement following predation of previous nesting attempts, as observed in Brewer’s sparrows *Spizella breweri* (Chalfoun & Martin 2010), pinyon jays *Gymnorhinus cyanocephalus* (Marzluff 1988), northern cardinals *Cardinalis cardinalis* (Kearns & Rodewald 2013) and great grey shrikes *Lanius excubitor* (Antczak *et al.* 2005).

Learning about nest site placement from personal experience may be costly, especially in species that have a limited number of breeding attempts and hence few opportunities for learning. Therefore, given that this extended phenotypic trait is likely to be associated with fitness and hence under selection, it is also likely to have a heritable component. Nest site selection and nesting behaviours have been shown to be heritable in non-avian taxa, e.g. painted turtles *Chrysemys picta* (McGaugh *et al.* 2010), natricine snakes *Tropidonophis mairii* (Brown & Shine, 2007), oldfield mice *Peromyscus polionotus* and deer mice *P. maniculatus* (Weber, Peterson, & Hoekstra, 2013). Elements of nest placement and construction in some bird species, such as colony size (Serrano & Tella 2007; Roche *et al.* 2011) and nest size (Møller 2006; Jarvinen *et al.* 2017) have been shown to be heritable. However, the height of nest placement has received very little comparable attention. The only previous study to have investigated the heritability of nest height in passerines found no evidence of heritability (Yeh *et al.* 2007), although this may be due to constraints on the availability of preferred nest heights within the study area.

The aim of this study was to use data from a long-term (25-years) study to investigate nest placement in long-tailed tits. Long-tailed tits form socially monogamous breeding pairs and together construct their nests. They invest a substantial amount of time and energy in nest building with the first nest of the season taking pairs an average of 38 days to build (McGowan *et al.* 2004). They construct dome nests, mainly consisting of moss, lichen, spiders’ silk and feathers. Like many passerine species the primary cause of nest failure is predation, accounting for 72% of nest failures (Gullett *et al.* 2014). Following nest predation, individuals frequently attempt to breed again provided it is not too late in the breeding season to do so (MacColl & Hatchwell 2002). Within our study site, corvids (Eurasian jays *Garrulus glandarius*, Eurasian magpies *Pica pica* and carrion crows *Corvus corone*) are the main nest predators, accounting for an average of 68.4% of all identified nest predators. Mammalian predators observed depredating long-tailed tit nests in our study site include weasels *Mustela nivalis*, stoats *Mustela ermine* and wood mice *Apodemus sylvaticus*, and grey squirrels *Sciurus carolinensis*. Previous work on long-tailed tits showed that nests at heights > 2m are significantly more likely to be depredated than those nesting < 2m from the ground (Hatchwell, Russell, *et al.* 1999), presumably exerting a significant selection pressure on long-tailed tits to place their nests low down. Nest vulnerability to different predator types is likely to vary consistently with height from the ground, e.g. high nests are unlikely to be depredated by small mammals and are more likely to be depredated by corvids. However, the relative rate of nest predation by the two categories of nest predator varies between years, so a degree of behavioural plasticity and an effect of breeding experience on choice of nest height would be expected. Indeed, there is very substantial and persistent variation within and between years in the height at which nests are placed.

We began by confirming previous work showing that the probability of breeding success is related to nest height in long-tailed tits. We then tested whether nest height influenced predator type, expecting high nests to be more vulnerable to avian predators, and low nests more vulnerable to mammalian predators. Also, we investigated whether nest height is a repeatable trait in long-tailed tits. Observations suggest that pairs choose a nest site together, but breeders often change partners in successive years, and even within years (Hatchwell *et al.* 2000) so we examined the repeatability of nest placement within pairs and within each sex individually. We then used an animal model approach (Kruuk 2004) to examine whether nest height was a heritable trait. Finally, we investigated the effects of breeding density and experience of predation on subsequent nest placement. Increased breeding density was predicted to force individuals to nest in undesirable locations more frequently, while personal experience of predation was predicted to affect birds’ choice of nest height in a subsequent breeding attempt. More specifically, following predation by mammalian predators pairs were predicted to nest higher, whilst those breeders whose nests were depredated by corvids were expected to nest lower.

## **2.2 Methods**

### **2.2.1 Study system**

A population of long-tailed tits was studied from 1994 to 2018 in the Rivelin Valley, Sheffield (53˚23′ N, 1˚34′ W). Each year 33 -114 breeding attempts by 25-72 pairs of long-tailed tits were monitored. Long-tailed tits form non-territorial, socially monogamous pairs each breeding season, but between breeding attempts divorce is frequent both within and between years (Hatchwell *et al.* 2000). More than 95% of birds in the study population were ringed under licence with a unique colour-ring combination and BTO ring; when ringed a blood sample (5-30µl) was taken from the brachial vein under licence for genetic analysis. Given the high site fidelity following their first breeding year, any unringed adults caught during the breeding season were assumed to be first-year breeders that have dispersed from outside the field site (McGowan *et al.* 2003). Nestlings were ringed under licence 11 days after hatching with a unique colour-ring combination and a BTO ring and a blood sample was taken.

The study site comprises 2.5 km2 of deciduous woodland, hedgerows, farmland, scrub, private gardens and a golf course. Nests are mainly discovered during the building phase through observation of pairs. It is estimated that > 95% of nests within the study site are found each year with undetected nesting attempts typically failing early in the breeding cycle (Sharp *et al.* 2008). Nests are constructed by both the breeding male and female, and for each breeding attempt a new nest was constructed. Once found, nests were checked every two days to gain accurate measures of first egg date, and either fledging or failure date. For inaccessible nests (> 5m) these dates were estimated by observation of the breeding pair’s behaviour. In cases where the nest and/or its contents were destroyed by a predator we were able to confidently establish the predator type in a substantial number of cases (54%) by the amount of damage to the nest; when a corvid was the predator the top of the nest was usually ripped off, whereas when the predator was a small mammal they usually entered the nest through the entrance hole or made another small hole in the nest (Hatchwell, Russell, *et al.* 1999).

The nest height and the type of vegetation that the nest was located in were recorded for each breeding attempt. Nest height was measured using a simple triangulation method, and nest height ranged from 0.3m to 30m (mean = 3.63m, N *=* 1137). For a number of years (1998-2011, inclusive) nest height was recorded as one of three categories (< 2m, 2 - 5m, > 5m). In the remaining years of the study, nest height was recorded in much finer-scale categories; nests < 2m were recorded to the nearest 0.1m and nests heights > 2m were recorded to the nearest 0.5m. Nests were found in a variety of vegetation types, most frequently bramble *Rufus fruticosus* (27.6%), gorse *Ulex europaeus* (15.9%), holly *Ilex aquifolium* (15.3%), birches *Betula* spp*.* (11.1%) and conifers (9.7%; for a complete list see Table S1.1). We categorised nest vegetation into two categories - thorny (those with thorns or spikes on their stems or leaves) and non-thorny – with the former providing more of a deterrent to predators than the latter. We found nest height and vegetation type were closely linked, with just 11% (N = 1081) of nests in the low height category in non-thorny vegetation, and 10% (N = 406) of medium and high nests in thorny vegetation. In addition, we found that for the nests in the low nest category there was no significant difference in the likelihood of predation between the thorny and non-thorny nest categories in the low nest height category (χ2 = 0.41, df = 1, *P* = 0.52, N = 1081). Therefore, we consider only nest height in our analyses.

### **2.2.2 Molecular analyses and pedigree construction**

Genomic DNA was extracted from all blood samples and individuals were genotyped at 19 autosomal microsatellite loci (Simeoni *et al.* 2007; Adams *et al.* 2015). In addition, individuals were sexed using two sex-typing markers, the *P2D-P8* and *Z-002A* markers (Griffiths *et al.* 1998; Dawson *et al.* 2010). Long-tailed tits have a fairly low rate of promiscuity, with around 10% of all offspring being the product of extrapair paternity (Green & Hatchwell 2018), but no intraspecific brood parasitism (Hatchwell *et al.* 2002). To determine the genetic sire of individuals we used a likelihood approach performed in CERVUS version 3.0.7 to determine whether the social male was the genetic father and to identify full-siblings (Marshall *et al.* 1998; Kalinowski *et al.* 2007; Green & Hatchwell 2018). All adult males present in the population in an individual’s birth year were considered as a possible father and each year was analysed separately. For each year, simulations were run with 10000 offspring, assuming that 95% of candidate fathers were sampled, 96.7% of loci were sampled (calculated from allele frequencies), and that 1.88% of loci were mistyped (calculated by comparing mother-offspring genotypes). The genetic father was assigned by reference to the delta scores calculated by CERVUS, where the social father or an alternative male achieved 95% confidence and had < 2 allelic mismatches they were assigned as the genetic father. For nestlings where the social male or an alternative male failed to reach this threshold they were classified as genetic father unknown.

Occasionally, unringed yearlings that appeared to be immigrants were offspring fledged within the study site from inaccessible nests. To address this problem, we used SPAGeDi version 1.5 (Hardy & Vekemans 2002) to calculate the relatedness of unringed yearlings to females that were known to have fledged unringed broods in the previous year using the Queller & Goodnight (1989) method. This allowed us to assign nine additional individuals to a mother. We then used CERVUS to assign paternity, as above, identifying the sire of all but one individual.

### **2.2.3 Statistical analysis**

All statistical analyses were conducted in R 3.5.3 (R Core Team 2018).

*2.2.3.1 Does nest height affect the likelihood that a nest will be depredated?*

To determine whether broad-scale nest height categories experienced differential predation and different predator types (mammals or corvids), we used a Pearson’s chi-squared test in the *stats* package (R Core Team 2018). We also constructed generalised linear models using the glm function (R Core Team 2018) to examine the effect of fine-scale nest height categories on predation probability and the likelihood of predation by different predator types.

*2.2.3.2 Does breeding density affect nest height?*

To investigate the relationship between nest height and breeding density, we used the number of nests found in each year as a proxy for breeding density and built two models with nest height as the response variable. One model was a linear regression model where nest height was a continuous variable using the lm function in the *stats* package (R Core Team 2018). In the other, broad-scale nest height categories were used in a cumulative link model using the *ordinal* package (Christensen 2019). The number of nests built within the same year, first egg date and an interaction between these two terms were the explanatory variables in both models.

*2.2.3.3 Which sex decides on nest location?*

We estimated the repeatability of individual (broad-scale analyses: N = 571 males,, N = 592 females; fine-scale analyses: N = 272 males, N = 282 females) and pair (broad-scale analysis: N = 843; fine-scale analysis: N = 426) nest height choices using Bayesian generalised linear mixed-effects models in the *MCMCglmm* package (Hadfield 2010). We ran separate models for each group (male, female or pair) to avoid pseudoreplication of nest attempts. These models included only identity as a random effect and did not include any fixed effects. Given the skew of fine-scale nest heights, this variable was log-transformed to normalise distribution. Repeatability was estimated as the variance explained by either male, female or pair identity divided by the total variance (Lessells & Boag, 1987). The models of broad-scale nest height category were multinomial generalised linear mixed-model, so 1 was added to the total variance for the probit link function (Nakagawa & Schielzeth 2010).

*2.2.3.4 Is nest height a heritable trait?*

To analyse the heritability of nest height, we constructed mixed-effects animal models that used pedigree-derived estimates of relatedness to estimate additive genetic covariance using the *MCMCglmm* package (Hadfield 2010). Multinomial mixed-effects models were constructed for analysis of broad-scale nest height categories using the ordinal family. For log-transformed fine-scale nest height values linear mixed-effect models using the gaussian family were constructed (for further details of model specification and implementation see Appendix S1.2). Nest height was modelled as a function of the first egg date (relative to 1st March) and age of the individual as fixed effects. In addition to the genetic pedigree derived relatedness estimate, we included year, pair ID, natal nest ID, dam and social sire as random effects. Most female breeders in our study population are immigrants, so the natal nest was known for both individuals in a pair infrequently; therefore, we tested each sex separately. In addition, we partitioned within individual variation in nest location into additive genetic effects and permanent environment effects by using individual ID as a random factor (Kruuk 2004; Wilson *et al.* 2010). Permanent environment effects are any that contribute to permanent differences between individuals. These could include non-additive genetic effects, such as dominance, epistasis, or early environmental effects (Kruuk 2004). We calculated the heritability estimate as the sex-specific additive genetic variance over the total phenotypic variance. For broad-scale nest height analysis 1 was added to the total phenotypic variance due to the probit link function (Nakagawa & Schielzeth 2010).

*2.2.3.5 How does prior experience of breeding adults of different types of nest predators affect nest placement?*

Finally, to investigate whether prior experience altered the birds’ choices of location we analysed the change in nest height between the first and second nesting attempt within the same year following nest predation. We used the change in nest height as the independent variable which was categorised as either: ‘higher’, nesting within a higher nest height category, ‘lower’, when moving to a lower nest height category, or ‘same’, when they remained in the same height category. Using cumulative link models in *ordinal* package (Christensen 2019) we tested whether the changes in nest height were correlated with the previous predator type and the birds’ age, whilst controlling for the previous nest height category. In addition, we tried to account for relative density of each predator type in that year, by calculating the ratio of corvid predations to the total number of predation events where the predator type was identified for each year. This value was also included in the models as a fixed effect. As breeding pairs frequently divorce between nesting attempts within the same year, we ran the models separately for males (N = 121), females (N = 106) and pairs (N = 95) to prevent pseudoreplication of data. We also completed a linear regression with a subset of data using the actual change in nest heights (N = 67 females; N = 75 males; N = 57 pairs); this model contained the same explanatory variables as the previous analysis.

## **2.3 Results**

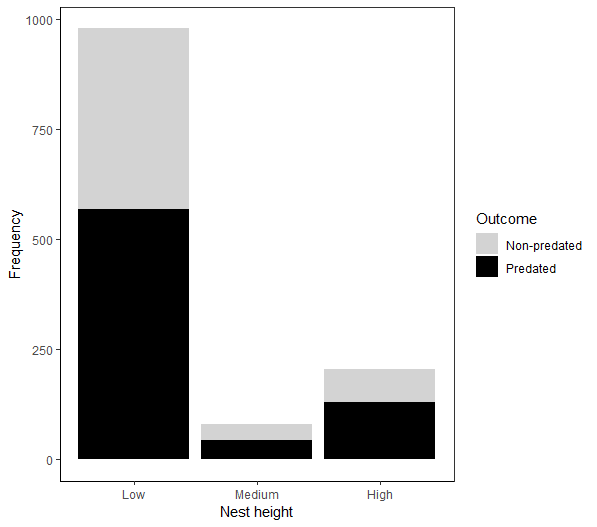
### **2.3.1 Does nest height affect the likelihood that a nest will be depredated?**

During the period of study, 79% of breeding attempts failed (N = 1613), and of these 74% failed due to predation. For depredated nesting attempts where the predator was identified, we found that 68.4% of nest were depredated by corvids (N = 510), the rest being depredated by mammals (31.6%). Nest height varied greatly from 0.3 to 30m but the distribution was highly skewed (mean ± SD = 3.75 ± 4.82m, median = 1.50 m). This meant that in terms of the broad scale nest height categories there were many more low nests than high nests, although there were also almost three times as many high nests than nests in the intermediate nest height category (low nests: N = 1109; medium nests: N = 106; high nests: N = 301).

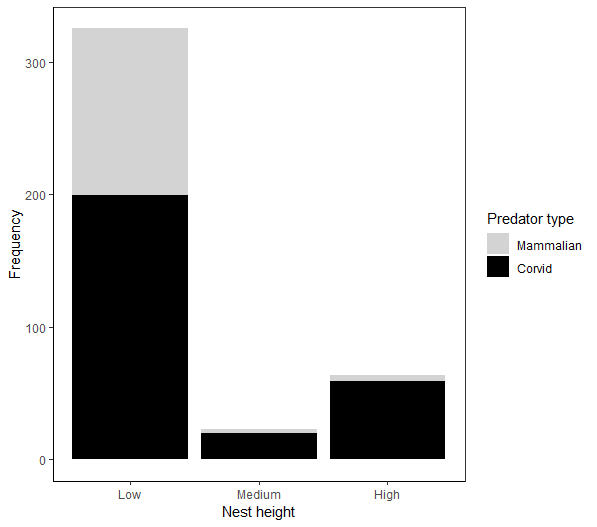
Low and medium nests were significantly less likely to be predated than higher nests (χ2 = 10.6, df = 2, *P* < 0.05, N = 1462, Fig 2.1). However, medium and high nests (>2m) were significantly less likely to be predated by small mammals (χ2 = 32.4, df = 2, *P* < 0.05, N = 478, Fig 2.2). Using the fine-scale nest height data, nest height was significantly associated with predation (estimate ± SD = 0.07 ± 0.02, *P* < 0.001) and predator type (estimate ± SD = 0.22 ± 0.06, *P* <0.001) in the same directions as for broad-scale categories.

### **2.3.2 Does breeding density affect nest height?**

Breeding density, measured as the number of nests built in a year (which is closely correlated with population size), was not significantly correlated with nest height (fine-scale nest height linear regression: estimate ± SD = 0.009 ± 0.03, *P* = 0.78; broad-scale nest height cumulative link regression: estimate ± SD = 0.02 ± 0.02, *P* = 0.23). First egg

****

**Figure 2.1.** Frequency of nesting heights from the ground of long-tailed tits in the three height categories (low = < 2m, medium = 2 – 5m, high = > 5m). The frequency of predation of nests within these height categories is indicated by the black bars.

****

**Figure 2.2.** Frequency of long-tailed tit nest predation by a known nest predator in the three height categories (low = < 2m, medium = 2 – 5m, high = > 5m). The frequency of predation by corvids within these height categories is indicated by the black bars, and the frequency of mammalian predation is shown by the grey bars.

date was correlated with broad-scale nest height categories (estimate ± SD = 0.06 ± 0.03, *P* < 0.05), with more nests falling into the higher category later in the season. However, there was no correlation between date and fine-scale nest height (estimate ± SD = 0.04 ± 0.06, *P* = 0.45). In neither model was there a significant interaction between the number of nests built in a year and the first egg date (fine-scale nest height linear regression: estimate ± SD = 0.0004 ± 0.0008, *P* = 0.64; broad-scale nest height cumulative link regression: estimate ± SD = -0.0002 ± 0.0003, *P* = 0.51), indicating that any seasonal change in nest height was not related to breeding density.

### **2.3.3 Which sex decides on nest location?**

There was a low repeatability of fine-scale nest height in both sexes and in pairs, although all lower bounds of the confidence intervals were close to zero (pairs: *r* [95% CI] = 0.07 [0.0002, 0.20]; males: *r* [95% CI] = 0.09 [0.0002, 0.18]; females: *r* [95% CI] = 0.11 [0.0004, 0.22]). However, we show that repeatability of nest height category did not differ from zero in either sex (males: 95% CI = 0, 0.19; females: 95% CI = 0, 0.20), nor for pairs (95% CI = 0, 0.23). Thus, there is no strong evidence in favour of either sex being the principal driver of nest placement, and nor is it a repeatable pair-level trait.

### **2.3.4 Is nest height a heritable trait?**

The heritability estimates for fine-scale nest heights were low, but significant, in both sexes although the lower bounds of the confidence intervals were close to zero (Tables 2.1 and 2.2). In contrast, the heritability estimates for broad-scale nest height did not differ significantly from zero (Tables 2.3 and 2.4). Permanent environmental effects and pair ID explained a negligible amount of the variation in nest height (Tables 2.1, 2.2, 2.3 and 2.4). Natal nest ID, dam ID and social sire explained only small amounts of variation

**Table 2.1.** Posterior modes, 95% confidence intervals (CI) and effective sample sizes of the complete animal model fitting the fine-scale nesting height categories in females long-tailed tits.

|  |  |  |  |
| --- | --- | --- | --- |
| **Fixed effects** | **Posterior mode** | **CI** | **Effective sample size** |
| Intercept | 0.18 | -0.62; 1.34 | 2000 |
| First egg date | 0.014 | -0.005; 0.035 | 2000 |
| Age | -0.12 | -0.32; 0.019 | 2000 |
| **Random effects** |  |  |  |
| VA | 0.002 | 0.0002; 0.16 | 2000 |
| VPE | 0.002 | 0.0002; 0.15 | 1906 |
| VPair ID | 0.002 | 0.0002; 0.14 | 2000 |
| VNatal nest ID | 0.001 | 0.0002; 0.13 | 2000 |
| VYear | 0.002 | 0.0002; 0.16 | 2000 |
| VSocial Sire | 0.001 | 0.0002; 0.14 | 2000 |
| VDam | 0.001 | 0.0001; 0.14 | 2000 |
| **Derived estimates** |  |  |  |
| *h2* | 0.002 | 0.0001; 0.15 | 2000 |

VA, additive genetic variance;VPE, variance associated with permenant experimental effects;VPair ID, variance associated with pair;VNatal nest ID, variance associated with natal nest; VYear ,variance associated to the year; VSocial Sire, variance associated with the social sire; VDam variance associated with year of breeding nest; *h2*, heritability estimate.

**Table 2.2.** Posterior modes, 95% confidence intervals (CI) and effective sample sizes of the complete animal model fitting the fine-scale nesting height categories in males long-tailed tits.

|  |  |  |  |
| --- | --- | --- | --- |
| **Fixed effects** | **Posterior mode** | **CI (95%)** | **Effective sample size** |
| Intercept | -0.32 | -1.05; 0.26 | 2000 |
| First egg date | 0.02 | 0.01; 0.04 | 2000 |
| Age | 0.01 | -0.19; 0.13 | 2000 |
| **Random effects** |  |  |  |
| VA | 0.002 | 0.0002; 0.22 | 2167 |
| VPE | 0.002 | 0.0003; 0.18 | 2000 |
| VPair ID | 0.003 | 0.0003; 0.30 | 2000 |
| VNatal nest ID | 0.003 | 0.0002; 0.24 | 2141 |
| VYear | 0.002 | 0.0002; 0.07 | 2000 |
| VSocial Sire | 0.002 | 0.0002; 0.24 | 2000 |
| VDam | 0.002 | 0.0002; 0.22 | 2000 |
| **Derived estimates** |  |  |  |
| *h2* | 0.002 | 0.0002; 0.20 | 1816 |

VA, additive genetic variance;VPE, variance associated with permenant experimental effects;VPair ID, variance associated with pair;VNatal nest ID, variance associated with natal nest; VYear ,variance associated to the year; VSocial Sire, variance associated with the social sire; VDam variance associated with year of breeding nest; *h2*, heritability estimate.

**Table 2.3.** Posterior modes, 95% confidence intervals (CI) and effective sample sizes of the complete animal model fitting the broad-scale nesting height categories in females long-tailed tits.

VA, additive genetic variance;VPE, variance associated with permenant experimental effects;VPair ID, variance associated with pair;VNatal nest ID, variance associated with natal nest; VYear ,variance associated to the year; VSocial Sire, variance associated with the social sire; VDam variance associated with year of breeding nest; *h2*, heritability estimate.

|  |  |  |  |
| --- | --- | --- | --- |
| **Fixed effects** | **Posterior mode** | **CI (95%)** | **Effective sample size** |
| Intercept | -4.17 | -6.14; -1.40 | 2000 |
| First egg date | 0.037 | -0.003; 0.08 | 2000 |
| Age | -0.35 | -0.94; 0.14 | 2000 |
| **Random effects** |  |  |  |
| VA | 0.014 | 0; 2.49 | 2000 |
| VPE | 0.008 | 0; 2.13 | 2000 |
| VPair ID | 0.017 | 0; 2.29 | 2000 |
| VNatal nest ID | 0.003 | 0; 1.71 | 2000 |
| VYear | 0.020 | 0; 3.21 | 2000 |
| VSocial Sire | 0.013 | 0; 1.69 | 2188 |
| VDam | 0.010 | 0; 1.90 | 2000 |
| **Derived estimates** |  |  |  |
| *h2* | 0.002 | 0; 0.40 | 2000 |

|  |  |  |  |
| --- | --- | --- | --- |
| **Fixed effects** | **Posterior mode** | **CI (95%)** | **Effective sample size** |
| Intercept | -5.12 | -9.46; -3.73 | 2000 |
| First egg date | 0.07 | 0.03; 0.12 | 2000 |
| Age | -0.08 | -0.57; 0.34 | 2000 |
| **Random effects** |  |  |  |
| VA | 0.022 | 0; 3.68 | 2000 |
| VPE | 0.008 | 0; 2.29 | 2000 |
| VPair ID | 2.15 | 0; 7.27 | 2000 |
| VNatal nest ID | 0.017 | 0; 3.37 | 2000 |
| VYear | 0.90 | 0; 4.10 | 2000 |
| VSocial Sire | 0.008 | 0; 2.06 | 2000 |
| VDam | 0.018 | 0; 2.50 | 2000 |
| **Derived estimates** |  |  |  |
| *h2* | 0.002 | 0; 0.34 | 2000 |

**Table 2.4.** Posterior modes, 95% confidence intervals (CI) and effective sample sizes of the complete animal model fitting the broad-scale nesting height categories in males long-tailed tits.

VA, additive genetic variance;VPE, variance associated with permenant experimental effects;VPair ID, variance associated with pair;VNatal nest ID, variance associated with natal nest; VYear ,variance associated to the year; VSocial Sire, variance associated with the social sire; VDam variance associated with year of breeding nest; *h2*, heritability estimate.

in nest height, indicating no effect of early experience or ‘natal nest imprinting’ on future nest placement (Tables 2.1, 2.2, 2.3 and 2.4). The distribution of nest heights did not differ significantly between years, but within years there was a significant increase in nest height, although this trend was apparent only in males (Tables 2.1, 2.2, 2.3 and 2.4). Finally, individuals tended to reduce the height of their nests with age, but this trend was not significant in either sex (Tables 2.1, 2.2, 2.3 and 2.4).

### **2.3.5 How does prior experience of breeding adults of different types of nest predators affect nest placement?**

Within the same breeding season, there was no evidence that individuals changed their nest height in a predictable direction following experience of nest predation (Table 2.5). We established that the previous predator type had no significant effect on the height category of the subsequent nest (female: estimate ± SD = -0.36 ± 0.48, *P* = 0.45; male: estimate ± SD = 0.034 ± 0.46, *P* = 0.99; pair: estimate ± SD = -0.29 ± 0.51, *P* = 0.57). The percentage of corvid predation varied substantially between years, from 39% to 100% (mean ± SD = 70.3% ± 17.7%). However, this variation was not related to the nest height category of second nesting attempts (female: estimate ± SD = 2.00 ± 1.46, *P* = 0.17; male: estimate ± SD = 0.006 ± 1.33, *P* = 0.99; pair: estimate ± SD = -2.18 ± 1.51, *P* = 0.15; Table 2.5). Nor did the age of the individual have a significant effect on changes in nest height (female: estimate ± SD = -0.12 ± 0.17, *P* = 0.48; male: estimate ± SD = -0.24 ± 0.18, *P* = 0.19).

As in the broad-scale analysis, previous nest predation experience had no significant effect on fine-scale height of subsequent nests (female: estimate ± SD = 1.59 ± 2.28, *P* = 0.49; male: estimate ± SD = 3.63 ± 1.96, *P* = 0.07; pair: estimate ± SD = 1.62 ± 2.45, *P* = **Table 2.5.** The change in nest height for male long-tailed tits given different predator types and previous nest height category.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Previous height category | | |
| Change in Height | Predator type | Low | Medium | High |
| Lower | Mammal | - | 0 | 1 |
|  | Corvid | - | 3 (37.5%) | 11 (55%) |
| Same | Mammal | 20 (59%) | 0 | 0 |
|  | Corvid | 36 (62%) | 2 (25%) | 9 (45%) |
| Higher | Mammal | 14 (41%) | 0 | - |
|  | Corvid | 22 (38%) | 3 (37.5%) | - |

0.51). Nor did the ratio of corvid predators to mammalian predator affect the nest height category of second nesting attempts (female: estimate ± SD = -2.75 ± 7.33, *P* = 0.71; male: estimate ± SD = 4.08 ± 6.04, *P* = 0.50; pair: estimate ± SD = -2.08 ± 7.79, *P* = 0.79). The age of the individual also had no significant effect on the change in nest height (female: estimate ± SD = -0.58 ± 0.71, *P* = 0.42; male: estimate ± SD = -0.93 ± 0.75, *P* = 0.22).

## **2.4 Discussion**

The height at which long-tailed tit nests are built affects breeding success, a pattern that has held throughout this long-term study (Hatchwell, Russell, *et al.* 1999). Therefore, it was expected that breeders would be under selection pressure to place their nests lower because they are more likely to be successful. However, we found only weak evidence that nest height was a repeatable or heritable trait. Fine-scale nest height was repeatable with very low heritability, but analysis of the larger sample of broadly categorised nest heights revealed no such effect. Nest height was not significantly related to the age of individual breeders and nor did it differ significantly between years. Within the same breeding season, individuals did not change their nesting height in response to the type of predator that depredated eggs or chicks, and nor was there an effect of relative predator density on nest heights within years. Finally, breeding density did not appear to influence nest height, indicating that preferred nest sites were not limiting.

Nest height is an extended phenotypic trait that appears to have very low heritability. Behavioural traits linked to reproduction are often thought to have a high degree of phenotypic plasticity to allow for adaptation to changing environmental conditions, which is thought to explain partially low to moderate estimates of heritability for these traits (mean = 0.31, median = 0.23; Stirling, Réale, & Roff 2002). Our estimates of heritability are substantially lower than this average. McGaugh *et al*. (2010) also show a very low heritability of nesting site vegetation cover in painted turtles (*h2*= 0.043). Natricine snakes showed a moderate correlation between nest site location between mothers and daughters (*r2* = 0.24; Brown & Shine, 2007), although this study was unable to separate genetic and non-genetic mechanisms (e.g. natal imprinting).

We also found no indication of imprinting because the location of an individual’s natal nest did not appear to influence placement of their own nests. These findings are in line with the only other previous study of nest height heritability, in dark-eyed juncos *Junco hyemalis* (Yeh *et al.* 2007), which established that nest height was not a heritable trait and that the height of a daughter’s nest was not significantly related to natal nest height. In juncos, it is likely that there was a limited choice of their preferred high nesting sites, but we think that such constraints are unlikely to operate in our study. First, the diversity of nest sites used by long-tailed tits in terms of height and supporting vegetation, coupled with the absence of territoriality except in close proximity to nests (Hatchwell, Russell, *et al.* 1999), means that there are no obvious limits in terms of nest site availability across our study site, except in open fields and grassland. Secondly, we find no evidence of competition for nest sites because the frequency of high nests did not covary with the number of nests built in a given year.

In our study, heritability analyses for males and females were based on large samples of individuals, but since both sexes contribute to nest-building in long-tailed tits and observations suggest that nest placement is a joint decision, it may be more appropriate to view nest height as a joint phenotype of the breeding pair. However, pairs are relatively short-lived in long-tailed tits due to mortality or divorce (Hatchwell *et al.* 2000) and the majority of females were immigrants into the population with their natal nest being unknown, giving us a relatively small sample of pairs in which the pedigree of both sexes was known. Nevertheless, we did account for pair identity in our analyses and it appeared to have low explanatory power, with repeatability using pair identity lower than within each sex. Furthermore, our results do not allow us to conclude whether one sex is more important than the other in deciding nest placement because repeatabilities and heritability estimates were not consistently higher for one sex over the other. Indeed, all heritability estimates had lower confidence intervals that were close to zero in the fine-scale categories or were zero in the broad-scale categories, which indicates that pair identity and the possibility that nest site is a joint phenotype would not have influenced our results substantially. Therefore, overall, we suggest that despite the selection pressure exerted by nest predators, nest height is, at most, a very weakly heritable trait in long-tailed tits.

Breeding performance has been found to improve age(Forslund & Part 1995). One of the reasons for this, may be a general improvement in skills, such as foraging, chick provisioning, nest building or nest placement (Nol & Smith 1987). In our study, nest height was not significantly associated with the age of individuals, although there was a general trend in both sexes that older individuals nested lower. This trend may indicate that those individuals that survive to breed in multiple breeding seasons learn that lower nesting sites are safer. However, few individuals in the population survive more than two years (the upper quartile of both males and females in this study was 2 years old), which may not give most individuals enough opportunities to acquire such experience. Seychelles warblers *Acrocephalus sechellensis* were shown to alter their nest site selection based upon age and experience from successful breeding attempts (Komdeur 1996). We investigated whether individuals were able to improve with age and experience of nest failure and found little evidence of learning. Further investigation should concentrate on the effects of breeding success on nest site selection.

The substantial difference in the ratio of corvid to mammalian predators between years may indicate that the most favourable nesting height change between years according to the predator assemblage (Forstmeier & Weiss 2004). If this was the case we would expect long-tailed tits to adjust their nest height following experience of nest predation and/or have some mechanisms to assess relative predator density (Marzluff 1988). Other bird species change their behaviour in response to nest predator presentations or playback of calls (Eggers *et al.* 2006; Emmering & Schmidt 2011; Zanette *et al.* 2011) and following nest predation (Marzluff 1988; Antczak *et al.* 2005; Chalfoun & Martin 2010; Kearns and Rodewald 2013). Chalfoun and Martin (2010) showed that individuals were more likely to alter their nesting height following predation. They also showed that changes in nest height were not uni-directional, which may prevent predators from learning this response and thus hinder the formation of a specific search image by the predator (Martin 1988; Bond and Kamil 1999). This may explain why long-tailed tits did not appear to adjust nest height in response to relative predator densities, and why following direct experience of predation individuals still made a seemingly arbitary choice of subsequent nest elevation.

It is important to note that while there was a significantly greater predation risk for high nests, there was also much variation between breeding seasons in the relative frequency of predation by corvids and mammals, suggesting that retaining phenotypic plasticity to adapt to current environmental conditions, including predator threats, would be the best strategy for breeding long tailed-tits. Furthermore, given the diversity of nest predators that long-tailed tits must contend with and the different search strategies they might employ, corvids being visual predators and their mammalian predators being olfactory predators, it may be that the predictability of nest predation is low and may eliminate the option of ‘safe’ nesting sites. Therefore, although we found that lower nesting sites are significantly less likely to be depredated than higher nesting sites, the high annual mortality rate of long-tailed tits (*c*.45%; McGowan *et al.* 2003; Meade & Hatchwell 2010) means that each individual breeder will gain experience of predation risk at relatively few nests. Therefore, biologically, the observed difference in predation risk with nest height may have little impact on an individual bird’s personal experience. Previous studies have also shown that when there is a great diversity in nest predators the predictability of nest predation is low and nest site selection strategies appear not to be adaptive (Filliater *et al.* 1994; Brua 1999; Dion *et al.* 2000; Emmering *et al.* 2018).

Nest height tended to increase through the breeding season for males, but not for females. Corvids are visual predators, so long-tailed tits may be more likely to switch to nest higher in the trees when there is increased vegetation cover following leaf emergence to conceal these nests better. However, this change in nesting height may not be related to predation risk *per se*. For example, northern cardinals also increase their nest height through their breeding season despite this trend not being linked to breeding success (Filliater *et al.* 1994).

We conclude that nest height in long-tailed tits is a relatively plastic trait, with low heritability. It appears that even with first-hand experience of predation they appear to randomly select the height at which to build their next nest. This strategy of maintaining the variation in nest height may prevent predators from developing a search image for their nests and hence may reduce the chances of them finding future nesting attempts. In addition, given the high probability of predation and variation in predator type it may be that selection on long-tailed tits to favour a particular nesting height is not sufficiently consistent to result in predictable decision rules about nest placement.

# **Chapter 3.**

# **Do environmental conditions affect incubation period and hatching success in a temperate passerine?**

**Summary**

The timing of breeding often has a profound influence on reproductive success in birds living in seasonal environments. Timing is typically investigated with reference to first egg date, but the timing of hatching and hence the period of peak nestling food demand will also be influenced by the length of the incubation period. Despite the incubation period being traditionally thought of as a relatively fixed trait within species we find a surprising degree of flexibility in duration of incubation. The aim of this study was to use a 25-year dataset to investigate the abiotic and biotic factors influencing incubation duration and hatching success in long-tailed tits (*Aegithalos caudatus*), a species exhibiting substantial variation in incubation duration. We also assess the consequences of variation in the length of incubation periods for the risk of nest predation. We found support for our predictions that later breeding attempts, drier conditions and larger clutches were associated with shorter incubation periods. Larger clutches were also more resilient to increases in incubation duration associated with rainy conditions. Surprisingly, warmer ambient conditions were associated with longer incubation periods. Weather conditions did not influence hatching success suggesting that adverse conditions are buffered by nests or by parental behaviour. We also show that longer incubation periods are likely to be costly, however, due to increased exposure to nest predators, and because longer incubations are associated (albeit weakly) with increased risk of hatching failure.**3.1 Introduction**

Reproductive success of birds is often dependent upon timing of breeding (Daan & Tinbergen 1997; Houston & McNamara 1999), with individuals that breed earlier in the season typically having higher fitness (Perrins 1970; Both 2010). For many birds living in seasonal environments, it is critical for reproduction to be timed to match temporally ephemeral food resources, with hatching expected to coincide with the peak of food abundance (Perrins 1970; Monros *et al.* 1998; Naef-Daenzer *et al.* 2004; Simmonds *et al.* 2017). Many studies have focused on changes to clutch initiation date (e.g. Visser *et al.* 1998; Charmantier *et al.* 2008; Schaper *et al.* 2012). However, other mechanisms for altering the timing of hatching exist. Females may adjust the length of the egg laying period by altering clutch size, or by increasing the intervals between laying (Haftorn 1981; Nilsson & Svensson 1993; Simmonds *et al.* 2017). Alternatively, the length of the incubation period could be adjusted by beginning incubation prior to clutch completion, by delaying the start of incubation (Haftorn 1981; Nord & Nilsson 2011; Álvarez & Barba 2014a; Simmonds *et al.* 2017), or by changing nest attentiveness once incubation has started (Martin *et al.* 2007; MacDonald *et al.* 2013; Coe *et al.* 2015).

Incubation is typically an energetic and time-consuming component of avian reproduction  (Tatner & Bryant, 1993; Williams, 1996; DuRant, Hopkins, Hepp & Romero 2013) due to the relatively high temperatures (34-40˚C) at which eggs must be maintained for optimal development (Webb 1987; DuRant, Hopkins, Hepp & Walters 2013). Increased investment means that incubating parents have less energy and time to spend on self-maintenance (Stearns 1989; Reznick *et al.* 2000; Zera & Harshman 2001), especially in species in which one parent is solely responsible for incubation (Deeming 2002). Consequently, incubation can reduce parental body condition (Tombre & Erikstad 1996; Hanssen *et al.* 2005) and immune function (Knowles *et al.* 2009), thereby lowering fitness by reducing adult survival (Visser & Lessells 2001) and future reproductive success (Reid *et al.* 2000a; Hanssen *et al.* 2005).

The ability of incubating parents to maintain suitable conditions may also be affected by environmental factors. For example, lower ambient temperatures can alter the nest microclimate, causing eggs to cool at a faster rate and reach lower temperatures when left unattended (Reid *et al.* 2000b). This means that an incubating parent must expend more energy re-heating cooled eggs and maintaining eggs at the optimal temperature for embryo development during incubation, potentially having negative effects on hatching success (Jarvinen 1993; Sheaffer & Malecki 1996; Skinner *et al.* 1998). Heavy rain also leads to reduced hatching success and nest failure in some passerine species (Wesołowski *et al.* 2002). In addition, rainfall increases the energy required for incubation as it increases heat loss from eggs via increased conductance and reduced insulating properties of damp nest materials (Reid *et al.* 2002; Hilton *et al.* 2004; Heenan 2013). Eggs that experience low or fluctuating temperatures not only suffer from reduced hatching success, but also from slower embryonic growth (Olson *et al.* 2006), production of poorer quality offspring (DuRant, Hopkins, Hepp, & Romero, 2013; Nord & Nilsson, 2011) and reduced long-term survival of offspring (Berntsen & Bech 2016). Thus, adverse weather conditions can have profound consequences for both the length of the incubation period and hatching success.

However, there are ways in which negative environmental impacts may be mitigated, for example, by building well-insulated nests (Deeming & Gray 2016) or changing incubation behaviour (Conway & Martin 2000b; Amininasab *et al.* 2016). Also, clutches of different sizes have different thermal properties, with larger clutches cooling more slowly but also taking longer to re-heat (Reid *et al.* 2000b; Cooper *et al.* 2005). Thus, relatively large clutches in tree swallows *Tachycineta bicolor* have shorter incubation periods (Ardia *et al.* 2006), although other studies have not found any effect of clutch size on the incubation period (Sandercock, 1997; Siikamiiki, 1995; Szekely, Karsai, & Williams, 1994; Reid *et al.*, 2000a; Wiebe & Martin, 2000). However, these studies are done at different elevations, latitudes and have different focal taxa so given this variation caution must be taken when interpreting the causal reason for this effect, or lack there of.

We investigated the effects of timing of breeding and environmental conditions on the incubation period and hatching success in the long-tailed tit *Aegithalos caudatus*. Long-tailed tits are well suited for this study because there is much natural variation in the length of time between their last egg being laid and the hatching of their eggs, henceforth referred to the incubation period (range 12 to 26 days), the causes and consequences of which are unknown. The length of the incubation period is heavily influenced by parental behaviour; as females may delay the start of incubation following clutch completion or may alter their behaviour once incubation has begun. Incubation is by females alone, who spend about 65% of daylight hours on the nest, while males occasionally bring food to incubating females (Hatchwell, Fowlie, *et al.* 1999). Long-tailed tits are cooperative breeders, but helpers aid only in the feeding of nestlings and fledglings (Gaston 1973; Hatchwell *et al.* 2004), and are not present at the nest during incubation. Breeding pairs also build complex nests that require a large investment in time and energy (McGowan *et al.* 2004). Their nests are domed with a small entrance hole, constructed of moss and fibres bound together with spiders’ silk, covered on the outside with flakes of lichen, and lined with up to 2,500 feathers (Hansell 1996; McGowan *et al.* 2004). This structure suggests that nests play an important role in protecting eggs and nestlings from predators and/or adverse environmental conditions. Moreover, nest predation rates during incubation are high (Gullett 2014), so an extension of the incubation period could significantly increase the risk of offspring predation.

In this study, we investigated incubation period, which includes the time during which female incubate their clutch of eggs and also any delay between clutch completion and the start of incubation. We first used a long-term (25 year) dataset to test whether ‘incubation’ period was associated with weather variables (temperature and precipitation), timing of breeding (clutch completion date and attempt number), clutch size and female age. We predicted that the incubation period would be longer in cold and wet weather conditions. Also, birds would try to match hatching to peak food abundance to provision nestlings with, so incubation periods should be shorter later in the season and in replacement nests. Clutch size was predicted to alter the length of the incubation period, although our prediction is two-tailed. Larger clutch sizes could increase the incubation period because more energy is required to incubate larger clutches. Alternatively, in line with the thermal inertia hypothesis, larger clutch size may decrease the length of the ‘incubation’ period because they help retain heat and therefore remain at a higher temperature during female foraging bouts. We expected that older females would be more experienced and able to incubate more efficiently.

Second, we tested whether the incubation period and environmental variables affected the viability of the embryos, measured as hatching success. We predicted that a shorter incubation period and better environmental conditions, i.e. warm and dry weather, would be associated with greater hatching success.

## **3.2 Methods**

### **3.2.1 Study system**

Long-tailed tits were studied between 1994 and 2018 in the Rivelin Valley, Sheffield (53˚23′ N, 1˚34′ W). Each year the study site contained 25-72 pairs that had 33-114 monitored breeding attempts; *c.* 95% of breeders were ringed with a British Trust for Ornithology ring and a unique combination of two colour rings. A very small proportion of nests, estimated to be < 5%, are not found in each year but these undetected nesting attempts typically fail early in the breeding cycle (Sharp *et al.* 2008). Long-tailed tits are single-brooded, but breeding attempts frequently fail prior to hatching and if there is sufficient time remaining pairs will initiate another breeding attempt (MacColl & Hatchwell 2002). Nests were routinely monitored every two days, and daily around the time of hatching, to obtain accurate reproductive parameters, i.e. first egg date, clutch size, hatching date, and either fledging or failure date.

*Measuring incubation period*

We measured incubation period as the number of days between clutch completion and hatching (Nilsson & Smith 1988; Wiebe & Martin 2000; Martin 2002; Martin *et al.* 2007; Rohwer *et al.* 2015; Bueno-Enciso *et al.* 2017). This includes the period during which females incubated eggs, and it also includes any delay in the start of incubation, which does sometimes occur in long-tailed tits. Therefore, our procedure for determining incubation period was as follows. The date the first egg of each clutch was laid (hereafter referred to as first egg date) was recorded as a Julian date, where 1st March is set as day 1. Long-tailed tits lay a single egg per day, around dawn, and we counted the number of eggs in a clutch on or after the day the 12th egg would have been laid; this is the largest clutch size (N *=* 2) recorded in the 26 years of our study. From this we calculated the Julian date of clutch completion. The assumption that females commenced incubation only after their clutch was complete is justified by observations that females do not start incubation until their last egg was laid (Glen 1985; BJ Hatchwell pers obs) and also by the observation that hatching is typically synchronous. We confirmed that short incubation periods were not a consequence of incubation starting prior to clutch completion by examining whether nestling size hierarchy was greater in broods with shorter incubation periods, which would be the expected result of early incubation and hence asynchronous hatching (Slagsvold *et al.* 1995; Stenning 2008). We calculated asynchrony as the mass of the heaviest nestling in a brood minus the mass of the lightest nestling in a brood, divided by the mean mass of the brood (Kluen *et al.* 2011). There was no significant difference in the degree of asynchrony of broods in the lower (range: 12 to 15 days) and upper (range: 17 to 26 days) quartiles of incubation period (F(1,73) = -0.03, *P* = 0.20). Hatching date was recorded by checking nests at daily intervals from the earliest expected date of hatching; given that eggs could hatch shortly after one nest-check and hence up to one day before being checked again, the hatch dates for all accessible nests were accurate to within one day.

We did not routinely record the date on which females started incubation for all nests, but we were able to obtain some estimate of how frequent delayed incubation was by noting whether the eggs were warm to the touch when eggs were counted 12 (N *=* 103), 11 (N *=* 35) or 10 (N *=* 22) days after the first egg date. For this sub-sample of breeding attempts (N *=* 160), 13 females had not begun incubation when the nest was checked. Of the remaining 147 breeding attempts we were able to calculate the maximum potential delay in starting incubation as the difference between the day the last egg was laid and the day the eggs were checked. We then compared this to the delay in hatching (number of days beyond the shortest incubation in our study) and found that 87.1% of them had a greater delay in hatching than could be accounted for by simply delaying the start of incubation. This indicates that whilst some of the longer incubation periods are due to delays in the start of incubation, most are indeed due to a prolonged incubation period. Importantly, given that any delay in the start of incubation is likely to be attributable to poor weather, any effect would be consistent with our hypothesis that adverse environmental conditions prolong the incubation period and extend the period of predation risk.

To account for inter-annual variation in the timing of breeding, for each breeding attempt we calculated relative first egg date and relative clutch completion date from the number of days between the dates of these events for a given nest and the earliest first egg and earliest incubation start dates recorded in that year.

Only breeding attempts for which first egg date, clutch size and hatch date information was available were used in analyses, leaving 372 breeding attempts by 289 females remaining in our dataset. The incubation period for these breeding attempts ranged from 12 to 26 days (mean ± SD = 16.4 days ± 1.56, median = 16 days; Fig S1).

*Measuring hatching success*

Brood size on day 11 was used to estimate the number of eggs that had successfully hatched. Long-tailed tits have a low chick mortality rate due to starvation between hatching and day 11 (2.3%; Hatchwell *et al.* 2004), so it is likely that a small brood size on day 11 is due to hatching failure rather than chick mortality; this was typically confirmed by the presence of unhatched but intact eggs in the nest on day 11. Hatching success was variable (range = 12.5% - 100%) but high on average (mean = 81.8%; median = 88.9%). Note that since some nests were predated between hatching and day 11 our sample size for this analysis was smaller than for incubation periods (N = 230).

### **3.2.2 Weather data**

Weather data for 1994-2018 was obtained from Weston Park Meteorological Station (Museums Sheffield, 2018), located 5km east of the centre of the study site and at a similar elevation (131m above sea level compared with mean field site altitude of 168m). Temperatures at the weather station are significantly positively correlated with those recorded at the field site (Gullett, *et al.* 2014), and were used because on-site temperature data were not available for all years. The close proximity of the weather station to the study site also means that the difference in precipitation between the weather station and field site is minor (Gullett *et al.* 2014).

We calculated mean daily temperature, mean daily minimum temperature, mean daily maximum temperature, mean daily rainfall, and the proportion of rainy days during the incubation period of each nest (N = 372). Initially, we defined the proportion of rainy days in three ways: (i) the proportion of days with any rain (> 0 mm), (ii) the proportion of days with > 0.35mm of rain (which excludes the least rainy 10 % of days), and (iii) the proportion of days with > 3mm of rain (which excludes the least rainy 75 % of days). However, mean daily rainfall during the incubation period, and all three measures of the proportion of rainy days were closely correlated (Pearson’s correlation: *r* ≥ 0.78, df = 370, *P* < 0.001 in all cases). Similarly, minimum and maximum temperatures were highly correlated with mean temperature (Pearson’s correlation: *r* ≥ 0.87, df = 370, *P* < 0.001 in both cases). Therefore, we used only the mean temperature and the proportion of rainy days (> 0 mm) in our main statistical models. These variables were significantly negatively correlated (Pearson’s correlation: *r* = -0.47, df = 370, *P* < 0.001), but this collinearity was well within the threshold to which information theoretic approaches are robust (VIF < 2; Freckleton 2011). The proportion of rainy days variable was used instead of the mean rainfall as it provides a better indicator of daily rainfall patterns, which we considered more likely to affect the nest’s insulation quality through the incubation period than total rainfall. However, the analysis was also conducted using mean daily maximum temperature, mean daily minimum temperature, mean daily rainfall and the proportion of days with > 3mm of rain and the results were qualitatively similar (Tables S2.1, S2.2 & S2.3).

### **3.2.3 Statistical analysis**

All statistical analyses were conducted in R 3.3.1 (R Core Team 2018). We used an information theoretic approach to model selection and constructed all possible models given our predictor variables but retained the random effect in all models. We used the Akaike’s Information Criterion corrected for small sample size (AICc) to compare model fit (Burnham & Anderson 2002). We report the results of all models within 2 AICc points of the model with the lowest AICc value. We used the MuMIn package (Barton, 2018) to calculate marginal and conditional pseudo-R2 using the methods described by Nakagawa and Schielzeth (2013), and the model-averaged estimates (mean and 95% confidence intervals). Where the 95% confidence intervals for an effect size did not span zero, the effect could be considered statistically significant (Burnham & Anderson 2002).

*Factors affecting incubation period*

To investigate factors affecting incubation period we constructed restricted maximum likelihood linear mixed models using the lmer function in the *lme4* package (Bates *et al.* 2014). incubation period was modelled as a function of the proportion of rainy days, mean daily temperature, relative incubation start date, clutch size, attempt (whether it was a first or replacement nest as a binary factor) and female age (in years from ringing as a nestling for philopatric recruits and assuming immigrant recruits were yearlings when first ringed); year was included as a random factor to take variation across years into account. We also included six interactions, although only one interaction was ever present in any single model. The six interactions were between mean daily temperature and the proportion of rainy days, mean daily temperature and relative incubation start date, mean daily temperature and clutch size, proportion of rainy days and incubation period start date, proportion of rainy days and clutch size, and clutch size and incubation period start date. All continuous variables were scaled and centred. Female identity was not included in the models because 77.7% of the data points came from unique females and no single female contributed more than 1.36% of the data to the analysis (Gullett *et al.* 2015). In addition, we separately investigated the repeatability of incubation period in females using the rptR package (Nakagawa & Schielzeth 2010) and found that it was not repeatable (R = 0 ± 0.03, *P* = 1).

*Factors affecting hatching success*

We built generalized linear mixed-effects models with a binomial error structure and logit link of hatching success using the glmer function in the *lme4* package (Bates *et al.* 2014). Hatching success was modelled as a function of incubation period, proportion of rainy days, mean daily temperature, pre-hatching period start date, clutch size, attempt and female age, including year as a random factor. In addition, we included the same six interactions as in the duration of incubation period models. All continuous variables were scaled and centred. Again, female identity was not used as a random factor given the high percentage of unique individuals in the dataset (81.1%).

*Analysis of predation during the incubation period*

The likelihood of predation during the pre-hatching period was assessed using a Mayfield estimate (Mayfield 1975). This was calculated as total number of losses divided by the total number of nest days, giving a daily nest predation measure. In this calculation, we assumed that the predation risk was equal throughout the season and across years, so all nests contributed equally to the calculation of daily nest predation rate. To quantitatively assess how variation in incubation period affected predation risk we used the model of hatching delay to predict how focal predictors, such as relative incubation start date, changed hatching delay and then use the daily nest predation rate to infer predicted changes in likelihood of nest predation.

## **3.3 Results**

### **3.3.1 Factors affecting incubation period**

An information theoretic approach to modelling incubation period identified three well-supported models (Table S2.5). These models explain a moderate amount of variation (conditional R2 = 34.5%; marginal R2 = 17.2%) with the random effect of year explaining half of this variation. The incubation period start date was present in all models, with pairs breeding later having shorter incubation periods (Table 3.1; Fig 3.1a). Weather variables were also important as they were present in all three top models with incubation periods increasing at higher temperatures (Table 3.1; Fig 3.1b) and as the proportion of rainy days increased (Table 3.1; Fig 3.1c). Larger clutch sizes were associated with shorter incubation periods (Table 3.1; Fig 3.1d). All the top models also

**Table 3.1.** The effects of clutch size, relative incubation start date, mean temperature, proportion of rainy days, female age and attempt on the duration of the incubation period in long-tailed tits. Shown are model-averaged parameter estimates and 95% confidence intervals for each fixed effect, and variance for the random effect from the three best fitting models. The model-averaged *R*2LMM(m) and *R*2LMM(c) were 17.4% and 35.1% respectively.

|  |  |  |
| --- | --- | --- |
| Fixed Effects | Slope ± 1 SE | 95% confidence intervals |
| Intercept | 16.35 ± 0.16 | - |
| Clutch size | -0.49 ± 0.08 | **-0.63; -0.34** |
| Relative incubation start date | -0.59 ± 0.12 | **-0.83; -0.36** |
| Mean temperature | 0.30 ± 0.13 | **0.03; 0.56** |
| Proportion of rainy days | 0.26 ± 0.11 | **0.04; 0.48** |
| Clutch size\* proportion of rainy days | -0.21 ± 0.08 | **-0.36; -0.06** |
| Female age | 0.02 ± 0.05 | -0.08; 0.12 |
| Attempt | -0.04 ± 0.13 | -0.29; 0.21 |
| Random Effect | **Variance ± 1 SE** |  |
| Year | 0.47 ± 0.68 | - |

A close up of a map

Description automatically generated

**Figure 3.1.** Duration of incubation period (days) in relation to (a) relative incubation start date, (b) mean temperature, proportion of rainy days and (d) clutch size. Points have been offset so that overlapping points can be better seen. The solid lines indicate the predicted values from model-averaged parameters and dashed lines indicate the standard error. In (c) due to an interaction between proportion of rainy days and clutch size lines represent the model-averaged parameters when the clutch size was set to the lower quartile value (9 eggs; blue), to the upper quartile value (10 eggs; red), to the minimum value (4 eggs; black) and to the maximum value (12 eggs; grey).

contained an interaction term between clutch size and proportion of rainy days, which indicates that the effect of increased rainfall on incubation period was lessened by having a larger clutch (Fig 3.1b). Breeding attempt and female age were each present in two of the four top models (Table S2.5), suggesting that the incubation period was shorter for replacement nests and younger females, but these effects were negligible (Table 3.1).

### **3.3.2 Factors affecting hatching success**

Three models were retained in the top subset (Table S2.6), although these models explain only a small amount of variation in hatching success (conditional R2 = 7.2%; marginal R2 = 4.9%). The duration of incubation periods had a negative effect on hatching success (Table 3.2; Fig 3.2a). Greater hatching success was also associated with larger clutch sizes (Fig 3.2b), younger females (Fig 3.2c) and first breeding attempts (Table 3.2), all of which were present in each of the top set of models. Lower mean temperatures and more rainy days were associated with greater hatching success, although only in one of the three models (Table S2.6) and their effects were negligible (Table 3.2).

### **3.3.3 Effects of incubation period on nest predation risk**

Through a typical breeding season, the incubation period is predicted to decrease from 17.0 to 14.1 days (Fig 3.1a). Given a daily nest predation rate during incubation of 0.02 (based on 540 nests where clutches were completed and incubation started, of which 27.4% were depredated during incubation) this reduction in incubation period equates to predation risk reducing by 6%, from 0.34 to 0.28. For the observed range of mean

**Table 3.2.** The effects of incubation period, clutch size, female age, attempt, mean temperature and proportion of rainy days on hatching success in long-tailed tits. Shown are model-averaged parameter estimates and 95% confidence intervals for each fixed effect, and variance for the random effect from the three best fitting models. The model-averaged *R*2LMM(m) and *R*2LMM(c) were 4.90% and 7.20 % respectively.

|  |  |  |
| --- | --- | --- |
| Fixed Effects | Slope ± 1 SE | 95% confidence intervals |
| Intercept | 1.66 ± 0.09 | - |
| Incubation period | -0.26 ± 0.06 | **-0.39; -0.14** |
| Clutch size | 0.19 ± 0.07 | **0.06; 0.32** |
| Female age | -0.15 ± 0.05 | **-0.26; -0.05** |
| Attempt | -0.32 ± 0.15 | **-0.61; -0.03** |
| Mean temperature | -0.01 ± 0.04 | -0.10; 0.07 |
| Proportion of rainy days | 0.01 ± 0.04 | -0.06; 0.08 |
| Random Effect | **Variance ± 1 SE** |  |
| Year | 0.08 ± 0.28 | - |

A close up of a map

Description automatically generated

**Figure 3.2.** Hatching success (proportion) in relation to (a) the length of the incubation period, (b) clutch size and (c) female age. Points have been offset so that overlapping points can be better seen. The solid lines indicate the predicted values from model-averaged parameters and dashed lines indicate the standard error.

temperatures during incubation from 5.5˚C to 13.9˚C, the model’s predicted change in the duration of the incubation period is an increase from 15.5 to 16.9 days (Fig 3.1b), which represents a change in predation risk of 3%, from 0.31 to 0.34. Similarly, the proportion of rainy days experienced during the incubation period ranged from 0 to 1, resulting in a change of incubation period from 15.7 to 16.8 days and a small increase in predation risk from 0.31 to 0.34. Clutch size ranged from 4 to 12 eggs in our sample of nests, which corresponds to a reduction in the incubation period from 18.7 to 15.3 days, under mean precipitation levels, with each additional egg resulting in the incubation period being shortened by 0.43 days and predation risk decreasing by 6%, from 0.37 to 0.31.

## **3.4 Discussion**

Variation in the incubation period of long-tailed tits over this 24-year study was influenced by clutch size, clutch completion date, mean daily temperature, and proportion of rainy days. Incubation period was longer for smaller clutches, for breeding attempts initiated earlier in the breeding season, under warmer temperatures, and when the proportion of rainy days was higher. However, the effect of rainy days varied with clutch size because larger clutch sizes were less affected by increases in the proportion of rainy days. In addition, incubation periods were shorter in replacement nesting attempts and when females were younger, although these effects were negligible. Changes in incubation durations influence the risk of nest predation during incubation with each additional day of incubation leading to predation probabilities increasing by 0.02. Modelling indicated that timing of breeding and clutch size are the most influential factors moderating nest predation risk by influencing incubation durations. Models of hatching success had limited explanatory power, but we found evidence that greater hatching success was associated with shorter incubation periods, and to a lesser extent younger females, larger clutch sizes and first breeding attempts. Mean temperature and rainfall had minimal effects on hatching success.

Shorter incubation periods towards the end of the breeding season may be advantageous by allowing chick provisioning to occur closer to the peak abundance of insects, especially of caterpillars that constitute the major component of nestling diet at this time of year (Gullett *et al.*, 2014). Caterpillar abundance in the Rivelin Valley typically peaks around 23 May, i.e. during the long-tailed nestling period, and chicks in relatively early and relatively late nests are provisioned with fewer caterpillars (Gullett, 2014). Other woodland passerines that predominantly provision offspring with caterpillars also appear to alter incubation behaviour in order to better match the date of caterpillar peak abundance, e.g. blue tit *Cyanistes caerulerus* (Visser, Noordwijk, Tinbergen, & Lessells, 1998), European pied flycatcher *Ficedula hypoleuca* (Both & Visser, 2005) and great tit *Parus major* (Simmonds, Sheldon, Coulson, & Cole, 2017). Adult long-tailed tits have more variable diets and the reduction in length of the incubation period later in the season could also be due to increased abundance of other insects that enables incubating females to increase daytime nest attendance (Dewey & Kennedy, 2001; Duncan Rastogi, Zanette, & Clinchy, 2006) and hence reduce incubation periods (Lyon & Montgomerie, 1985; Martin, 2002; Martin, Auer, Bassar, Niklison, & Lloyd, 2007). In addition, the rate at which males provision females on the nest during the incubation period could increase later in the breeding season leading to greater female nest attentiveness and hence shorter incubation periods, as has been shown in other passerines (Eikenaar, Berg, & Komdeur, 2003; Martin & Ghalambor, 1999; Matysioková, Cockburn, & Remeš, 2011).

Laying a larger clutch is thought to be costly in most passerine species, as increased brood sizes may increase the activity around the nest leading to increased predation (Skutch 1949; Johnsgard 1973; Perrins 1977; Martin *et al.* 2000). This likely cost may be partially compensated for by the reduction in incubation period with larger clutches; an increase in clutch size by one egg was associated with a reduction in incubation period of approximately half a day. Ardia *et al.* (2006) found that larger clutches had shorter incubation periods in tree swallows *Tachycineta bicolor*, and it has been suggested that under temperate or cold conditions it is better to have a larger clutch size as this increases thermal inertia, thus reducing cooling rates relative to smaller clutches (Reid *et al.* 2000b; Cooper *et al.* 2005). This relationship between clutch size and incubation period contrasts with experimental evidence that increased clutch sizes do not reduce incubation duration (Székely *et al.* 1994; Siikamiiki 1995; Sandercock 1997; Reid *et al.* 2000a; Wiebe & Martin 2000), a discrepancy that may be due to females being unable to cover and effectively incubate the whole clutch in experimental treatments. Our results represent natural variation where females would presumably be able to incubate the whole clutch effectively. Further experimental work would be required to confirm this explanation.

The interaction between clutch size and the proportion of rainy days indicated that larger clutch sizes seem to be particularly advantageous when the proportion of rainy days increased. During periods of rainfall the nest is likely to lose heat via increased conductance through damp nesting materials (Heenan, 2013; Hilton *et al.*, 2004; Reid *et al.*, 2002), so our result may indicate larger clutches were able to buffer the effects of rainfall because a smaller clutch surface area to volume ratio would reduce exposure to the damp nesting materials or humid and cold air. However, it is also important to consider the potential impact of weather on the behaviour of incubating birds; for example, any effects of increased conductance may be confounded by females adjusting their nest attentiveness during periods of heavy rainfall and storms (Fu *et al.*, 2017; MacDonald *et al.*, 2013).

Contrary to our predictions higher ambient temperatures were associated with longer incubation periods. This result is especially surprising given that previous studies have found that other species, including cavity-nesting species whose nests tend to be well insulated (Massaro, Stanbury, & Briskie, 2013) are likely to delay the start of incubation in colder ambient temperatures thus increasing the duration of incubation (blue tits: Kluen *et al.*, 2011; great tits: Monros, Belda, & Barba, 1998). A likely mechanism for higher temperature increasing the duration of the incubation period is adjustment of incubation behaviour under different ambient temperatures. For example, incubating females may leave the nest for shorter periods in cold conditions (Amininasab *et al.*, 2016; Voss, Reed Hainsworth, & Ellis-Felege, 2006; Walters, Webber, Jones, & Volker, 2016), and therefore eggs may remain within the optimal incubation temperature range for a greater proportion of the day. However, in other studies, a greater proportion of the day is spent incubating when ambient temperatures are higher (Ardia, Pérez, & Clotfelter, 2010; MacDonald *et al.*, 2013; Morton & Pereyra, 1985; Simmonds *et al.*, 2017), perhaps because lower costs of re-warming and heating the eggs allow females to incubate for longer before they need to forage again. Regardless of the mechanism generating the positive association we observed between temperature and incubation duration in long-tailed tits, this effect suggests that climate change will not only lead to changes in the timing of breeding (Gullett *et al.*, 2013), but may lead to an increase in the incubation period of long-tailed tits, potentially increasing the risk hatching is mis-timed relative to peak availability of caterpillars (Burgess *et al.*, 2018).

Weather variables were found to have marginal negative consequences, as they had negligible effects on hatching success and led to only a small change in the probability of nest predation, through increased exposure time. We found that the changes in the incubation duration, and therefore relative risk of exposure due to weather variables was relatively small, compared with changes due to clutch size and timing of breeding. However, further studies should aim to quantify if changes in weather conditions affect predator behaviour, and therefore predation risk. The negligible effect of temperature on hatching success is somewhat surprising given that other studies have found that changes in ambient can affect egg viability (Bessinger *et al.*, 2005; Ardia *et al.*, 2006). Contrary to our predictions and other studies (MacDonald *et al.*, 2013; Martin *et al.*, 2017), rainfall had only a negligible effect on hatching success and lower hatching success tended to be associated with lower rainfall. These negligible changes in hatching success suggests that long-tailed tits may be able to buffer the effects of weather conditions, either through females altering their incubation behaviour or through adaptation of nest construction. Further investigation is required to test these hypotheses.

Other factors were found to have important impacts on hatching success. First, hatching success decreased as the incubation period increased, a finding consistent with previous studies of blue tits (Kluen *et al.*, 2011; Nord & Nilsson, 2011), tree swallows (Lombardo, Bosman, Faro, Houtteman, & Kluisza, 1995), and great tits (Diez‐Méndez, Rodríguez, Álvarez, & Barba, 2019). These results indicate that whilst birds may delay hatching to allow it to better coincide with the peak of food abundance there is a cost to this strategic decision if it reduces hatching success. The relationship between hatching success and incubation period could be the result of either decreasing egg viability with time or increased fluctuation in egg temperature causing a reduction in egg viability. Support for the incubation inefficiency explanation is equivocal. An experimental study of house wrens *Troglodytes aedon* that used cross-fostering of eggs to extend or reduce the length of time females had to incubate a clutch had no effect on hatching success (Sakaluk, Thompson, & Bowers, 2018), suggesting that the inefficient incubation hypothesis is unlikely. However, females in lower body condition incubate for longer and have reduced hatching success due to lower incubation temperatures (Hepp *et al.*, 2006) and increased incubation recesses (Bueno-Enciso, Barrientos, & Sanz, 2017). In addition, Nord and Nilsson (2011) found that when incubation temperature was low, the incubation period was extended and the hatching success was lower. These mechanisms are not mutually exclusive, and either could result in the observed decrease in long-tailed tit hatching success.

We also found a weak, positive relationship between hatching success and clutch size. This contrasts with experimental studies reporting lower hatching success with increased clutch sizes (Reid *et al.*, 2000b; Siikamiiki, 1995), which was presumed to be attributable to energetic constraints on successful incubation of experimentally enlarged clutches, as already discussed. The contrast between our observational findings and previous experimental studies may be explained by better quality females having larger clutch sizes and being better able to maintain the incubation temperatures (Hepp *et al.*, 2006), leading to higher hatching success.

Finally, we found that hatching success was lower for older females. Previous work on putative age effects is equivocal. Some studies report increasing hatching success with age, e.g. prothonotary warblers *Protonotaria citrea* (Blem, Blem, & Barrientos, 1999), while others have found no effect, e.g. European starlings *Sturnus vulgaris* (Komdeur, Oorebeek, Van Overveld, & Cuthill, 2005), blue tit (Lambrechts *et al.*, 2012) and house sparrows *Passer domesticus* (Stewart & Westneat, 2013). Our results are consistent with senescence, which has been widely reported in passerine reproductive performance (Monaghan, Charmantier, Nussey, & Ricklefs, 2008; Robertson & Rendellt, 2012; Jankowiak & Wysocki, 2016), but it should be noted that in previous studies, albeit based on smaller sample sizes, we have not detected senescence in long-tailed tit life history traits (Hatchwell *et al.*, 2004; Meade & Hatchwell, 2010).

In conclusion, our long-term analysis showed that mean daily temperature, proportion of rainy days, clutch size and relative pre-hatching period start date explained a large amount of variation in the incubation period of long-tailed tits. In contrast, we found marginal effects of environmental conditions on hatching success. Finally, long incubation periods were likely to be costly due to reduced hatching success and increased exposure to predation risk.

# **Chapter 4.**

# **Is nest insulation quality affected by variation in ambient temperature and rainfall?**

**Summary**

Incubation is an energy consuming process which leads to trade-offs arising between maintenance of egg temperature and self-maintenance. Many species build nests to contain offspring and these nests may also reduce the energetic costs of incubation by buffering changes in environmental conditions and reducing the cooling rate of eggs when the parent is not incubating. This study aimed to quantify the ability of nests to buffer changes in ambient temperature and rainfall in long-tailed tits (*Aegithalos caudatus*). This was achieved by observing the effect of different ambient temperatures and simulated rainfall conditions on nest insulation quality under laboratory conditions. Nests were subsequently dissected to determine whether nest composition influenced nest insulation quality under these different conditions and to observe seasonal variation in nest composition. We found that neither ambient temperature nor simulated rainfall significantly altered nest insulation quality. Furthermore, nest composition was not significantly related to nest insulation quality under the different treatments, although the mass of moss in a nest significantly affected their water absorption. In addition, we found that nest insulation quality was not significantly affected by the timing of building, but total nest mass and the mass of feathers declined through the breeding season. These results indicate that long-tailed tit nests can buffer changes in ambient temperature and rainfall, although the mechanism for this remains unclear.

## **4.1 Introduction**

Incubation is key to hatching and breeding success in avian species. The temperature at which eggs are incubated affects offspring growth and development, with offspring incubated at higher temperatures, and with fewer fluctuations in temperature, having higher fitness (Martin & Wiebe 2006; Olson *et al.* 2006; Kluen *et al.* 2011; Nord & Nilsson 2011; DuRant, Hopkins, Hepp & Walters 2013; Berntsen & Bech 2016). Therefore, optimal incubation behaviour is fundamental to the success of a breeding attempt and future reproductive success of the offspring (Gorman & Nager 2004). However, incubation is costly behaviour (Williams, 1996) and therefore leads to trade-offs between maintaining an adequate nesting environment and self-maintenance (Martin 2002). When parents leave the nest to forage this can leave eggs exposed to ambient conditions. This is particularly likely in the 43% of bird species which exhibit uniparental incubation behaviours because the incubating sex must frequently leave the nest to forage, unless regularly fed by their partner (Deeming 2002; Martin *et al.* 2007).

Leaving the nest and ceasing incubation are energetically costly in a temperate environment because eggs rapidly lose heat to their surrounding environment (Reid *et al.* 2002). The rate of this heat loss largely determines the energy requirements of incubation, as the energy needed to re-warm the eggs to the optimal temperature is much greater than that required to maintain this temperature (Reid *et al.* 2002). Therefore, nest insulation quality is particularly important when the bird leaves the nest because a well-insulated nest can decrease the rate at which eggs lose heat and reduce the amount of energy required to re-heat the eggs once the bird returns to the nest to incubate. Deeming and Gray (2016) investigated the relationship between nest insulation quality and nest attentiveness for 11 passerines, finding that those species with better insulated nests spend less time incubating their eggs. In addition to reducing the amount of energy required for incubation, intraspecific studies have shown that those individuals which build better insulated nests have better condition offspring (Lombardo *et al.* 1995; Álvarez *et al.* 2013; de Zwaan & Martin 2018).

While better insulated nests are advantageous in reducing the energy required for incubation, building such nests requires increased time and energy expenditure, mainly in terms of collection of materials (for review see Mainwaring & Hartley 2013). Lee and Lima (2016) found that it was better for species with high predation rates to invest less in construction and to be able to quickly build a re-nest. Therefore, a number of other factors are likely to affect the amount of energy individuals are willing to invest in nest construction, e.g. the body condition of the individual and/or their partner (Soler *et al.* 1998; Soler *et al.* 2007). In crested tits *Parus cristatus* male quality predicts whether they aid their female when nest building, with higher quality males building more than lower quality males (Lens *et al.* 1994). Male help also depended on the timing of building, with males building more later in the breeding season as this shortened the time between the starting of building and laying. Thus, seasonal variation in nest building investment may result from greater time constraints on nest building later in the season. Some species reduce the quality of nests built later in the breeding season (McGowan *et al.* 2004; de Zwaan & Martin 2018), and in extreme cases, time constraints can lead to re-use of old nests despite the potential costs of increased ectoparasites (Brown & Brown, 1986; Møller 1990) and nest predation (Otterbeck *et al.* 2019).

Given the costs of nest building, we may expect that it would be beneficial to adjust nest structure to prevailing ambient conditions. Nest composition varies with local weather conditions (Deeming *et al.* 2012; Campbell *et al.* 2018), latitude (Rohwer & Law 2010; Crossman *et al.* 2011; Mainwaring *et al.* 2012; Mainwaring, Deeming, *et al.* 2014; Rohwer *et al.* 2015) and altitude (Kern & Van Riper 1984; Altamirano *et al.* 2019). Weather conditions will also affect the ability of incubating parents to maintain their own body condition and suitable thermal conditions for developing embryos. For example, lower ambient temperatures cause eggs to cool at a faster rate and reach lower temperatures when left unattended (Reid *et al.* 2000a), but well-insulated nests buffer temperature changes and reduce the energetic requirements of incubation (Lombardo 1994; Deeming *et al.* 2012; Deeming & Gray 2016). However, additional work is required to determine the direct effects of ambient temperature on nest insulation quality.

Higher rainfall could also increase heat loss from the eggs via increased conductance and reduced insulating properties of damp nest materials (Reid *et al.* 2002; Hilton *et al.* 2004; Heenan 2013; Deeming & Campion 2018). However, only one study has previously investigated the effects of simulated rainfall on the whole nest (Deeming & Campion 2018). Nest materials may be selected for their waterproofing qualities (Deeming 2002). For example, mosses may be used as a building material due to their ability to retain moisture, thereby preventing the nest cup from becoming waterlogged (Wesołowski *et al.* 2002; Wesołowski & Wierzcholska 2018). In addition, lichen has also been suggested to have hydrophobic properties which may allow nests to repel water (Hansell 2000). However, the abilities of these materials to achieve these functions when incorporated into nests have not been experimentally tested. Previous studies which have investigated nesting materials have generally tested each material separately (Reid *et al.* 2002; Hilton *et al.* 2004), rather than the nest as a whole.

Long-tailed tits (*Aegithalos caudatus*) are an ideal study species to investigate the impact of environmental conditions on nest insulation quality. They invest a large amount of time and energy when building their nests (Hansell 1996). They construct enclosed nests composed of moss, lichen and spiders’ silk, and lined with feathers. Their wide distribution across the Palearctic means that during the incubation period they can experience a wide range of ambient temperatures and rainfall, and previous research shows that they are able to adapt their nest-building behaviour to environmental conditions (McGowan *et al.* 2004). They also suffer from a high nest predation rate (Gullett *et al.* 2013) and therefore are likely to experience trade-offs in relation to predation pressures. For example their extended investment in nest building may increase the amount of activity around the nest, increasing the likelihood of being noticed by predators, which may predate the nest or even the breeding pair. However, building a well-insulated nest may allow them to alter incubation behaviour in order to reduce activity around the nest and the length of the incubation period, hence reducing the risk of depredation. This study aims to investigate whether long-tailed tits build nests that buffer the effects of temperature and rainfall, potentially leading to reduced costs of incubation and shorter incubation periods.

We investigated the effect of temperature and simulated rainfall on nest insulation quality in long-tailed tits, predicting that nest contents would cool more rapidly at lower temperatures and with increasing ‘rainfall’. We then investigated how nest composition was related to nest insulation quality under different conditions. We predicted that the mass of feathers would determine nest insulation quality, a larger mass of moss would increase the capacity of nests to absorb water, while lichen would extend the time it takes for nests to reach saturation. Finally, we investigated whether nest insulation quality, feather mass and total nest mass changed in nests constructed through the breeding season and between years.

## **4.2 Methods**

### **4.2.1 Study system**

Long-tailed tit nests were collected between 2016 and 2018 from a population of long-tailed tits, which were monitored throughout their breeding season in the Rivelin Valley, Sheffield (53˚23′ N, 1˚34′ W). Within the population *c.* 95% are individuals are ringed under licence with a unique combination of colour rings and a metal BTO ring. Long-tailed tits are a single-brooded species but their nests frequently fail due to predation (72%; Gullett *et al.* 2013). If a breeding attempt fails relatively early in a season, pairs may build a new nest (MacColl & Hatchwell 2002). Nests were located through observation of the pairs and were found mainly during the building phase. Some breeding attempts remain undetected each year but this is estimated to be a very small percentage of nests (< 5%) that fail early in the breeding cycle (Sharp *et al.* 2008). We checked nests every two days to gain an accurate measure of the date on which first eggs were laid. Each breeding attempt was monitored until it fledged or failed. In a majority of cases we were able to identify predators as being either small mammals (which usually enter the nest through the entrance hole) or corvids (which rip the top from the nest) (Hatchwell, Russell, *et al.* 1999).

### **4.2.2 Nest collection and storage**

Fifty undamaged nests were collected in 2016 (N = 3), 2017 (N = 21) and 2018 (N = 26) following abandonment (6%), fledging (36%), or after eggs or chicks had been depredated by a mammal (58%). Following other studies of nests’ thermal properties (Mainwaring *et al.* 2012; Mainwaring, Deeming, *et al.* 2014), collected nests were stored in a freezer at -20˚C for at least 7 days to kill any invertebrates. After freezing, nests were stored at room temperature for at least a week prior to the beginning of the experiment to allow them to thoroughly defrost and dry.

### **4.2.3 Testing the effects of ambient temperature on nest insulation quality**

To investigate the effects of ambient temperature on nest insulation quality we measured insulation quality of a sample of 24 nests (all from 2018) under two different temperature conditions, 6˚C and 15˚C. These temperatures were chosen because they are close to the extremes of the mean ambient temperatures experienced during long-tailed tits’ incubation periods (range of means: 5 - 14 ˚C, N = 351 nests from long-term data, Chapter 3). Each nest was exposed to both 6˚C and 15˚C treatments, using controlled temperature rooms, and the order of treatments was randomly assigned.

Nest insulation quality was measured using standard procedures (McGowan *et al.* 2004; Mainwaring *et al.* 2012). Three iButtons (DS1923-F5 iButtons, Homechip Ltd, UK) were heated to 70˚C (by placing them in water at that temperature for two minutes and then drying). Two were then placed inside the nest on top of the nest lining in the bottom of the nests where the eggs would normally lie; the iButtons were fixed with duct tape so they were not touching each other. The other iButton was left outside, approximately 20cm from the nest. The iButtons were then allowed to cool for 45 minutes, recording the temperature every 20 seconds during this cooling period.

To measure nest insulation quality we first used the nlsList function in the nlme package (Pinheiro & Bates 2000) in R 3.5.3 (R core team 2018) to generate cooling curves (Fig 4.1) from the iButton data based on the equation for Newton’s law of cooling (McGowan *et al.* 2004; Mainwaring *et al.* 2012):

Temperature of the iButton = ambient temperature + [*B* ] (Eqn 4.1)

A screenshot of a cell phone

Description automatically generated

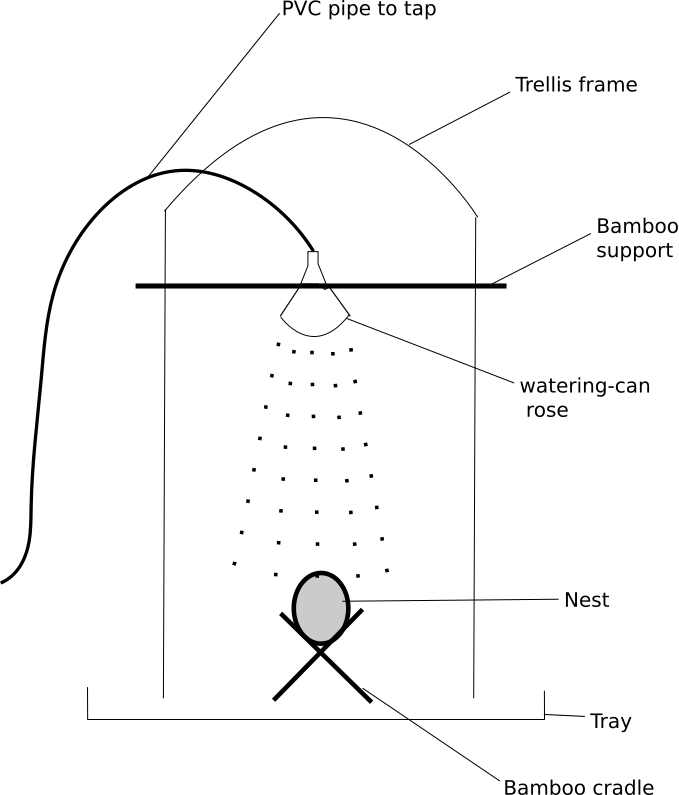
**Figure 4.1.** Example of the iButton temperature trajectories used to generate cooling coefficients. The two iButtons inside the nest (blue and orange) cools at a slower rate than the control iButton outside the nest (grey).

Where *B* is the difference between the temperature recorded by the iButton in the nest and ambient temperature, *C* is the cooling rate coefficient and *t* is the time elapsed since the experiment began measured in seconds.

We then calculated the nest insulation coefficient as the cooling rate coefficient of the iButton outside the nest minus the cooling rate coefficient of an iButton inside the nest; higher values indicate greater nest insulation quality. We calculated a separate insulation coefficient for each iButton that was inside the nest; these were significantly correlated (Pearson’s correlation: *r* = 0.81, df = 23, *P* < 0.001) and we used the average of these two measures in all analyses.

### **4.2.4 Simulating rainfall and measuring nest insulation quality**

We experimentally tested the effects of simulated rainfall on nest insulation quality, with each nest (N = 50) being subjected to three conditions: dry, damp and saturated. Nests experienced the treatments in a randomly assigned order and in analysis were grouped by the first treatment they experienced to test for effects of the order of treatment. Once each treatment was complete, nests were left at room temperature to dry, i.e. had returned to within 5% of their original dry mass before the next trial was initiated (mean drying time for damp nests ± SD = 34.4 ± 17.2 hours; mean drying time for saturated nests = 68.3 ± 19.1 hours). In preliminary trials on 11 nests using a purpose-built shower (Fig 4.2) we found that nests were saturated, i.e. no additional water could be absorbed, when they had gained an additional 110% of their dry mass. We classified damp nests as those that had gained an additional 60% of their dry mass (i.e. had absorbed approximately half the amount of water required to saturate the nest). Nests were weighed to the nearest 0.001g using an electronic balance (EMB 200-3; Kern & Sohn, Balingen, Germany) after



**Figure 4.2**. Experimental setup for wetting nests. The total height of the trellis was 180cm and the watering can rose was 115cm from the nest.

standing for two minutes to allow them to shed any surface water, so nests did not drip during the weighing or during subsequent testing of nest insulation quality. We were able to wet nests to within 5% of their target mass. The dry mass of nests ranged from 16.2g to 40.8g (mean ± SD = 28.5g ± 6.19).

We also recorded the length of time it took each nest to reach this target mass and noted if any water had entered the nest lining. The insulation quality of nests when subjected to each treatment was determined using the iButton methods described above for assessing the impact of temperature on nest incubation quality using a temperature-controlled room maintained at 6˚C.

### **4.2.5 Investigating the effects of nest composition on insulation quality**

After measuring nest insulation quality, a subset of 24 nests (2016 = 3 nests; 2017 = 21 nests) were dissected and separated into the main components used by long-tailed tits for their nests, i.e. lichen, moss, other structural materials (such as grass, small twigs, spiders’ silk), and feathers (McGowan *et al.* 2004). We also had a miscellaneous category for other material, such as egg fragments, faecal sacs and any debris that could not be classified into one of the four main categories. Once separated the materials in each category were weighed using an electronic balance (EMB 200-3; Kern & Sohn, Balingen, Germany) to the nearest 0.001g. In addition, all the feathers were removed and weighed for an additional 15 nests from 2018.

### **4.2.6 Statistical analysis**

All statistical analyses were conducted in R version 3.5.3 (R Core Team 2018). Both in the temperature and rainfall experiment analyses, nest insulation qualities were modelled using a linear mixed effects model employing the lmer function in the lmerTest package to test for the significance of fixed factors with Satterthwaite’s method (Kuznetsova *et al.* 2017). Nest insulation quality was modelled as a function of treatment (fixed factor), order of treatment (fixed factor), a treatment and order of treatment interaction term (fixed factor), and nest ID as a random factor. Year was also included as a random effect in the rainfall experiment analysis. In the rainfall experiment nine nests were built by four pairs, so pair identity was included as a random effect in the rainfall experiment analysis.

We then investigated the effect of nest composition on nest insulation quality under the different treatments using the lmer function in the lme4 package (Bates *et al.* 2014). For this analysis, we used an information theoretic approach to model selection and constructed all possible models. We used this approach as it allowed us to compare models with interactions between treatment and total nest mass, as well as interactions between treatment and the mass of each material type (feathers, moss, lichen and other structural materials), although only one interaction was present in each of the models. We used the Akaike’s Information Criterion corrected for small sample size (AICc) to compare model fit (Burnham & Anderson 2002). Models within 2 AICc points of the model with the lowest AICc value were considered a good fit. In addition to total nest mass, the mass of each material type and treatment, we also included order of treatment as a fixed factor and nest identity as a random factor in the analysis. We used the MuMIn package (Barton 2018) to calculate marginal and conditional pseudo-R2 using the methods described by Nakagawa and Schielzeth (2013) and used the lmerTest package to test for the significance of fixed factors using Satterthwaite’s method (Kuznetsova *et al.* 2013).

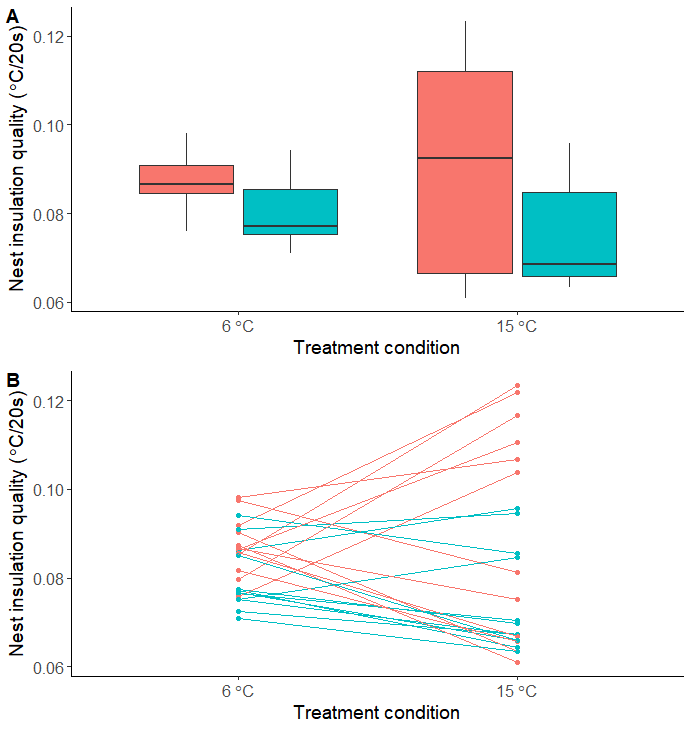
This approach was also used to investigate whether composition affected the time taken to gain the amount of water required for each wet treatment. We modelled the time taken to gain the required mass of water as a function of total nest mass, the mass of each material type, treatment, order of treatment as fixed factors and nest identity as a random factor. Again, we modelled all possible interactions between treatment and total nest mass and the mass of each material type, although only one interaction was ever present in any one model. We also investigated whether the proportion of each material affected nest insulation quality and time taken to reach treatment condition and results were qualitatively similar (for results see Tables S3.1 and S3.2).

To investigate nests constructed throughout the breeding season and between years in terms of nest insulation quality, total nest mass and feather mass, we performed separate linear models using the lm function in the *stats* package (R Core Team 2018). We constructed three linear models to investigate how three response variables- nest insulation quality, total nest mass and feather mass- changed through the breeding season, using first egg date as a proxy for timing of breeding. Three additional linear models were constructed to test whether there were any differences in nest mass, feather mass and nest insulation quality between years.

## **4.3 Results**

### **4.3.1 Testing the effects of ambient temperature on nest insulation quality**

We found no significant difference in the insulation quality of experimental nests at 6˚C and 15˚C (F(1,22) = 0.02, *P* = 0.89; Fig 4.3). The order of treatment significantly affected the nest insulation quality (F(1,22) = 7.33, *P* < 0.05) with those that experienced the hot treatment first having a lower nest insulation quality than those that first experienced the cold treatment. However, there was no significant interaction between the treatments and order that the nests experienced the treatments (F(1,22) = 1.43, *P* = 0.25; Fig 4.3).



**Figure 4.3.** Nest insulation coefficients for all nests (N = 24) under the two treatment conditions; 6˚C and 15˚C. Higher numbers indicate a better insulation quality. In both A and B, orange represents the nests that experienced 6˚C followed by 15˚C and green represents those that experienced 15˚C followed by 6˚C. A - The midline in the boxes represents the median and the box represents the interquartile range, with top line of the box representing the 75th percentile and the bottom line representing the 25th percentile. The bottom of the vertical line represents 25th percentile - 1.5 x interquartile range, while the top of the vertical line represents 75th percentile + 1.5 x interquartile range. B - The line between two points represents the change in an individual nest.

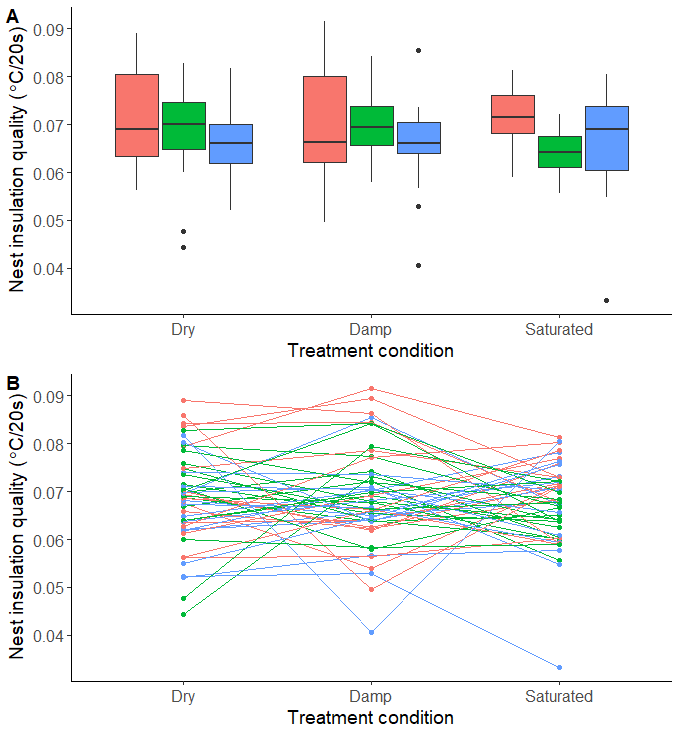
### **4.3.2 Testing the effects of simulated rainfall on nest insulation quality**

Water penetrated the inner cup of a minority of nests in the saturated condition (37.5%, N = 50) and never in the damp condition. Dry, damp and saturated nests did not differ significantly in their insulation quality (F(2,102) = 0.34, *P* = 0.71; Fig 4.4), and the order in which nests were exposed to treatments had no significant effect on their insulation quality (F(2,61) = 2.93, *P* = 0.06). Finally, there was no significant interaction between treatment and order (F(4,102)  = 1.02, *P* = 0.41).

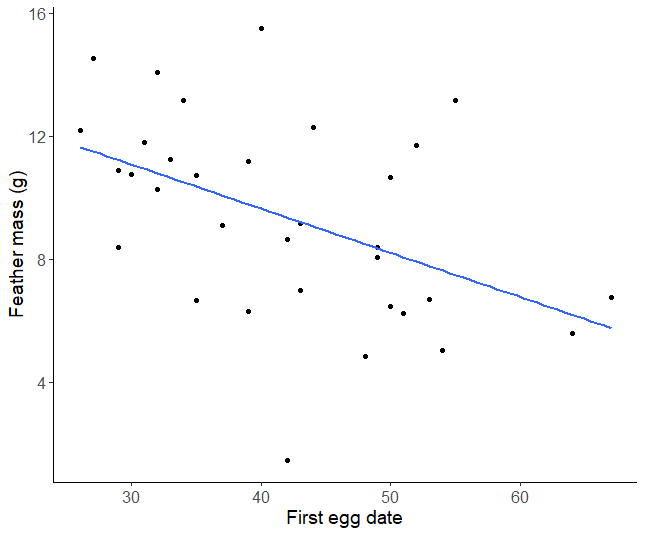
### **4.3.3 Nest composition**

As expected, we found that feather mass declined significantly in nests built throughout the breeding season (F(1,32) = 8.98, *P* < 0.01; Fig 4.5), as did total nest mass (F(1,32)  = 7.14, *P* < 0.05). However, there was no significant effect of timing of breeding on nest insulation quality (F(1,32) = 0.82, *P* = 0.37), and there is only a marginal decline in the proportion of feathers (F(1,32) = 3.22, *P* = 0.08). Nest insulation quality, total nest mass and feather mass did not differ significantly between years (nest insulation quality: F(2,38) = 0.68, *P* = 0.51; total nest mass: F(2,38)  = 0.14, *P* = 0.87; feather mass: F(2,38) = 1.06, *P* = 0.36), although there was a marginally significant difference in the proportion of feathers between years (F(2,38) = 2.97, *P* = 0.06).

When examining the relationship between nest insulation quality and nest composition, the null model with the lowest AICc value was the model containing only total nest mass, with no other model within 2 AICc points (Table S3.3). However, despite total nest mass being present in one of the top models, it was not found to have a significant effect on nest insulation quality (Table 4.1).



**Figure 4.4.** Nest insulation coefficients for all nests (N = 50) under the three treatment conditions; dry, damp (water added until nest mass increased by 60%), saturated (water added until nest mass increased by 110%). Higher numbers indicate a better insulation quality. In both A and B, orange represents the nests that experienced the dry condition first, green represents those that experienced the saturated condition first and blue represents those that experienced the damp condition first. A - The midline in the box represents the median and the box represents the interquartile range, with top line of the box represents the 75th percentile and the bottom line representing the 25th percentile. The bottom of the vertical line represents 25th percentile - 1.5 x interquartile range, while the top of the vertical line represents 75th percentile + 1.5 x interquartile range. B - The line between two points represents the change in an individual nest.



**Figure 4.5.** Relationship between long-tailed tit nest feather mass (N= 41) and the date the first egg was laid. The line represents the best fit line for a linear model.

**Table 4.1.** The effects of total nest mass on nest insulation quality in long-tailed tits. Shown are model-averaged parameter estimates, 95% confidence intervals and *P* value for total nest mass, and variance for nest identity (the random effect) from the best fitting models. The model-averaged *R*2LMM(m) and *R*2LMM(c) were 1.23% and 1.23%, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed Effects | Slope ± 1 SE | 95% confidence intervals | *P* value |
| Intercept | 0.06 ± 0.003 | - | - |
| Total nest mass | 0.00009 ± 0.0001 | -0.00011; 0.00018 | 0.67 |
| Random Effect | **Variance ± 1 SE** |  |  |
| Nest ID | 0.00 ± 0.00 | - | - |

**Table 4.2.** The effects of total nest mass, feather mas, moss mass, other structural mass and treatment on the time taken to gain the amount of water needed for treatment. The asterisk (\*) indicates an interaction between two factors. Shown are model-averaged parameter estimates, 95% confidence intervals and *P*-value for total nest mass, and variance for nest identity (the random effect) from the best fitting models. The model-averaged *R*2LMM(m) and *R*2LMM(c) were 75.9% and 75.9% respectively.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed Effects | Slope ± 1 SE | 95% confidence intervals | *P*-value |
| Intercept | 44.49 ± 50.64 | - | - |
| Moss mass | -17.48 ± 6.04 | **-29.65; -5.32** | **<0.05** |
| Treatment | -238.17 ± 64.00 | **-367.53; -108.82** | **<0.05** |
| Treatment \* total nest mass | 12.19 ± 2.18 | **7.79; 16.59** | **<0.05** |
| Total nest mass | 5.60 ± 3.02 | -0.44; 11.64 | 0.07 |
| Feather mass | -8.23 ± 5.86 | -19.88; 3.42 | 0.39 |
| Other structural material mass | -1.66 ± 3.35 | -8.30; 4.99 | 0.62 |
| Random Effect | **Variance ± 1 SE** |  |  |
| Nest ID | 0.00 ± 0.00 | - | - |

The time taken for nests to gain the prescribed amount of water differed between the damp and saturated conditions, and within each treatment, the time taken to gain this weight was associated with the total nest mass, with heavier nests taking longer to wet (Table 4.2). This make intuitive sense because these nests had to absorb more water. However, there was also a significant negative correlation between the time taken to gain the amount of water for the treatment and mass of moss (Table 4.2), i.e. more mossy nests were able to absorb water faster. In contrast, the mass of feathers and other structural materials were retained in the top model set but did not have a significant effect (Table 4.2 and S3.4).

## **4.4 Discussion**

The insulation quality of long-tailed tit nests was not significantly affected by ambient temperature. Furthermore, it was not altered by the addition of water via simulated rainfall, with neither damp nor saturated nests having a significantly lower insulation quality than dry nests. Similarly, neither nest mass, nor the mass of individual nesting materials were found to be important in determining nest insulation quality under any of the experimental conditions. However, we showed that the time to absorb the amount of water required for the treatments increased with the nest mass, although the mass of moss decreased the length of time required to saturate the nest. Nest composition varied through the breeding season with nests built later in the season having a lower total mass and a lower mass of feathers, although the proportion of feathers did not decrease, and insulation quality was not lowered significantly.

In our experiment under controlled conditions, nest insulation quality did not differ at two ambient temperatures, indicating that long-tailed tit nests’ ability to reduce the cooling of nests contents was not affected by ambient temperature. Foraging bouts increase in duration with increasing ambient temperatures in other passerine species (Conway & Martin 2000b; Amininasab *et al.* 2016), which is attributed to the lower cooling rate of eggs at higher temperatures. Our results suggest that similarly in long-tailed tits the cooling rate of nest contents was dependent upon ambient temperature, so shorter off-bouts would be expected under cooler ambient conditions. However, further work on how female incubation behaviour is altered by changes in ambient temperature is needed to test this hypothesis.

Likewise, we established that nest insulation quality did not decrease when nests were wet. Even when nest material was saturated with water under controlled conditions, nest insulation quality was not significantly diminished, suggesting that such adverse environmental conditions were buffered by nests. In some respects, this was a surprising result because studies of the open-cup nests of several passerines (e.g. Heenan 2013; Deeming & Campion 2018) have found that nest insulation quality was significantly lowered by dampening nests and nesting materials, especially feathers, are known to be less effective insulators when they are wet (Reid *et al.* 2002; Hilton *et al.* 2004). To the best of our knowledge, this is the first study to find that the insulation quality of nests was not compromised by dampening, a finding that may be due to the methods of wetting nests because most previous studies have submerged nests or materials in water to saturate them (e.g. Heenan, 2013; Reid et al., 2002; Hilton et al., 2004; but see Deeming & Campion, 2018). It was noticeable when running our experiments that water only entered the nest cup in a minority of nests in the saturated condition. Thus, in a majority of cases, the nest structure was able to absorb and retain the water so that the interior lining of the nest was not dampened,and insulation quality was preserved. This matches anecdotal observations in the field, where the cups of intact long-tailed tit nests become wet only during exceptionally heavy or prolonged rainfall (C.G.H. pers. obs.). Although, there may be fundamental differences between nests *in situ* and these nests which have been removed and tested in the lab, so this should be bore in mind when interpreting our results. It may be expected in fact that nests *in situ* would perform better than those in lab - as those in the lab have been removed from the substrate they were held in, frozen and when tested do not have any over head substrate to deflect some of the water. Any of these may cause nests to performance sub-optimally under testing conditions, although we find that these nests are still able to buffer the effects of simulated rainfall. Future studies should however aim to test nests *in situ* to be able to observe any potential differences.

We expected that the amount of specific nesting materials would influence nest insulation quality under different treatment conditions. However, nest mass had only a minor effect on nest insulation quality, and none of the material types, including feathers, was retained in the top model set. This was surprising given that previous studies (McGowan *et al.* 2004; Windsor *et al.* 2013; Mainwaring, Deeming, *et al.* 2014) that have investigated nest insulation quality, using dry nests, had shown that it was significantly correlated with the mass of insulating materials in the nest cup. One possible explanation for this is that although feathers are expected to be the main source of nest insulation, other nesting materials found in long-tailed tit nests may also provide some insulation because they are used for this function in other species, e.g. moss, some lichen species and dry grass (Hilton *et al.* 2004; Mainwaring, Deeming, *et al.* 2014). It is also notable that the feathers in long-tailed tit nests line the entire inner surface of the nest, including the roof of the dome, and preen oil confers a hydrophobic property to feathers (Rijke & Jesser 2011). Therefore, another possibility is that it is only feathers in the nest cup itself that provide insulation and the feathers in the dome of the nest are performing another function, such as preventing water from entering the nest cup. In addition to the thermoregulatory function, feathers can also serve as a sexual signal in a number of bird species (Maynard fSmith & Harper, 2003). In addition, we expected to find that moss would retain water and prevent the nest cup from becoming waterlogged (Wesołowski *et al.* 2002; Wesołowski & Wierzcholska 2018), but the mass of moss was not significantly related to the nest insulation quality under damp or saturated conditions. As in the case of feathers, it may be that only the moss in the dome of the nest performs this function. Future studies should investigate this possibility by dissecting the nest cup and dome of the nest separately.

The mass of lichen present on the nest was predicted to affect the time taken to saturate nests, as it has previously been suggested that lichen reduces the amount of water penetrating the nest (Hansell 2000). However, while the mass of lichen was correlated with the time taken to saturate the nest, it had only a minor effect. Thus, lichen may be present on the outside of the nest to act as disruptive camouflage (Hansell 1996), rather than to resist wetting. We did find that the amount of time taken to saturate nests was correlated to the mass of moss, nests with a higher mass of moss being saturated faster than those with lower mass. Biddle *et al.* (2019) examined the effect of rainfall on nests of 19 passerine species and showed that nests with a larger proportion of moss absorbed more water.

The shape of long-tailed tit nests may explain why no particular nesting material is associated with insulation quality, unlike the open-cup nesters previously tested (Heenan 2013; Deeming & Campion 2018). Enclosed nests were previously thought to play an important role in reducing predation of nests but a recent meta-analysis reported that this was not the case (Martin *et al.* 2017). Furthermore, Lamprecht and Schmolz (2004) establish that the shape of the nest altered the rate of heat loss - enclosed nests greatly reduced the amount of heat lost to the environment when compared with open nests, presumably due to the reduction of air movement around the eggs (Heenan & Seymour 2011; Wilson *et al.* 2017). Therefore, the shape of a long-tailed tit nest may be more important in determining its insulative properties rather than the materials composing it.

In conclusion, our experiments, conducted under controlled laboratory conditions, show that long-tailed tit nests appear to buffer nest contents against variation in ambient temperature and rainfall. This suggests that whilst the females are away from their nests, nests reduce the cooling rates of egg under all but the most extreme weather conditions, thereby reducing the amount of energy required for incubation.

# **Chapter 5.**

# **The effects of environmental conditions on incubation behaviour.**

**Summary**

Incubation of eggs within the optimal temperature range is vital for the successful development of embryos. However, incubation is energetically costly for the incubating parent, with changes in environmental conditions likely to influence these costs. Therefore, incubation behaviour is expected to be sensitive to environmental factors. We investigated the effects of seasonal changes and weather conditions on female incubation behaviour in long-tailed tits (*Aegithalos caudatus*). We found that incubation behaviour –measured by nest attentiveness, and the duration of incubation bouts and recesses – were significantly affected by temperature and diurnal cycles. Nest attentiveness and the duration of incubation bouts decreased through the day and were highest at intermediate temperatures, while the duration of incubation recesses increased through the day and were lowest at intermediate temperatures. Females incubating older embryos had higher nest attentiveness and shorter incubation recesses, and those incubating larger clutches took longer incubation recesses. We also found, as expected, that there was a significant negative relationship between nest attentiveness and the duration of incubation periods. We conclude that ambient temperature and other factors influence incubation behaviour in long-tailed tits, with likely consequences for reproductive success.

## **5.1 Introduction**

Parental incubation is vital in most bird species, with the notable exception of megapodes, because the poikilothermic nature of eggs means that they require an external heat source to maintain them at the optimal temperature for embryonic development. Eggs kept outside the optimal temperature range (<24˚C or >40.5˚C) have slow embryonic development and developmental abnormalities can emerge, with extended time outside this range leading to embryo death (Webb 1987; DuRant, Hopkins, Hepp & Walters 2013). As a result, the microclimate in which embryos develop can affect the quantity (Reid *et al.* 2000a, 2000b; DuRant, Hopkins, Hepp & Romero 2013) and quality (Lombardo *et al.* 1995; Tombre & Erikstad 1996; Larsen *et al.* 2003; Dawson *et al.* 2005) of offspring produced. It can also have long-term effects on the survival of these offspring to sexual maturity (Berntsen & Bech 2016) and fecundity (Gorman & Nager 2004). Therefore, optimal incubation behaviour is vital to the success of a breeding attempt and long-term reproductive success.

Incubation is a costly behaviour (Vleck 1981; Williams, 1996; Monaghan & Nager 1997) that may lower female survival and increase predation risk (Visser & Lessells 2001). Consequently, trade-offs occur between maintaining an adequate nesting environment and self-maintenance (Martin 2002). For example, when females leave the nest to forage, their eggs are exposed to ambient conditions. This is particularly common in species which exhibit uniparental incubation behaviours (43% of avian species exhibit uniparental care; Deeming 2002) as the incubating individual must frequently leave the nest to forage. Unless regularly fed by their partner (Deeming 2002), for example in pied flycatchers *Ficedula hypoleuca* (Lifjeld, Slagsvold & Stenmark, 1987) and snow bunting *Plectrophenax nivalis* (Lyon & Montgomerie, 1985). The energetic costs of incubation behaviour may also be influenced by many factors, including weather conditions (MacDonald *et al.* 2013; Coe *et al.* 2015; Amininasab *et al.* 2016), food availability (Nilsson & Smith 1988; Chalfoun & Martin 2007), rate of male provisioning of the female (Hatchwell, Fowlie, *et al.* 1999), female quality (Soler *et al.* 2001; Ardia & Clotfelter 2007), clutch size (Reid et al., 2000b; Wiebe & Martin, 2000) and nest thermal properties (Demming & Gray, 2016).

Evidence for a linear relationship between ambient temperature and nest attentiveness is equivocal, with some studies finding that females had lower nest attendance in low ambient temperatures (e.g. house wrens *Troglodytes aedon* in Voss *et al.* 2006; tree swallows *Tachycineta bicolor* Ardia *et al.* 2009; tree swallows *Tachycineta bicolor* in Coe *et al.* 2015) and others finding that nest attendance decreased with increasing temperature (e.g. tree swallows *Tachycineta bicolor* and black-capped chickadees *Poecile atricapillus* in Voss *et al.* 2006; blue tits *Cyanistes caeruleus* in Amininasab *et al.* 2016). However, this inconsistency may partially be explained by there being a non-linear relationship between incubation behaviour and ambient temperature. In their conceptual model of incubation, Conway and Martin (2000b) argued that at low ambient temperature females should favour self-maintenance because low temperatures raise the costs of incubation, and thus females’ food requirements. Therefore, low nest attendance with increased incubation recesses for foraging would be favoured at low temperatures. At high ambient temperatures, the eggs may require cooling, rather than heating, for their temperature to remain within the optimal range. This may be achieved either through females reducing the amount of time they spent at the nest or, in the case of extremely high temperature, through active cooling of eggs. Most commonly females will stand over the eggs to shade them from solar radiation in the hottest part of the day i.e. crowned plovers *Vanellus coronatus* (Brown & Downs, 2003) and hoopoe larks *Alaemon alaudipes* (Tieleman, van Noordwijk & Williams, 2008). Therefore, nest attendance is expected to peak at intermediate temperatures, with evidence of this trend being found in orange-crowned warblers *Vermivora celata* (Conway & Martin 2000b), Carolina chickadee *Poecile carolinensis* (Walters *et al.* 2016) and blue tits *Cyanistes caeruleus* (Bambini *et al.* 2018).

Rainfall may also increase nest conductance, as wet nesting materials are likely to provide poor insulation, leading to increased cooling of eggs and increased incubation costs. Coe *et al*. (2015) reported that high rainfall caused tree swallows *Tachycineta bicolor* to decrease the duration and number of incubation recesses and increase the duration of incubation bouts. In other studies, however, birds have been found to extend their incubation recesses under inclement weather conditions (Wiebe & Martin 2000; MacDonald *et al.* 2013). Thus, it is again plausible that there is a non-linear relationship between environmental conditions, in this case rainfall, and incubation behaviour.

Incubation behaviour may also be affected by seasonal changes due to changes in temperature, food availability and changes in photoperiod. Few studies have assessed the effects of changes in day length on incubation behaviour (but see Wheelwright & Beagley 2005). It is plausible that the shortening of nights between the onset of breeding and the summer solstice reduces investment in incubation. This pattern may arise because in diurnally foraging species there is no trade-off between incubation and foraging during the night so incubation of the clutch during the night is relatively constant compared to incubation during the day (Ricklefs & Brawn 2013). A meta-analysis of data recorded across a range of latitudes, and thus a range of day lengths, indicate that females compensate for shorter nights by spend an increased proportion of daylight hours incubating (Álvarez & Barba 2014b).

The variation in incubation behaviour caused by these environmental factor is known to influence the duration of the incubation period (Lyon & Montgomerie 1985; Nilsson & Smith 1988; Reid *et al.* 2002; Martin *et al.* 2018) and hatching success (Webb 1987; Nord & Nilsson 2011), as the incubation behaviour is closely linked to the egg temperature with eggs having to be kept with the optimal temperature range (Webb 1987) to allow embryonic development. For example, the amount of time that the eggs spent below 26˚C was positively correlated with the length of the incubation period in tree swallows (Coe *et al.* 2015).

We investigated the effects of environmental conditions and timing of breeding on incubation behaviour in long-tailed tits *Aegithalos caudatus*. Our previous findings (see Chapter 3) show that the variation in the duration of incubation period in this species can be explained by the timing of breeding (i.e. relative incubation start date), clutch size and weather conditions (i.e. mean rainfall and the proportion of rainy days). Also, the durations of the incubation period were negatively correlated with hatching success. This study aimed to investigate whether observed changes in incubation period duration with seasonal and environmental conditions are due to changes in female incubation behaviour, and whether female incubation behaviour also had consequences for hatching success. In addition, we aimed to test the effect of nest insulation quality (see chapter 4) on incubation behaviour and the duration of the incubation period. Female long-tailed tits incubate alone with males bringing food only occasionally (Hatchwell, Fowlie, *et al.* 1999), so we expected that females would experience trade-offs between maintenance of egg temperature and their own self-maintenance, especially under inclement weather conditions. We measured incubation behaviour in three ways: nest attentiveness per hour, and the mean duration of incubation bouts and recesses initiated within an hour. We predicted that females would have higher nest attendance and/or longer incubation bouts at lower temperatures and in dry conditions (i.e. low rainfall). We also expected that females incubating larger clutches would have prolonged incubation recesses, because larger clutches are also expected to cool at a slower rate. Finally, our previous study found that the incubation periods were shorter towards the end of the breeding season and we expect that this may be due to females increasing nest attentiveness later in the breeding season.

## **5.2 Methods**

### **5.2.1 Study system**

Incubation behaviour was observed in a population of long-tailed tits in the Rivelin Valley, Sheffield, UK (53˚23′ N, 1˚34′ W) between 2016 and 2018. Approximately 95% of the population was ringed under licence with a BTO ring and a unique combination of two colour rings; adult immigrants were captured and ringed at the beginning of each breeding season and nestlings were ringed 11 days after hatching.

Breeding attempts were located by observation of pairs and were typically located at the nest building stage. A very small percentage of nests, estimated to be < 5%, are not found in each year but these undetected nesting attempts typically fail early in the breeding cycle (Sharp *et al.* 2008). Following the discovery of a nest, it was visited every other day until the clutch or brood had been depredated or fledged. The identity of the pair, first egg lay date, clutch size, incubation period, hatch date and fledge date were all recorded, as described in Chapter 3.

### **5.2.2 Recording incubation behaviour**

Incubation activity was recorded for 48 breeding attempts (N = 14 in 2016, N = 17 in 2017, N = 17 in 2018) by 40 different females. Activity was monitored for 48 to 96 hours (mean ± SD = 67.4 ± 15.5 hours) using a temperature probe (Tinytag Plus 2 TGP-4020 and Thermistor Probe PB-5009-0M6, Gemini Data Loggers, UK). Recordings were taken when the embryos were aged between 1 and 12 days (with day 1 being the day on which the last egg was laid). Temperature probes were inserted through the wall of the nest, and then laid along the inner side of the lining at about the same level as the eggs. The temperature probe took a measurement every 20 seconds. Methods used are similar to those used by Macdonald *et al.* (2013). While the loggers were in nests, a subset (N = 25) were video recorded for a two-hour period. Nests were filmed from 5m, using a video camera (Sony Handycam DCR-SR58) mounted on a tripod, after cameras were placed at the nests birds were observed for 30 minutes to check that their natural behaviour was not disturbed. Recordings were subsequently used to confirm that the temperature loggers accurately reflected the incubation bouts and recesses of females.

### **5.2.3 Measurement of start of incubation, incubation period and hatching success**

We assumed that females commenced incubation on the day the last egg was laid, as occurs in a large majority of nests (Chapter 3). To account for inter-annual variation in the timing of breeding we calculated the ‘relative start of incubation date’ as the number of days between the start of incubation for a given nest and the earliest start of incubation date recorded that year. We calculated the length of the incubation period as the number of days between clutch completion and hatching (Nilsson & Smith 1988; Wiebe & Martin 2000; Martin 2002; Martin *et al.* 2007; Rohwer *et al.* 2015; Bueno-Enciso *et al.* 2017; see Chapter 3). ‘Hatching success’ was calculated as brood size on day 11 of the nestling period (day of hatching = day 0) divided by clutch size. Nestling mortality through starvation is rare so the number of chicks on day 11 is a good measure of hatched eggs (Hatchwell *et al.* 2004). For further details on the measurement of the length of the incubation period and hatching success see Chapter 3.

### **5.2.4 Measurement of nest insulation quality**

Nest insulation quality was determined for a subset of nests (N = 16) under laboratory conditions. Two heated iButtons (~70˚C) were placed inside the nest and one heated iButton outside of the nest and allowed to cool to ambient temperature (6˚C) for 45 minutes. All iButtons recorded the temperature every 20s. We measured nest insulation quality using a logistic model to calculate the cooling rate of each iButton (McGowan *et al.* 2004; Mainwaring *et al.* 2012), as detailed in equation 4.1. For further details of protocols and calculations of nest insulation quality see chapter 4.

### **5.2.5 Analysis of incubation behaviour**

Raven Pro 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY, USA) was used in conjunction with Rhythm 1.1 (Cornell Laboratory of Ornithology, Ithaca, NY, USA) to analyse data from the temperature loggers. Rhythm detected incubation bouts and recesses using the following scheme: an initial change in temperature of at least 0.5˚C at a rate of 0.2˚C/min, which is sustained for a minimum of 6 minutes. We found that using 6 minutes prevented the detection of false incubation recesses caused by changes in temperature when the bird moved around the nest, although this did mean that some shorter incubation bouts and recesses were not selected by Rhythm. This was corrected by selecting these bouts when data were visually inspected in Raven Pro 1.4 (Figure S4.1; Mills & Cooper 2005). In addition, minor adjustments to on and off-bouts were made as necessary (Mills & Cooper 2005; Ospina *et al.* 2015). These data were then summarised using R 3.3.1 (R Core Team 2018). We extracted the time the female first left the nest in the morning, both the exact time and the number of minutes after sunrise, and the time the female last entered the nest in the evening, both the exact time and the number of minutes before sunset. The duration of the active day was calculated as the number of hours between the female first leaving the nest in the morning and last entering the nest of an evening. We calculated a daily nest attentiveness (the % of time a female spent in the nest within an active day) for each full day recorded, i.e. excluding the days when the logger was inserted into or removed from the nest. We then calculated a mean of these values to give a mean nest attentiveness for each female. From each recording day the following measurements were also taken for every hour between the bird first exiting the nest in the morning (or the probe first being placed in the nest) and last entry in the evening (or the probe being removed from the nest): (a) nest attentiveness (% of time a bird spent in the nest in a given hour), (b) mean duration of incubation bouts (if an incubation bout was initiated at 0950h and the female incubated for 20 mins then this whole bout would be included in the hour 9 mean. Bouts were recorded to nearest minute), (c) mean duration of incubation recesses (to nearest minute), (d) number of incubation bouts initiated, and (e) number of incubation recesses.

The videos were transcribed by noting the time (to the nearest minute) of the female arrival and departure from the nest. We calculated the mean nest attentiveness, mean duration of incubation bouts and recesses, and the number of incubation bouts and recesses over the video-ed period for both the temperature and video data, so each video recording was treated as an individual data point. We used a Pearson’s correlation to validate the temperature logger recordings, finding that all measures of incubation behaviour were significantly positively correlated between the temperature logger and video recordings (coefficients varied from 0.77 to 0.95; Table S4.1).

### **5.2.6 Weather data**

In 26 out of 48 recordings, temperature data were recorded using a Tinytag logger located within 0.5m of the nest which took recordings every 20 seconds. However, in 22 cases these data were not available so environmental temperature data from temperature loggers (iButtons DS1922L - F5) located within 1m of the nest were used, these loggers recording the temperature every 15 mins. An average of these temperatures was calculated to give a mean hourly ambient temperature for each nest during the recording period.

Hourly rainfall data were obtained from Weston Park Meteorological Station (Museums Sheffield 2018), located 5km east of the centre of the study site and at a similar elevation (131m above sea level compared with mean field site altitude of 168m). The close proximity of the weather station to the study site also means that the difference in precipitation between the weather station and field site is minor (Gullett *et al.* 2014).

### **5.2.7 Statistical analysis**

All statistical analyses were conducted in R 3.3.1 (R Core Team 2018). We used lmer function in the *lme4* package (Bates *et al.* 2015). An informatic theoretic approach to model selection was used, meaning all possible models were constructed from our predictor variables but the random effects were retained in all models. Akaike’s Information Criterion corrected for small sample size (AICc) was used to compare model fit (Burnham & Anderson, 2002), and all models within two AICc points of the model with the lowest AICc value were reported. Model averaging was used to calculate parameter estimates, 95% confidence intervals, marginal and conditional R2 values(R2LMM(m) and R2LMM(c); Nakagawa & Schielzeth, 2013).

Variation in incubation behaviour in relation to various predictors was investigated by constructing restricted maximum likelihood linear mixed models using the lmer function in the *lme4* package (Bates *et al*. 2015). We analysed factors affecting three aspects of incubation behaviour - hourly nest attentiveness, and the mean duration of incubation bouts and incubation recesses which were initiated within an hour (model details are summarised in Table 4.2). Hourly nest attentiveness (proportion of time spent in the nest) was arcsine square-root transformed, whilst the duration of incubation bouts and recesses where log10-transformed to normalize the data. Transformed response variables were modelled as a function of the time of recording (hour), hourly rainfall, mean hourly temperature, day length (the number of hours between sunrise and sunset), relative incubation start date, embryo age, clutch size and attempt (i.e. whether it was a first or replacement clutch). We also included mean temperature2 and rainfall2 as previous research suggested that the relationship between ambient temperature and incubation behaviour was best described using a quadratic relationship (Conway & Martin 2000b). Nest identity, female identity and year were also included in all models as random factors. The variance inflation factors (VIF) for all variables present in the models were checked for collinearity, and were within the threshold to which information theoretic approaches are robust (i.e. VIF < 2; Freckleton 2011), apart from collinearity between day length and the relative incubation start date. Therefore, no model contained both day length and the relative incubation start date.

We also used linear mixed models to investigate the relationship between duration of the active day and mean daily rainfall, daily rainfall2, mean daily temperature, mean daily temperature2, day length, relative incubation start date, embryo age, clutch size and attempt. The relationship between the time at which a female last entered the nest in the evening (in minutes before sunset) and the same predictors was also investigated using linear mixed models. Again, nest identity, female identity and year were included as random factors, and day length and relative incubation start date had a high collinearity so were not included in the same models. The time the female first left the nest in the morning (in minutes after sunrise) was also investigated using the same fixed and random effects as in the previous models, except it included the nightly mean temperature and mean rainfall from the previous night, rather than mean daily temperature and rainfall. The response variable in this case, the time the female first left the nest, was log10-transformed to normalize the data.

Finally, we investigated whether the length of the incubation period and hatching success were affected by incubation behaviour. We constructed two linear mixed models using the lmer function in *lme4* (Bates *et al.* 2015) with the duration of the incubation period as the response variable, one model containing mean nest attentiveness as a fixed effect and the other containing nest insulation quality. Female identity and year were used as random factors in the first model, whilst the second model used only year as a random effect because all observations were conducted on different females. Generalised linear mixed models with a binomial error structure and logit link using the glmer function (*lme4;* Bates *et al.* 2015) were used to investigate the effects of mean nest attentiveness and nest insulation quality on hatching success. Two models were built: the first included mean nest attentiveness, and the second contained nest insulation quality. As above, in the first model both female identity and year were included as random effects, whereas for the second model only year was included because all observations were from different females. I also constructed a linear mixed model using the lmer function (*lme4;* Bates *et al.* 2015) to investigate the effect of nest insulation quality on nest attentiveness. In this model again only year was used as a random effect.

## **5.3 Results**

### **5.3.1 Incubation behaviour**

Daytime incubation began (i.e. females first left the nest) between 0452h and 0847h (BST) and ended (last entry into the nest) between 1705h and 2043h. This variation in the start and end of daytime incubation meant that the length of this active day varied greatly between recordings (range: 10.3–14.9 hours; mean ± SD = 12.84 ± 1.08 hours). Variation in the length of the active day was primarily explained by day length (Table 5.1). Other variables - embryo age, mean daily temperature, mean daily temperature2, mean rainfall and attempt - were present in the top model set (i.e. those within two AICc points of the best model), but for these variables the 95% confidence intervals indicated that zero effects could not be excluded (Tables 5.1 and S4.3). For the timings of first incubation recess or last entry in the day (measured relative to sunrise and sunset, respectively), embryo age, temperature, rainfall and day length were retained in the top model set but the 95% confidence intervals of their parameter estimates indicated that zero effects could not be excluded (Tables 5.1, S4.4 and S4.5). Attempt number was also retained in the top set of models for the timings of the last entry to the nest but again 95% confidence intervals indicated that zero effects could not be excluded (Tables 5.1 and S4.4). In the top set of models explaining the timings of the first incubation recess, clutch size and the relative incubation start date were also retained but zero effects could not be excluded (Tables 5.1 and S4.5).

Nest attentiveness varied greatly between breeding attempts from 25.3% to 76.6% (mean ± SD = 59.0 ± 9.8%; median = 60.8%), as did the length of incubation bouts (mean ± SD = 22.9 ± 5.5 mins; median = 22.5 mins; range = 1–128 mins) and length of incubation recesses (mean ± SD = 16.7 ± 13.5 mins; median = 13.1 mins; range = 2–439 mins).

**Table 5.1.** The results of linear mixed models (LMMs) investigating the effects of embryo age, mean daily temperature, mean daily rainfall, clutch size, relative incubation start date, attempt and day length on duration of the active day, time of last entry before sunset and time of leaving after sunrise in long-tailed tits. Shown are model-averaged parameter estimates and 95% confidence intervals (CI) for fixed effects, variance for the random effects, R2LMM(m) andR2LMM(c) from the best fitting models. 95% CI in bold highlight which indicate that variables have non-zero effects.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Response variable*** | *Active Day (N = 89)* | | *Last entry before sunset (N = 137)* | |  | *Leaving after sunrise (N = 131)* | | |
| Fixed Effects | **Slope (SE)** | **95% CI** | **Slope (SE)** | **95% CI** |  | **Slope (SE)** | **95% CI** |
| **Intercept** | -2.58 (2.47) | - |  | - | **Intercept** | 1.98 (0.44) | - |
| **Embryo age** | -0.04 (0.06) | -0.15; 0.08 | 0.37 (1.16) | -1.91; 2.66 | **Embryo age** | 0.03 (0.02) | -0.01; 0.07 |
| **Mean daily temperature** | -0.46 (0.96) | -2.34; 1.42 | 17.8 (44.6) | -70.3; 106 | **Mean nightly temperature** | 0.72 (0.62) | -0.53; 1.99 |
| **Mean daily temperature2** | -0.28 (0.63) | **-**1.53; 0.97 | -0.19 (0.38) | -32.2; 191 | **Mean nightly temperature2** | 0.94 (0.62) | -0.31; 2.15 |
| **Mean daily rainfall** | 0.33 (0.48) | -0.62; 1.27 | -5.00 (13.5) | -31.6; 21.6 | **Mean nightly rainfall** | 0.60 (0.42) | -0.23; 1.45 |
| **Attempt** | 0.06 (0.18) | -0.29; 0.42 | - | - | **Clutch size** | -0.003 (0.005) | -0.01; 0.007 |
| **Day length** | 1.07 (0.18) | **0.71; 1.43** | - | - | **Day length** | 0.009 (0.01) | -0.01; 0.03 |
|  |  |  |  |  | **Relative incubation start date** | -0.002 (0.003) | -0.01; 0.03 |
| Random Effect | **Variance (SE)** |  | **Variance (SE)** |  |  | **Variance (SE)** |  |
| **Nest ID** | 0.067 (0.23) |  | 194 (13.6) |  |  | 2.56E-08 (7.81E-05) |  |
| **Female ID** | 0.23 (0.48) |  | 153 (12.2) |  |  | 0.20 (0.45) |  |
| **Year** | 3.17E-11 (1.694E-06) |  | 24.5 (3.80) |  |  | 0.005 (0.04) |  |
| **R2LMM(m)** | 0.42 |  | 0.04 |  |  | 0.03 |  |
| **R2LMM(m)** | 0.66 |  | 0.26 |  |  | 0.13 |  |

**Table 5.2.** The results of linear mixed models (LMMs) investigating the effects of time, embryo age, mean temperature, clutch size, relative incubation start date, mean rainfall, attempt, female age and day length on hourly nest attentiveness, mean hourly duration of incubation bouts and mean hourly duration of incubation recesses in long-tailed tits. Shown are model-averaged parameter estimates and 95% confidence intervals (CI) for fixed effects, variance for the random effects, R2LMM(m) andR2LMM(c) from the best fitting models. 95% CI in bold highlight which indicate that variables have non-zero effects.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Response variable*** | *Hourly nest attentiveness (N = 1573)* | | *Mean hourly duration of incubation bouts (N =1666)* | | *Mean hourly duration of incubation recesses (N = 1742)* | |
| Fixed Effects | **Slope (SE)** | **95% CI** | **Slope (SE)** | **95% CI** | **Slope (SE)** | **95% CI** |
| **Intercept** | 0.77 (0.10) | - | 2.97 (0.16) | - | 2.01 (0.32) | - |
| **Time** | -0.004 (0.002) | **-0.008; -0.0006** | -0.01 (0.004) | **-0.02; -0.003** | 0.008 (0.004) | **0.0010; 0.015** |
| **Embryo age** | 0.03 (0.006) | **0.01; 0.04** | 0.003 (0.003) | **-0.003; 0.01** | -0.04 (0.01) | **-0.07; -0.02** |
| **Mean temperature** | -1.21 (0.37) | **-1.94; -0.37** | -4.29 (0.74) | **-5.75; -2.84** | 2.53 (0.75) | **1.06; 4.00** |
| **Mean temperature2** | -0.97 (0.27) | **-1.50; -0.43** | -1.65 (0.58) | **-2.79; -0.52** | 0.65 (0.58) | -0.49; 1.79 |
| **Clutch size** | -0.003 (0.003) | -0.008; 0.003 | 0.008 (0.006) | -0.003; 0.02 | 0.08 (0.03) | **0.02; 0.14** |
| **Mean rainfall** | -0.0006 (0.001) | -0.003; 0.002 | 0.0008 (0.002) | -0.004; 0.004 | -0.01 (0.03) | -0.06; 0.04 |
| **Relative incubation start date** | -0.0006 (0.0004) | -0.001; 0.0005 | -0.002 (0.001) | -0.004; 0.0004 | 0.0010 (0.0024) | -0.004; 0.006 |
| **Day length** | 0.0008 (0.003) | -0.004; 0.006 | 0.009 (0.007) | -0.005; 0.02 | - | - |
| **Attempt** | 0.01 (0.009) | -0.01; 0.03 | 0.10 (0.05) | -0.001; 0.21 | - | - |
| **Female age** | 0.007 (0.005) | -0.004; 0.02 | 0.01 (0.009) | -0.006; 0.03 | - | - |
| Random Effect | **Variance (SE)** |  | **Variance (SE)** |  | **Variance (SE)** |  |
| **Nest ID** | 0.005 (0.07) |  | 0.03 (0.15) |  | 0.03 (0.16) |  |
| **Female ID** | 0.008 (0.09) |  | 6.71E-9 (5.29E-5) |  | 0.04 (0.19) |  |
| **Year** | 0.0005 (0.02) |  | 2.17E-9 (2.53E-5) |  | 0.001 (0.03) |  |
| **R2LMM(m)** | 0.06 |  | 0.06 |  | 0.09 |  |
| **R2LMM(m)** | 0.27 |  | 0.16 |  | 0.29 |  |

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Description automatically generated**

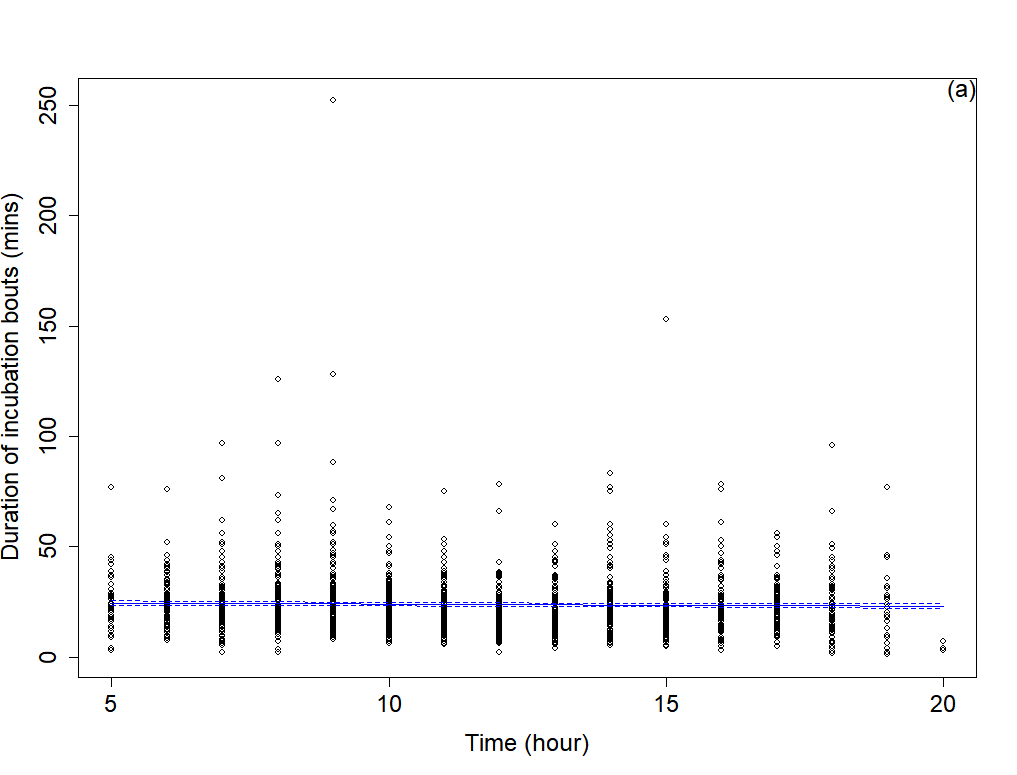
**Figure 5.1.** Female long-tailed tit hourly nest attentiveness (proportion of time spent in the nest during one hour) in relation to (a) the time of day, (b) embryo age, and (c) the mean ambient temperature. The solid lines indicate the predicted values from model-averaged parameters and dashed lines indicate the standard error.

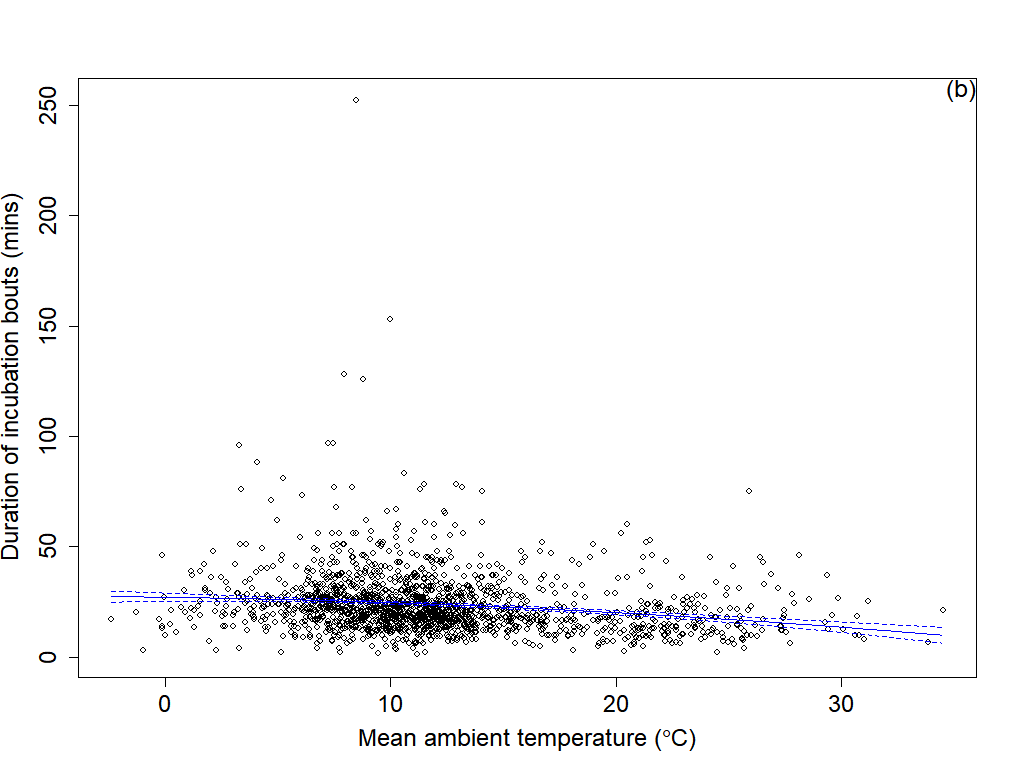
Both nest attentiveness (Tables 5.2 and S4.6; Fig 5.1a) and the duration of incubation bouts (Tables 5.2 and S4.7; Fig 5.2a) decreased through the day, whilst the duration of incubation recesses increased (Table 5.2 and S4.8; Fig 5.3a).

Nest attentiveness and the mean duration of incubation bouts were positively correlated with embryo age, although non-zero effects could only be excluded in the nest attentiveness model (Tables 5.2, S4.6 and S4.7; Fig 5.1b). The duration of incubation recesses declined as embryos aged (Tables 5.2 and S4.8; Fig 5.3b).

Mean ambient temperature effected all measures of incubation behaviour (Tables 5.2), with nest attentiveness (Fig 5.1c) and mean duration of incubation bouts being highest at intermediate temperatures (Fig 5.2b), whilst the mean duration of incubation recesses was shortest at intermediate temperatures (Fig 5.3c). In contrast, mean rainfall had relatively minor effects on measures of incubation behaviour, although rainfall was present in all of the top model sets (Tables S4.6, S4.7 and S4.8). It was negatively correlated with nest attentiveness and the duration of incubation recesses, whilst the duration of incubation bouts increased with increasing rainfall (Tables 5.2).

Females with larger clutches had longer incubation recesses than those with smaller clutches (Tables 5.2 and S4.8, Figure 5.3d). Those with larger clutches also tended to have longer incubation bouts and lower nest attentiveness, although the 95% confidence intervals indicated that zero effects cannot be excluded (Tables 5.2, S4.6 and S4.7). Greater nest attentiveness and longer incubation bouts were also associated with earlier incubation start dates, second breeding attempts, longer days and older females, although all these effects were minor (Tables 5.2, S4.6 and S4.7). Longer incubation recesses were weakly associated with later starts to incubation (Tables 5.2 and S4.8).

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**Figure 5.2.** The mean hourly duration of long-tailed tit incubation bouts (in minutes) in relation to (a) the hour of the day and (b) the mean hourly ambient temperature. The solid lines indicate the predicted values from model-averaged parameters and dashed lines indicate the standard error.

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**Figure 5.3.** The mean hourly duration of long-tailed tit incubation recess (in minutes) in relation to (a) the hour of the day, (b) embryo age, (c) the mean ambient temperature and (d) clutch size. The solid lines indicate the predicted values from model-averaged parameters and dashed lines indicate the standard error.

### **5.3.2 Incubation period and hatching success**

Mean nest attentiveness was correlated with the duration of the incubation period and hatching success (Table 5.3). As predicted, higher nest attentiveness associated was shorter incubation periods, but counter-intuitively, it was also associated with lower hatching success. Any effects of nest insulation quality on incubation period duration, hatching success and nest attentiveness were minor (Table 5.3).

## **5.4 Discussion**

We found that incubation behaviour, measured by nest attentiveness and the duration of incubation bouts and recesses, were associated with temperature and the timing within the day. Nest attentiveness and the duration of incubation bouts decreasing as the day progressed and being greater at intermediate temperatures. In contrast, the duration of incubation recesses increased through the day and were lowest at intermediate temperatures. Females incubating older embryos had higher nest attentiveness and shorter incubation recesses, while those with larger clutches were shown to take longer incubation recesses. All other factors – mean rainfall, relative incubation start date, attempt number, day length and female age – had a negligible influence on incubation behaviour although collinearity prevented the effects of day length being distinguished from the duration of the active day. We also found that incubation behaviour influenced the duration of the incubation period and hatching success, with higher nest attentiveness being associated with shorter incubation periods and lower hatching success.

**Table 5.3.** The results of linear mixed models investigating the effects of nest attentiveness and nest insulation on duration of the incubation period and general linear mixed models investigating the effects of duration of the incubation period, nest attentiveness and nest insulation quality on hatching success. Shown are model-averaged parameter estimates and 95% confidence intervals (CI) for fixed effects, variance for the random effects, R2LMM(m) andR2LMM(c) from the best fitting models. 95% CI in bold highlight which indicate that variables have non-zero effects.

|  |  |  |
| --- | --- | --- |
| Model effects |  |  |
| ***Incubation Period (N =32)*** |  |  |
| *Fixed effects* | Slope (SE) | 95% CI |
| Intercept | 2.99 (0.09) | - |
| Mean nest attentiveness | -0.33 (0.14) | **-0.62; 3.17** |
| *Random effects* | Variance (SE) |  |
| Year | 8.47E-05 (9.20E-03) |  |
| Female identity | 4.24E-03 (6.51E-02) |  |
| *R2(m)* = 0.14; *R2(c)* = 0.74 |  |  |
|  |  |  |
| ***Incubation Period (N = 21)*** |  |  |
| *Fixed effects* | Slope (SE) | 95% CI |
| Intercept | 2.59 (0.22) | - |
| Nest insulation quality | 2.93 (3.18) | -3.69; 9.56 |
| *Random effects* | Variance (SE) |  |
| Year | 0 (0) |  |
| *R2(m)* = 0.05; *R2(c)* = 0.05 |  |  |
|  |  |  |
| ***Hatching success (N = 24)*** |  |  |
| *Fixed effects* | Slope (SE) | 95% CI |
| Intercept | 5.13 (2.47) | - |
| Mean nest attentiveness | -6.06 (3.96) | **-14.68; -1.64** |
| *Random effects* | Variance (SE) |  |
| Year | 0.18 (0.43) |  |
| Female identity | 0.79 (0.89) |  |
| *R2(m)* = 0.03; *R2(c)* = 0.18 |  |  |
|  |  |  |
| ***Hatching success (N = 15)*** |  |  |
| *Fixed effects* | Slope (SE) | 95% CI |
| Intercept | 0.89 (2.18) | - |
| Nest insulation quality | 8.49 (32.56) | -60.67; 39.34 |
| *Random effects* | Variance (SE) |  |
| Year | 0 (0) |  |
| *R2(m)* = 0.001; *R2(c)* = 0.001 |  |  |
|  |  |  |
| ***Mean nest attentiveness (N = 21)*** |  |  |
| *Fixed effects* | Slope (SE) | 95% CI |
| Intercept | 0.82 (0.17) | - |
| Nest insulation quality | -3.29 (2.46) | -8.35; 2.68 |
| *Random effects* | Variance (SE) |  |
| Year | 0.002 (0.04) |  |
| *R2(m)* = 0.06; *R2(c)* = 0.34 |  |  |

Incubation behaviour had an important effect on both the duration of incubation period and hatching success. Just as in other intraspecific studies (Lyon & Montgomerie 1985; Nilsson & Smith 1988; Reid *et al.* 2002; Martin *et al.* 2018), high nest attentiveness was associated with shorter incubation periods in long-tailed tits, but counterintuitively, it was also correlated with lower hatching success. This is surprising given that we have previously demonstrated that shorter incubation periods were associated with greater hatching success (see chapter 3), and thus would expect that higher nest attentiveness to be positively associated with hatching success. However, nest attentiveness is only one factor that contributes to egg temperature; others include the location of eggs within clutches, clutch size and brood-patch temperature, all of which are argued to have a greater effect on egg temperature (Martin *et al.* 2018). The mechanism leading to lower hatching success when nest attentiveness is high is unclear and further detailed work would be required to investigate how those other factors vary with nest attentiveness and affect hatching success in long-tailed tits. We found that nest insulation quality appears to have only minor effects the nest attentiveness, the duration of the incubation period or hatching success. Deeming & Gray (2016) have shown that between species the quality of nest insulation influences female nest attentiveness, although our result indicates that this pattern might not hold within species.

We find that all aspects of incubation behaviour were affected by the time of day, with nest attentiveness and duration of incubation bouts decreasing throughout the day, and the duration of incubation recesses increasing. A relationship between time of day and incubation behaviour has been shown in other studies, which have suggested that increased nest attentiveness in the morning is due to lower ambient temperatures at that time (Haftorn 1979; Bambini *et al.* 2018). However, this is unlikely in our study because we accounted for ambient temperature in our analysis. An alternative possibility is that lower nest attendance in the afternoon is caused by females requiring more time to forage in order to gain the energy required for a full night of incubation.

All measures of incubation behaviour are correlated with ambient temperature, with nest attentiveness and the duration of incubation bouts peaking at intermediate temperatures, and incubation recesses being shortest at intermediate temperatures. Conway and Martin (2000b) argued that a low ambient temperature, incubation is more energetically expensive (Vleck 1981) so females require longer incubation recesses to gain the energy required to incubate the clutch. In contrast, at intermediate to high temperatures incubation is less energetically expensive meaning that females can to incubate longer, although at higher ambient temperatures these longer incubation bouts are unnecessary so females spend less time on the nest (Conway & Martin 2000b; Walters *et al.* 2016; Bambini *et al.* 2018). This may explain why incubation periods increased at higher temperatures in the long-term dataset (see chapter 3). If nest attentiveness is relatively high between 0 and 15˚C (Fig 5.1) and declines only above this temperature range, clutches that experienced relatively low ambient temperatures would be incubated more consistently and hence would develop faster than those experiencing higher ambient temperatures. This explanation is also consistent with higher nest attentiveness being linked to shorter incubation periods in this study.

Contrary to our predictions, the relationship between rainfall and incubation behaviour was linear, and explained only a negligible amount of variation in incubation behaviour. This result is somewhat surprising given that we previously found that longer incubation periods were associated with a greater proportion of rainy days (see chapter 3 for this result and chapter 6 for further discussion). However, in chapter 4 we found that nests effectively buffered any effects of rainfall on nest conductance. Moreover, while rainfall has been reported to affect incubation behaviour in open-nesting species (Fu *et al.* 2017) and those that feed by hawking insects in the air (Coe *et al.* 2015), it has no significant effect on incubation behaviour in blue tits either (Bambini *et al.* 2018). Therefore, the enclosed nests of long-tailed tits and the fact that they primarily glean insects from branches may explain why their incubation behaviour is not affected by rainfall.

As incubation progressed and embryos aged, we found that females increased nest attentiveness and reduced incubation recesses. This age-specific pattern of incubation has also been reported in other passerines, including dusky flycatchers *Empidonax oberholseri*, great tits *Parus major*, black-capped chickadees *Poecile atricapillus* and Carolina chickadees (Webb 1987; Cooper & Voss 2013; Walters *et al.* 2016). Cooper and Voss (2013) found that the cooling rates of embryos were increased with embryo age requiring females to adjust their incubation behaviour by shortening incubation recesses to maintain optimum embryo temperatures.

Larger clutches require more energy to incubate, so it is argued that incubation recesses need to be longer to recoup the necessary energy for incubation (Reid et al., 2000b; Wiebe & Martin, 2000). However, Reid *et al.* (2000b) showed that larger clutches also cooled at a slower rate, so the temperature of the eggs were on average higher than in smaller clutches, mitigating heat loss during these longer incubation recess. This would explain why larger clutches are associated with longer incubation recesses in this study.

Contrary to our expectations there were only minor seasonal changes in incubation behaviour, although we did find that the active day was a function of day length. Other studies have found that the active day is a function of day length (Wheelwright & Beagley 2005; Álvarez & Barba, 2014). However in these studies, the birds also adjusted their incubation behaviour with these changes in day length - in great tits, the mean duration of off-bouts was lowest at intermediate day lengths (Álvarez & Barba, 2014) and savannah sparrows *Passerculus sandwichensis* decrease their incubation bouts throughout the season (Wheelwright & Beagley 2005). We found no such adjustment in long-tailed tits, indicating that as the breeding season progresses nest attentiveness within a 24-hour period decreases in long-tailed tits due to the nights becoming shorter. However, although we verified that our measure is a good indication of when the female is present at the nest, we were unable to check whether she was actively incubating the eggs for this entire period. Future work should aim to place the thermistor probe in a fake egg to obtain more accurate timings for the duration of contact incubation.

Older females are more proficient than younger females at a number of reproductive behaviours in other species (Wheelwright & Schultz 1994; Forslund & Pärt 1995; Daunt *et al.* 1999), although senescence in later life may cause individuals to become less proficient at reproductive tasks (Robertson & Rendell 2001; Monaghan *et al.* 2008; Jankowiak & Wysocki 2016). In this study, individuals ranged in age from 1 to 5 years old, and we found negligible effects of female age on nest attentiveness and incubation bout duration. This may be because there were few older females in our dataset, although our result is in line with some previous work, studies that found no significant relationship between age and incubation behaviour (Wheelwright & Beagley 2005; Matysioková & Remeš 2010; Amininasab *et al.* 2016).

We confirm previous findings in a range of passerine species that incubation behaviour is driven by time of day and ambient temperature, with high nest attentiveness in the morning and at intermediate temperatures. Higher nest attentiveness and shorter incubation recesses were associated with older embryos. In addition, females with larger clutches took longer incubation recesses. Finally, we found evidence that incubation behaviour in long-tailed tits did impact on reproductive success, with nest attentiveness being significantly correlated with the duration of the incubation period and hatching success.

# **Chapter 6.**

# **General discussion**

## **6.1 Summary of results**

The amount of energy parents invest in a single reproductive attempt is likely to be influenced by many factors, including their own condition (Lens, Wauters & Dhondt 1994), their mate’s quality (Soler, Møller & Soler 1998; Mahr *et al.* 2012; Jelínek *et al.* 2016) and habitat quality (Hails & Bryant 1979). Investment decisions are then further complicated by variable environmental conditions, such as weather (Siikamiiki 1995; Nord & Nilsson 2012) and predation risk (Cresswell 2008). This thesis aimed to investigate the effects of variable environmental conditions on the duration of the incubation period and hatching success in long-tailed tits, and whether these effects can be ameliorated by changes in the placement and structure of nests, and incubation behaviour. Long-tailed tits were used as the study species due to their large investment in nest building and the variation noted in their nest placement, nest structure and incubation behaviour.

In chapter 2, I found that nest placement, specifically nest height, affected the likelihood that a breeding attempt was predated and the nest predator type that it is vulnerable to. However, nest height choice relative to predation risk was random in long-tailed tits, showing that direct experience and the relative abundance of nest predators does not influence nest height. In addition, heritability estimates and natal nest location explained little of the variation in nest height. However, I did find a seasonal trend in nest height, perhaps attributable to changes in vegetation over the season, because nest height was not influenced by breeding density so was not explained by limited availability of preferred nesting heights.

Then, in chapter 3, I found that timing of breeding, clutch size and weather conditions were associated with variation in the duration of the incubation period. Incubation periods were shorter later in the breeding season, for larger clutches, under higher ambient temperatures and lower rainfall. I also quantified the effect of a longer incubation period on the likelihood of predation - the predation risk decreased by 6% at the largest clutch size (12 eggs) and by the end of the breeding season. In addition, I found that at higher ambient temperatures and under an increased proportion of rainy days the likelihood of predation increases. Finally, I found that longer incubation periods were associated with lower hatching success, while younger females, those with larger clutches and first breeding attempts had higher hatching success.

In chapters 4 and 5, I investigated the possible mechanisms behind observed variation in the duration of the incubation period and hatching success, by observing the effects of seasonal and environmental conditions on nests and incubation behaviour. In chapter five, I found that incubation behaviour, as measured by nest attentiveness, was strongly correlated with the duration of the incubation period, with higher nest attentiveness being associated with shorter incubation periods. Given the seasonal decline in incubation periods reported in chapter 3, it was somewhat surprising, therefore, that there was negligible change in incubation behaviour over the breeding season. Furthermore, there was no significant effect of time of breeding on nest insulation quality or the proportion of feathers in nests, although nest mass and feather mass did decline through the breeding season (chapter four). The results from chapter five support the Conway and Martin (2000) model that suggests the relationship between ambient temperature and incubation behaviour is not linear: females spend more time incubating at intermediate temperatures (between 0 and 15˚C). This result is consistent with the relationship established in chapter three between incubation period duration and ambient temperature, which showed that incubation periods were shorter at lower ambient temperatures. In addition, I discovered that nest insulation quality was not affected by ambient temperature (chapter 4). I also found that simulated rainfall had no significant effect on the nest insulation quality (chapter 4), and rainfall had only a negligible effect on all measures of incubation behaviour (chapter 4). These are surprising results given that an increased proportion of rainy days lengthened the incubation period, albeit only marginally at typical clutch sizes (chapter 3).

## **6.2 Implications and future directions**

### **6.2.1 Weather effects**

Low ambient temperatures are generally thought to negatively impact egg temperature during incubation, because the energetic requirements for the incubating parent are increased (Bryan and Bryant 1999). Coe et al. (2015) demonstrated that the duration of the incubation period was significantly positively correlated with the amount of time the eggs spent below physiological zero temperature (26˚C) in tree swallows *Tachycineta bicolor*. Therefore, I expected that at higher ambient temperatures eggs would spend less time below this temperature and therefore higher ambient temperatures would be linked to shorter incubation periods. However, contrary to my prediction, higher ambient temperatures were correlated with longer incubation periods in long-tailed tits (chapter three). Following analysis of incubation behaviour, I showed that this relationship between ambient temperature and the incubation period is probably driven by incubation behaviour because females spend a greater proportion of their time incubating when ambient temperatures were relatively low (chapter five). Females have also been shown to incubate for longer at low ambient temperatures in a number of other passerine species (Voss *et al.* 2006; Amininasab *et al.* 2016; Walters *et al.* 2016). If this pattern of increased incubation period holds for other species it suggests that climate change will impact the duration of the incubation period, which in turn would alter hatching date. Much of the previous research on the effects of climate change have focused on the timing of the breeding initiation (e.g. Charmantier *et al.*, 2008; Schaper *et al*., 2012; Visser, van Noordwijk, Tinbergen, & Lessells, 1998) and some work on the duration of egg laying period (Haftorn 1981; Nilsson & Svensson 1993; Simmonds *et al.* 2017) in relation to peak food abundance. In line with this long-tailed tits have advanced their first egg date over the last 40 years (Gullet *et al.* 2013). However, my results may indicate that this relationship between ambient temperature and incubation period may mean that hatching date has not advanced as much as this. Pied flycatchers *Ficedula hypoleuca* and great tits *Parus major* have reduced the interval between laying and hatching of eggs with increasing spring temperatures (Both & Visser 2005; Visser *et al.* 2006), but this may not be the case in other species, such as the long-tailed tit. My results indicate that to fully understand the ability of birds to adapt in response to climate change, further research should investigate the relationship between changes in hatching date and peak food abundance, not just timing of laying. In addition, much of the work which has investigated the effects of phenology on timing of breeding has been done in pied flycatchers and great tits, these other two species are secondary cavity nesters which readily use nest boxes, which may mean they are affected and able to adapt in similar ways. The variation observed long-tailed tit incubation period duration may be partly due to their nesting ecology as they build enclosed nests (Figure 6.1). The utilisation of large databases like the BTO nest record scheme to analyse changes in timing of hatching may be required. Also, more detailed studies of variation the incubation period of cup nesting as detailed breeding behaviour is recorded less frequently in these species. For example, data from the BTO nest record scheme suggests that common blackbirds (*Turdus merula*) and song thrush (*Turdus philomelos*) are advancing their egg laying by as much as 9 days (BTO, 2020), further investigation of these species may reveal that this trait has greater plasticity than previously thought. This work should concentrate on the effects of ambient temperature on incubation behaviour and incubation period.



Figure 6.1. Long-tailed tits construct an enclosed nest, which they build in the forks of trees and in bushes. Photo credit: Caitlin Higgott.

Another predicted consequence of climate change is that mean rainfall will decrease during the spring and summer but the intensity of rainfall events will increase (UKCP, 2019). Rainfall can also potentially affect the duration of the incubation period and incubation behaviour, although this aspect of environmental conditions has received less attention than ambient temperature. In chapter 3, I found that an increased proportion of rainy days was associated with longer incubation periods. I predicted that such an association would either be due to the effects of rainfall on the conductance of the nest materials or on incubation behaviour. However, in chapter 4, simulated rainfall under controlled conditions had no significant impact on nest insulation quality even when the nests were completely saturated, which means that it is unlikely the observed increase in incubation period with rainfall was due to increased nest conductance. In addition, in chapter five, hourly incubation behaviour was not associated with mean hourly rainfall, a result that is contrary to studies that have found that incubation behaviour is affected by rainfall (Coe *et al.* 2015; Fu *et al.* 2017). This difference in results between this and previous studies may be due to differences in life-history traits between study species. It is also possible that whilst females exhibited only a slight decrease in their nest attentiveness with increased rainfall, following a more extended period of rainfall they may change their behaviour more substantially. Further investigation should be conducted into the effects of prolonged periods of rainfall on incubation behaviour. Another alternative explanation is that whilst time spent on the nest remains similar, the effectiveness of incubation may be reduced under increased rainfall. Variation in brood patch temperature and its impact on egg temperature have received little attention, although an interspecific study has indicated that higher brood patch temperatures are associated with shorter incubation periods (Deeming 2008). Brood patch temperature was not associated with duration of the incubation period in barn swallows *Hirundo rustic* (Hasegawa *et al.* 2016), although in that study only one measurement of the brood patch temperature was taken during the incubation period and only ambient temperature at the time of the recording was accounted for. Therefore, future studies should further investigate the effects of weather conditions on egg temperature, and to quantify any variation of brood patch temperature throughout the incubation period.

### **6.2.2 Seasonal effects**

Woodland passerines that primarily provision their offspring with caterpillars have been shown to adjust their lay dates and incubation behaviour so that the timing of hatching better coincides with peak caterpillar abundance (Visser *et al.* 1998; Both & Visser 2005; Simmonds *et al.* 2017). Incubation periods decreased through the breeding season in long-tailed tits (chapter 3), which would be predicted to result in the provisioning period being closer to the peak of food abundance for later broods. Over the course of the study of long-tailed tits in the Rivelin Valley, mean hatching date was 11th May and latest hatch date was 16th June. Previous work by Gullett (2014) indicated that caterpillar abundance peaked in the Rivelin Valley around 23rd May, with relatively few caterpillars remaining by 30th June. Gullett (2014) found that a majority of late nests (which fledged after the peak caterpillar abundance) were provisioned during the period close enough to the peak that the proportion of dietary caterpillars provided to nestlings was not affected, apart from a single nest which fledged very late. The reduction in incubation period towards the end of the breeding season may explain how females who start incubation later are able to match hatching to this period.

An increase in food availability during the incubation period later in the season may be important in allowing females to decrease the incubation period, because increased food availability has been shown to increase nest attentiveness in other species (Dewey & Kennedy 2001; Duncan Rastogi *et al.* 2006), However, as shown in chapter five, relative incubation start date and day length explained a negligible amount of the variation in incubation behaviour. In fact, the active day (the time between the female first exiting the nest in the morning and entering the nest for the last time in the evening) was determined by the number of daylight hours, and given that long-tailed tits finish breeding before the summer solstice, females must have spent less time incubating later in the breeding season (assuming that they incubate constantly through the night). Thus, a greater proportion of time in a 24-hour cycle would be spent away from the nest. It is possible that increased time away from the nest allows females to feed more, gaining more energy, and thus allows them to increase their brood patch temperature and incubate more effectively. On the other hand, Ardia and Clotfelter (2007) found that egg temperature was unaffected by insect availability during incubation in tree swallows*.* It should also be borne in mind that increased time spent foraging does not necessarily mean that individuals find more food, although studies have shown that increased time foraging can lead to individuals finding higher quality prey items (Lucas 1983; Lucas 1985; Tremblay *et al.* 2005). In future studies, it would be interesting to examine whether there is any relationship between egg temperature and female diet during incubation, this may be accomplished by collecting female faecal samples from the nest and using metabarcoding to identify the female’s diet.

Another potential explanation for the seasonal decrease in incubation period is fewer temperature fluctuations during incubation recesses because this would lead to more rapid development (Olson *et al.* 2006). Fewer temperature fluctuations during incubation recesses could be due to a combination of the consistency of nest insulation quality through the season (chapter 4; McGowan *et al.* 2004) and ambient temperatures generally increasing later in the breeding season. This would mean that nest contents should remain at a higher temperature when the female is not incubating. Again, it would be interesting in future studies of seasonal variation in incubation periods and incubation behaviour to examine fine-scale variation in egg temperature, especially during incubation recesses.

### **6.2.3 Nest predation**

The timing of breeding and weather conditions influenced the length of the incubation period, and hence the exposure of nests to the risk of predation (chapter three). Early nests and smaller clutches which experienced higher ambient temperatures and greater rainfall were all associated with longer incubation periods and hence a longer period of exposure to predators. In other species, larger clutches and broods increase predation risk because they require larger nests that may attract nest predators (Møller 1990; Soler *et al.* 1998), take longer to complete the clutch (Johnsgard 1973; Perrins, 1977), and require more activity around the nest when feeding nestlings (Skutch 1949; Martin *et al.* 2000). This study indicates that larger clutches may also mitigate this higher risk of predation, by reducing the length of the incubation period and hence exposure to predators. Changes in weather conditions may also affect predator behaviour and the availability of other prey. For example, rainfall is also associated with lower levels of nest predation in certain species (Morrison & Bolger 2002; Preston & Rotenberry 2006). The mode that nest predators use to detect nests may mean that different predator types are affected differently by these various factors, i.e. if rainfall decreases the number of visits that parents make to the nest, then there will be less activity for around the nest and reduce the chances of visual predators detecting the nest. In addition, in other taxa, rainfall has been shown to decrease the likelihood of nests being discovered by olfactory predators (Bowen & Janzen, 2005). I had no independent measures of predator abundance or activity with which to examine risk in a quantitative manner, but there is very likely to be variation within and between seasons. In future studies it would be interesting to explore the extent to which the actual threat of nest predation during incubation is mitigated and/or exacerbated by variation in environmental conditions.

The risk of nest predation was influenced by nest height with higher nests being more likely to be predated than lower nests (chapter two). However, in contrast to other work (Marzluff 1988; Martin & Martin 2001; Fontaine & Martin 2006; Peluc *et al.* 2008), pairs did not appear to change their nest height in reaction to personal experience of a nest predator or the relative abundance of nest predators. However, my measure of relative predator abundance was a coarse one based upon the relative number of losses caused by different nest predators within the whole study site. As suggested earlier, future studies should directly assess local predator abundance to better estimate the influence of predator density on nest site selection. The relative number of losses caused by a nest predator could be used by long-tailed tits as a source of public information because pairs do not defend exclusive territories and often wander widely during the breeding season. Thus, they routinely encounter other pairs and visit nests other than their own, giving them the opportunity to learn from other pairs and to base their future nest-site choice on public information about the probability of success in relation to nest sites. This use of public information to inform nest site choices has been reported in a number of passerines, such as collared flycatchers *Ficedula albicollis* (Doligez *et al.* 2002, 2004)*,* blue tits *Cyanistes caeruleus* (Parejo *et al*. 2007), northern cardinals *Cardinalis cardinalis* (Kearns & Rodewald 2013) and spotless starlings *Sturnus unicolor* (Parejo *et al.* 2008). It would be interesting for future studies to investigate whether the fate of breeding attempts that have previously been visited, or pairs that have been interacted with affect an individual’s future choices.

There was a high degree of behavioural plasticity in nest site selection, with nest height having low repeatability and a small genetic component, consistent with results from a previous study in dark-eyed juncos *Junco hyemalis* (Yeh *et al.* 2007). I suggest that this low heritability is attributable to the fact that all nests, regardless of height, have a substantial risk of predation. Moreover, changes in the relative prevalence of mammalian and avian predators between years means that inheritance, imprinting or copying of nest site choice would not necessarily lead to success.

I did establish that nest height was positively correlated with first egg date; this may be related to leaf emergence on trees, which makes nests in tree forks less visible to predators. On the other hand, the same is true of low nests situated in deciduous plants, such as hawthorn or dog-rose, although not for those placed in evergreen holly or bramble (both of which are typically < 2m from the ground). Climatic effects may also explain seasonal changes in nest height. Nesting low down early in the breeding season may reduce exposure to negative abiotic conditions, such as wind speed, which is lower closer to the ground (With and Webb 1993; Forstmeier and Weiss 2004). Nest microclimate may provide more consistent selection pressure than nest predation in shaping nest site selection in long-tailed tits. Future studies should aim to quantify variation in microclimate between vegetation types and at different nesting heights and investigate whether individuals select nest sites that have similar microclimates to their parents.

## **6.3 Conclusions**

In this thesis, I found that the incubation behaviour and duration of the incubation period of long-tailed tits were influenced by timing of breeding and weather conditions. Moreover, longer incubation periods reduced hatching success and increased exposure time to nest predators. Later breeding attempts were shown to have shorter incubation periods, which will likely be beneficial because hatching will occur closer to the peak of caterpillar abundance. The provisioning period coinciding with peak caterpillar abundance leads to the nestlings being heavier and having longer tarsi (Gullet, 2014). However, incubation behaviour is also established as an important driver of the duration of the incubation period, and I demonstrated that high ambient temperatures are associated with lower nest attentiveness and longer incubation periods. Previous studies have established that reducing incubation period is important for reducing phenological mismatch in other species (Both and Visser 2005; Visser *et al.* 2006), and thus this relationship between ambient temperature and incubation period could limit a female long-tailed tit’s ability to match timing of hatching with the peak of caterpillar abundance. As suggested earlier, provisioning offspring significantly outside of this peak in caterpillar abundance leads to the production of lighter offspring with shorter tarsi, which could affect their subsequent survival. In addition, I found that an increasing proportion of rainy days lengthened the incubation period. However, the mechanism for this remains unclear, because the results of chapters 4 and 5 indicate that the birds’ behaviour can buffer the effects of rainfall, as simulated rainfall did not affect their nest insulation quality and incubation behaviour was only marginally influenced by rainfall.

Choice of nest placement was not influenced by predation risk. I found behavioural plasticity in nest placement with little genetic influence. Experience of predation also appears to have little effect on nest placement, contrary to other studies (Marzluff 1988; Martin & Martin 2001; Fontaine & Martin 2006; Peluc *et al.* 2008). I attributed this to the high predation rate on all nests and variable nest predator types that makes no nesting height reliably better than any other.

Overall, breeding long-tailed tits exhibited behaviours that reduced the impact of variable abiotic conditions on their offspring, by building nests which buffer against changes induced by rainfall and increasing their incubating attentiveness at relatively low ambient temperatures. However, pairs did not appear to alter their behaviour, in terms of nest placement, dependent upon biotic conditions such as predation, perhaps due to the uncertainty surrounding these choices. Nevertheless, the evidence for the effectiveness of these changes in behaviour under variable abiotic conditions are mixed, as we find positive effects in relation to one environmental variable (ambient temperature) but not the other (rainfall). Increasing time spent incubating in low ambient temperatures does shorten the incubation period. This has positive knock-on effects, such as increased hatching success and a reduction in time exposed to nest predators. However, these positive changes in incubation period were not found with respect to variation in rainfall. In future, we should aim to better understand the relative importance of these factors and the interactions between them to fully understand their impacts. In conclusion, variation in nest-building and incubation behaviour can be an important way for individuals to mitigate changes in their environment in order to enhance their breeding performance.

# **Appendix 1**

**Table S1.1.** Summary of vegetation that nests were found in between 1994 and 2018 (N = 1425)

|  |  |  |
| --- | --- | --- |
| Nest location | N | Proportion |
| Bramble | 394 | 0.276 |
| Gorse | 226 | 0.159 |
| Holly | 218 | 0.153 |
| Birch | 158 | 0.111 |
| Conifer | 138 | 0.097 |
| Hawthorn | 86 | 0.060 |
| Oak | 33 | 0.023 |
| Sycamore | 27 | 0.019 |
| rose | 23 | 0.016 |
| elder | 13 | 0.009 |
| alder | 11 | 0.008 |
| berberis | 11 | 0.008 |
| privet | 10 | 0.007 |
| honeysuckle | 8 | 0.006 |
| ivy | 8 | 0.006 |
| larch | 7 | 0.005 |
| willow | 7 | 0.005 |
| hebe | 6 | 0.004 |
| ash | 5 | 0.004 |
| bracken | 5 | 0.004 |
| sapling | 5 | 0.004 |
| yew | 4 | 0.003 |
| blackthorn | 3 | 0.002 |
| fence | 3 | 0.002 |
| cedar | 2 | 0.001 |
| clematis | 2 | 0.001 |
| juniper | 2 | 0.001 |
| lime | 2 | 0.001 |
| beech | 1 | 0.001 |
| cherry tree | 1 | 0.001 |
| cotoneaster | 1 | 0.001 |
| heather | 1 | 0.001 |
| laurel | 1 | 0.001 |
| poplar | 1 | 0.001 |
| rhododendron | 1 | 0.001 |
| snowberry | 1 | 0.001 |

**S1.2. – Details of model implementation**

For the broad-scale nest height category models, given the ordinal nature of the data, we the fixed residual variance to 1. Whilst for random effects priors we used a weakly informative prior (V = 1, nu = 1000, alpha.mu = 0 and alpha.V = 1). In the case of the fine-scale nest height category models we also used weakly informative priors, setting V = 1 and nu = 0.002.

All MCMCglmm models were run for 1500100 iterations using a burn-in period of 1000 and a thinning interval of 5000. Our effective sample size was approximately 3000 for each parameter. The convergence of the MCMC chains was checked using the Heidelberg stationary test and were visually assessed. The autocorrelations of the posterior samples were also checked and were always below 0.1.

# **Appendix 2**

**Table S2.1.** The seven best fitting models (ΔAICc ≤ 2) that explain variation in the duration of the incubation period in long-tailed tits with two outliers of 23 and 26 days excluded. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| **Model configuration** | **AICc** | **ΔAICc** | **Wi** |
| Clutch size + relative incubation start date + mean temperature + proportion of rainy days + relative incubation start date \* mean temperature | 1256.22 | 0 | 0.22 |
| Clutch size + relative incubation start date + mean temperature + relative incubation start date \* mean temperature | 1256.43 | 0.21 | 0.20 |
| Clutch size + female age + relative incubation start date + mean temperature + proportion of rainy days + relative incubation start date \* mean temperature | 1256.83 | 0.61 | 0.16 |
| Clutch size + female age + relative incubation start date + mean temperature + relative incubation start date \* mean temperature | 1256.97 | 0.76 | 0.15 |
| Attempt + clutch size + relative incubation start date + mean temperature + proportion of rainy days + relative incubation start date \* mean temperature | 1257.73 | 1.51 | 0.10 |
| Attempt + clutch size + relative incubation start date + mean temperature + relative incubation start date \* mean temperature | 1258.02 | 1.81 | 0.09 |
| Attempt + clutch size + female age + relative incubation start date + mean temperature + proportion of rainy days + relative incubation start date \* mean temperature | 1258.13 | 1.92 | 0.08 |

**Table S2.2.** The best fitting models (ΔAICc ≤ 2) that explain variation in the duration of the incubation period in long-tailed tits with proportion of rainy days, and maximum and minimum mean daily temperatures rather than mean daily temperature. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Temperature Variable | Model configuration | AICc | ΔAICc | Wi |
| Maximum temperature | Clutch size + relative incubation start date + maximum temperature + proportion of rainy days + clutch size \* proportion of rainy days | 1306.33 | 0 | 0.31 |
|  | Clutch size + relative incubation start date + maximum temperature + proportion of rainy days + clutch size \* maximum temperature | 1307.00 | 0.67 | 0.22 |
|  | Clutch size + female age + relative incubation start date + maximum temperature + proportion of rainy days + clutch size \* proportion of rainy days | 1307.28 | 0.95 | 0.19 |
|  | Attempt + clutch size + relative incubation start date + maximum temperature + proportion of rainy days + clutch size \* proportion of rainy days | 1307.74 | 1.41 | 0.15 |
|  | Clutch size + female age + relative incubation start date + maximum temperature + proportion of rainy days + clutch size \* maximum temperature | 1308 | 1.67 | 0.13 |
| Minimum temperature | Clutch size + female age + relative incubation start date + proportion of rainy days + clutch size \* proportion of rainy days | 1306.09 | 0 | 0.41 |
|  | Attempt + clutch size + female age + relative incubation start date + proportion of rainy days + clutch size \* proportion of rainy days | 1306.46 | 0.37 | 0.34 |
|  | Clutch size + relative incubation start date + minimum temperature + proportion of rainy days + clutch size \* proportion of rainy days | 1307.02 | 0.93 | 0.26 |

**Table S2.3.** The best fitting models (ΔAICc ≤ 2) that explain variation in the duration of the incubation period in long-tailed tits with mean daily rainfall rather than proportion of rainy days. The table shows the models using mean daily temperature, maximum mean daily temperatures and minimum mean daily temperatures. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Temperature Variable | Model configuration | AICc | ΔAICc | Wi |
| Mean temperature | Clutch size + relative incubation start date + mean temperature + mean rainfall + clutch size \* mean rainfall | 1304.78 | 0 | 0.21 |
|  | Clutch size + relative incubation start date + mean temperature + mean rainfall + clutch size \* mean temperature | 1305.32 | 0.54 | 0.16 |
|  | Clutch size + female age + relative incubation start date + mean temperature + mean rainfall + clutch size \* mean rainfall | 1305.87 | 1.09 | 0.12 |
|  | Clutch size + relative incubation start date + mean rainfall + clutch size \* mean rainfall | 1306.00 | 1.22 | 0.12 |
|  | Attempt + clutch size + relative incubation start date + mean temperature + mean rainfall + clutch size \* mean rainfall | 1306.00 | 1.22 | 0.12 |
|  | Clutch size + female age + relative incubation start date + mean temperature + mean rainfall + clutch size \* mean temperature | 1306.16 | 1.38 | 0.11 |
|  | Attempt + clutch size + relative incubation start date + mean temperature + mean rainfall + clutch size \* mean temperature | 1306.51 | 1.73 | 0.09 |
|  | Attempt + clutch size + relative incubation start date + mean rainfall + clutch size \* mean rainfall | 1306.77 | 1.99 | 0.08 |
| Maximum temperature | Clutch size + relative incubation start date + maximum temperature + mean rainfall + clutch size \* maximum temperature | 1300.60 | 0 | 0.33 |
|  | Clutch size + female age + relative incubation start date + maximum temperature + mean rainfall + clutch size \* maximum temperature | 1301.32 | 0.72 | 0.23 |
|  | Attempt + clutch size + relative incubation start date + maximum temperature + mean rainfall + clutch size \* maximum temperature | 1301.79 | 1.19 | 0.18 |
|  | Attempt + female age + clutch size + relative incubation start date + maximum temperature + mean rainfall + clutch size \* maximum temperature | 1302.26 | 1.66 | 0.14 |
|  | Clutch size + relative incubation start date + maximum temperature + mean rainfall + clutch size \* mean rainfall | 1302.54 | 1.94 | 0.12 |
| Minimum temperature | Clutch size + relative incubation start date + mean rainfall + clutch size \* mean rainfall | 1306.00 | 0 | 0.33 |
|  | Attempt + clutch size + relative incubation start date + mean rainfall + clutch size \* mean rainfall | 1306.77 | 0.77 | 0.22 |
|  | Clutch size + female age + relative incubation start date + mean rainfall + clutch size \* mean rainfall | 1307.29 | 1.29 | 0.17 |
|  | Clutch size + relative incubation start date + mean rainfall + minimum temperature + clutch size \* mean rainfall | 1307.71 | 1.71 | 0.14 |
|  | Attempt + clutch size + female age + relative incubation start date + mean rainfall + clutch size \* mean rainfall | 1307.79 | 1.79 | 0.12 |

**Table S2.4.** The best fitting models (ΔAICc ≤ 2) that explain variation in the duration of incubation period in long-tailed tits with proportion of heavy rain days rather than proportion of rainy days. The table shows the models using mean daily temperature, maximum mean daily temperatures and minimum mean daily temperatures. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Temperature Variable | Model configuration | AICc | ΔAICc | Wi |
| **Mean temperature** | Clutch size + relative incubation start date + mean temperature + relative incubation start date \* mean temperature | 1308.20 | 0 | 0.11 |
|  | Clutch size + relative incubation start date + mean temperature + clutch size \* mean temperature | 1308.27 | 0.07 | 0.11 |
|  | Attempt + clutch size + relative incubation start date + mean temperature + relative incubation start date \* mean temperature | 1308.78 | 0.58 | 0.08 |
|  | Clutch size + female age + relative incubation start date + mean temperature + relative incubation start date \* mean temperature | 1308.94 | 0.74 | 0.08 |
|  | Clutch size + relative incubation start date + mean temperature + female age + clutch size \* mean temperature | 1309.03 | 0.83 | 0.07 |
|  | Clutch size + relative incubation start date + mean temperature + proportion of heavy rain days + clutch size \* mean temperature | 1309.08 | 0.88 | 0.07 |
|  | Clutch size + female age + relative incubation start date + mean temperature + proportion of heavy rain days + relative incubation start date \* mean temperature | 1309.13 | 0.93 | 0.07 |
|  | Attempt + clutch size + female age + relative incubation start date + mean temperature + relative incubation start date \* mean temperature | 1309.20 | 1.00 | 0.07 |
|  | Attempt + clutch size + relative incubation start date + mean temperature + clutch size \* mean temperature | 1309.30 | 1.10 | 0.06 |
|  | Attempt + clutch size + relative incubation start date + mean temperature + proportion of heavy rain days + relative incubation start date \* mean temperature | 1309.63 | 1.43 | 0.05 |
|  | Attempt + clutch size + female age + relative incubation start date + mean temperature + clutch size \* mean temperature | 1309.86 | 1.66 | 0.05 |
|  | Clutch size + female age + relative incubation start date + mean temperature + proportion of heavy rain days + clutch size \* mean temperature | 1309.86 | 1.66 | 0.05 |
|  | Clutch size + female age + relative incubation start date + mean temperature + proportion of heavy rain days + relative incubation start date \* mean temperature | 1309.89 | 1.69 | 0.05 |
|  | Attempt + clutch size + relative incubation start date + mean temperature + proportion of rainy days + clutch size \* mean temperature | 1309.96 | 1.76 | 0.05 |
|  | Attempt + clutch size + female age + relative incubation start date + mean temperature + proportion of heavy rain days + relative incubation start date \* mean temperature | 1310.05 | 1.85 | 0.04 |
| Maximum temperature | Clutch size + relative incubation start date + maximum temperature + clutch size \* maximum temperature | 1304.36 | 0 | 0.16 |
|  | Clutch size + relative incubation start date + maximum temperature + proportion of heavy rain days + clutch size \* maximum temperature | 1304.80 | 0.44 | 0.13 |
|  | Clutch size + female age + relative incubation start date + maximum temperature + clutch size \* maximum temperature | 1305.04 | 0.68 | 0.11 |
|  | Attempt + clutch size + relative incubation start date + maximum temperature + clutch size \* maximum temperature | 1305.45 | 1.09 | 0.09 |
|  | Clutch size + female age + relative incubation start date + maximum temperature + proportion of heavy rain days + clutch size \* maximum temperature | 1305.50 | 1.14 | 0.09 |
|  | Clutch size + relative incubation start date + maximum temperature + relative incubation start date \* maximum temperature | 1305.64 | 1.28 | 0.08 |
|  | Attempt + clutch size + relative incubation start date + maximum temperature + proportion of heavy rain days + clutch size \* maximum temperature | 1305.79 | 1.43 | 0.08 |
|  | Attempt + clutch size + female age + relative incubation start date + maximum temperature + clutch size \* maximum temperature | 1305.86 | 1.50 | 0.07 |
|  | Attempt + clutch size + female age + relative incubation start date + proportion of heavy rain days + maximum temperature + clutch size \* maximum temperature | 1306.20 | 1.84 | 0.06 |
|  | Clutch size + female age + relative incubation start date + maximum temperature + relative incubation start date \* maximum temperature | 1306.20 | 1.84 | 0.06 |
|  | Clutch size + relative incubation start date + maximum temperature + proportion of heavy rain days + relative incubation start date \* maximum temperature | 1306.34 | 1.98 | 0.06 |
| Minimum temperature | Clutch size + relative incubation start date | 1311.98 | 0 | 0.10 |
|  | Attempt + clutch size + relative incubation start date | 1312.01 | 0.03 | 0.10 |
|  | Clutch size + proportion of heavy rain days + relative incubation start date + proportion of heavy rain days \* relative incubation start date | 1312.59 | 0.61 | 0.08 |
|  | Attempt + clutch size + relative incubation start date + minimum temperature + relative incubation start date \* minimum temperature | 1312.67 | 0.69 | 0.07 |
|  | Clutch size + relative incubation start date + minimum temperature + relative incubation start date \* minimum temperature | 1312.68 | 0.70 | 0.07 |
|  | Attempt + clutch size + proportion of heavy rain days + relative incubation start date + proportion of heavy rain days \* relative incubation start date | 1312.72 | 0.74 | 0.07 |
|  | Attempt + female age + clutch size + relative incubation start date | 1312.87 | 0.89 | 0.06 |
|  | Attempt + clutch size + proportion of heavy rain days + relative incubation start date | 1312.90 | 0.92 | 0.06 |
|  | Clutch size + proportion of heavy rain days + relative incubation start date | 1313.07 | 1.09 | 0.06 |
|  | Clutch size + female age + relative incubation start date | 1313.19 | 1.21 | 0.06 |
|  | Attempt + clutch size + female age + relative incubation start date + minimum temperature + relative incubation start date \* minimum temperature | 1313.41 | 1.43 | 0.05 |
|  | Attempt + clutch size + female age + proportion of heavy rain days + relative incubation start date + clutch size \* proportion of heavy rain days | 1313.46 | 1.48 | 0.05 |
|  | Clutch size + female age + proportion of heavy rain days + relative incubation start date + clutch size \* proportion of heavy rain days | 1313.69 | 1.71 | 0.04 |
|  | Attempt + clutch size + relative incubation start date + clutch size \* relative incubation start date | 1313.76 | 1.78 | 0.04 |
|  | Attempt + clutch size + female age + proportion of heavy rain days + relative incubation start date | 1313.77 | 1.79 | 0.04 |
|  | Clutch size + relative incubation start date + minimum temperature | 1313.91 | 1.93 | 0.04 |
|  | Clutch size + relative incubation start date + clutch size \* relative incubation start date | 1313.92 | 1.94 | 0.04 |

**Table S2.5.** The three best fitting models (ΔAICc ≤ 2) that explain variation in the duration of the incubation period in long-tailed tits. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Clutch size + relative incubation start date + mean temperature + proportion of rainy days + clutch size \* proportion of rainy days | 1310.69 | 0 | 0.47 |
| Clutch size + female age + relative incubation start date + mean temperature + proportion of rainy days + clutch size \* proportion of rainy days | 1311.65 | 0.96 | 0.29 |
| Attempt + clutch size + relative incubation start date + mean temperature + proportion of rainy days + clutch size \* proportion of rainy days | 1312.02 | 1.33 | 0.24 |

**Table S2.6.** The three best fitting models (ΔAICc ≤ 2) that explain variation in the hatching success in long-tailed tits. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Attempt + clutch size + female age + incubation period | 918.05 | 0 | 0.54 |
| Attempt + clutch size + female age + incubation period + mean temperature | 919.59 | 1.54 | 0.25 |
| Attempt + clutch size + female age + incubation period + proportion of rainy day | 919.96 | 1.91 | 0.21 |

# **Appendix 3**

**Table S3.1.** The three best fitting models (ΔAICc ≤ 2) that explain variation in the time taken to gain the amount of water needed for treatment. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Total nest mass + proportion of feathers + proportion of moss + treatment + treatment\* total nest mass | 531.20 | 0 | 0.41 |
| Total nest mass + treatment + treatment\* total nest mass | 532.59 | 1.40 | 0.21 |
| Total nest mass + proportion of moss + treatment + treatment\* total nest mass | 532.72 | 1.53 | 0.19 |
| Total nest mass + proportion of feathers + proportion of moss + proportion of other structural material + treatment + treatment\* total nest mass | 532.77 | 1.57 | 0.19 |
|  |  |  |  |

**Table S3.2.** The effects of total nest mass, feather mas, moss mass, other structural mass and treatment on the time taken to gain the amount of water needed for treatment. The asterisk (\*) indicates an interaction between two factors. Shown are model-averaged parameter estimates, 95% confidence intervals and *P*-value for total nest mass, and variance for nest identity (the random effect) from the best fitting models. The model-averaged *R*2LMM(m) and *R*2LMM(c) were 73.37% and 73.44% respectively.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed Effects | Slope ± 1 SE | 95% confidence intervals | *P*-value |
| Intercept | 153.76 ± 140.68 | - | - |
| Treatment | -238.17 ± 67.22 | -367.53; -108.82 | <0.05 |
| Treatment \* total nest mass | 12.19 ± 2.29 | 7.79; 16.59 | <0.05 |
| Total nest mass | -0.16 ± 1.87 | -0.44; 11.64 | 0.93 |
| Proportion of feathers | -173.85 ± 175.97 | -19.88; 3.42 | 0.33 |
| Proportion of moss | -340.61 ± 248.79 | -29.65; -5.32 | 0.18 |
| Proportion of other structural material | -27.60 ± 77.35 | -8.30; 4.99 | 0.73 |
| Random Effect | **Variance ± 1 SE** |  |  |
| Nest ID | 5.95 ± 1.12 | - | - |

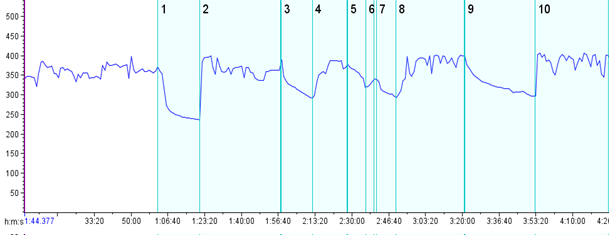
**Table S3.3.** The two best fitting models (ΔAICc ≤ 2) that explain variation in the nest insulation quality in long-tailed tits. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| NULL | -537.99 | 0 | 0.66 |
| Total nest mass | -536.62 | 1.37 | 0.34 |
|  |  |  |  |

**Table S3.4.** The three best fitting models (ΔAICc ≤ 2) that explain variation in the time taken to gain the amount of water needed for treatment. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Feather mass + moss mass + total nest mass + treatment + treatment\* total nest mass | 528.06 | 0 | 0.50 |
| Feather mass + moss mass + other structural material mass + total nest mass + treatment + treatment\* total nest mass | 529.12 | 1.06 | 0.29 |
| Moss mass + total nest mass + treatment + treatment\* total nest mass | 529.83 | 1.76 | 0.21 |
|  |  |  |  |

# **Appendix 4**

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**Figure S4.1.** Image from Raven Pro 1.4, temperature against time. Units of the y-axis are tenths of degrees rather than degrees, for example a temperature of 35.0˚ C is plotted as 350. Highlighted sections show selection of incubation bouts (orange) and incubation recesses (light green).

**Table S4.1.** Results of Pearson’s correlation between values extracted using temperature logger and those extracted from the video recordings.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variables** | **t** | **Df** | **p-value** | **r** |
| Nest attentiveness | 5.84 | 1, 23 | <0.05 | 0.77 |
| Total length of time spent on the nest | 8.02 | 1, 23 | <0.05 | 0.86 |
| Total length of time spent off the nest | 7.65 | 1, 23 | <0.05 | 0.85 |
| Mean duration of incubation bouts | 5.82 | 1, 23 | <0.05 | 0.77 |
| Mean duration of incubation recesses | 14.04 | 1, 23 | <0.05 | 0.95 |
| Number of incubation bouts | 6.41 | 1, 23 | <0.05 | 0.81 |
| Number of incubation recesses | 8.69 | 1, 23 | <0.05 | 0.88 |

**Table S4.2.** Summary of statistical models

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Level** | **Response variables** | **Transformation** | **Fixed effects** | **Random effects** |
| Hourly analyses | Nest attentiveness  Mean duration of incubation bouts  Mean duration of incubation recesses | Arc sine  Log  Log | Hour  Embryo age  Relative incubation start date  Day length  Clutch size  Attempt  Female age  Mean hourly ambient temperature  Hourly rainfall | Nest identity  Female identity  Year |
| Daily analyses | Duration of active day  Last entry to nest before sunset (number of minutes before sunset) | -  - | Embryo age  Relative incubation start date  Day length  Clutch size  Attempt  Female age  Mean daily ambient temperature  Mean daily rainfall | Nest identity  Female identity  Year |
|  | Time of first incubation recess (number of minutes after sunrise) | Log | Embryo age  Relative incubation start date  Day length  Clutch size  Attempt  Female age  Mean nightly ambient temperature  Mean nightly rainfall | Nest identity  Female identity  Year |
| Nest-level recordings | Incubation Period | Log | Mean nest attentiveness  . | Year  Female identity |
|  |  | Log | Nest insulation quality | Year |
|  | Hatching success | Log | Mean nest attentiveness  . | Year  Female identity |
|  |  | Log | Nest insulation quality | Year |
|  | Mean nest attentiveness | - | Nest insulation quality | Year |

**Table S4.3.** The ten best fitting models (ΔAICc ≤ 2) that explain variation in the duration of the active day. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Day length + embryo age | -220.06 | 0 | 0.16 |
| Day length + mean daily rainfall | -220.15 | 0.09 | 0.15 |
| Day length + embryo age + mean daily rainfall | -220.55 | 0.49 | 0.13 |
| Day length + mean daily temperature + mean daily temperature2 | -220.84 | 0.78 | 0.11 |
| Day length | -220.92 | 0.86 | 0.10 |
| Day length + mean daily rainfall + mean daily temperature + mean daily temperature2 | -221.24 | 1.19 | 0.09 |
| Attempt + day length + embryo age | -221.72 | 1.66 | 0.07 |
| Attempt + day length + mean daily rainfall | -221.75 | 1.69 | 0.07 |
| Attempt + day length | -211.95 | 1.90 | 0.06 |
| Day length + embryo age + mean daily temperature + mean daily temperature2 | -211.95 | 1.90 | 0.06 |

**Table S4.4.** The seven best fitting models (ΔAICc ≤ 2) that explain variation in the timing of last entry into the nest of an evening, in minutes before sunset. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Mean daily temperature + mean daily temperature2 | 1410.93 | 0 | 0.24 |
| Relative incubation start date + mean daily temperature + mean daily temperature2 | 1411.38 | 0.45 | 0.19 |
| Mean daily rainfall + mean daily temperature + mean daily temperature2 | 1412.05 | 1.12 | 0.14 |
| Null | 1412.16 | 1.23 | 0.23 |
| Embryo age + mean daily temperature + mean daily temperature2 | 1412.63 | 1.70 | 0.10 |
| Relative incubation start date + mean daily rainfall + mean daily temperature + mean daily temperature2 | 1412.81 | 1.88 | 0.10 |
| Embryo age | 1412.88 | 1.95 | 0.09 |

**Table S4.5.** The thirteen best fitting models (ΔAICc ≤ 2) that explain variation in the timing of first incubation recess of the morning, in minutes after sunrise. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Null | 473.43 | 0 | 0.11 |
| Embryo age | 473.48 | 0.05 | 0.11 |
| Mean nightly rain + mean nightly temperature + mean nightly temperature2 | 473.56 | 0.13 | 0.11 |
| Mean nightly temperature + mean nightly temperature2 | 473.64 | 0.21 | 0.10 |
| Mean nightly rain | 474.01 | 0.58 | 0.08 |
| Embryo age + mean nightly rain | 474.42 | 0.99 | 0.07 |
| Relative incubation start date + mean nightly temperature + mean nightly temperature2 | 474.59 | 1.16 | 0.06 |
| Relative incubation start date + mean nightly rainfall + mean nightly temperature + mean nightly temperature2 | 474.59 | 1.16 | 0.06 |
| Day length | 474.97 | 1.54 | 0.05 |
| Embryo age + relative incubation start date | 474.98 | 1.55 | 0.05 |
| Embryo age + mean nightly temperature + mean nightly temperature2 | 475.01 | 1.58 | 0.05 |
| Clutch size | 475.06 | 1.63 | 0.05 |
| Embryo age + mean nightly rainfall + mean nightly temperature + mean nightly temperature2 | 475.23 | 1.8 | 0.05 |

**Table S4.6.** The eight best fitting models (ΔAICc ≤ 2) that explain variation in hourly nest attentiveness. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Hour + embryo age + mean temperature + mean temperature2 | -128.82 | 0 | 0.28 |
| Hour + embryo age + relative incubation start date + mean temperature + mean temperature2 | -127.67 | 1.15 | 0.16 |
| Hour + embryo age + clutch size + mean temperature + mean temperature2 | -127.16 | 1.66 | 0.12 |
| Hour + embryo age + attempt + mean temperature + mean temperature2 | -127.00 | 1.82 | 0.11 |
| Hour + embryo age + attempt + relative incubation start date + mean temperature + mean temperature2 | -126.95 | 1.87 | 0.11 |
| Hour + embryo age + mean rainfall + mean temperature + mean temperature2 | -126.93 | 1.89 | 0.11 |
| Hour + embryo age + day length + mean temperature + mean temperature2 | -126.85 | 1.97 | 0.11 |

**Table S4.7.** The eight best fitting models (ΔAICc ≤ 2) that explain variation in the mean length of incubation bouts within an hour. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Hour + mean temperature + mean temperature2 | 2299.96 | 0 | 0.23 |
| Hour + attempt + mean temperature + mean temperature2 | 2301.00 | 1.04 | 0.14 |
| Hour + embryo age + mean temperature + mean temperature2 | 2301.04 | 1.08 | 0.13 |
| Hour + attempt + relative incubation start date + mean temperature + mean temperature2 | 2301.09 | 1.13 | 0.13 |
| Hour + relative incubation start date + mean temperature + mean temperature2 | 2301.73 | 1.77 | 0.09 |
| Hour + day length + mean temperature + mean temperature2 | 2301.74 | 1.78 | 0.09 |
| Hour + mean rainfall + mean temperature + mean temperature2 | 2301.75 | 1.79 | 0.09 |
| Hour + clutch size + mean temperature + mean temperature2 | 2301.77 | 1.81 | 0.09 |

**Table S4.8.** The eight best fitting models (ΔAICc ≤ 2) that explain variation in the mean length of incubation recesses within an hour. Shown are the Akaike’s Information Criterion corrected for small sample size (AICc), the difference in AICc and the model weight (Wi) for each model.

|  |  |  |  |
| --- | --- | --- | --- |
| Model configuration | AICc | ΔAICc | Wi |
| Hour + embryo age + clutch size + mean temperature + mean temperature2 | 2366.36 | 0 | 0.47 |
| Hour + embryo age + clutch size + mean rainfall + mean temperature + mean temperature2 | 2367.5 | 1.14 | 0.27 |
| Hour + embryo age + clutch size + relative incubation start date + mean temperature + mean temperature2 | 2367.54 | 1.18 | 0.26 |

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