Multi-brooding and breeding season length in the reed warbler

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This thesis is my own work and has not been submitted for the award of a higher degree elsewhere.

Summary

The causes and consequences of multi-brooding were assessed in an intensive nest monitoring study of the Eurasian reed warbler *Acrocephalus scirpaceus*, a species for which there is both evidence for earlier breeding and an extended season duration. Greater invertebrate availability was found to predict an increase in the probability of double brooding and to reduce the interval between broods. Given that invertebrate availability is likely to have increased as a result of warming temperatures, the relationship between invertebrate availability and double brooding supports the idea of an increased propensity to multi-brood driving extended breeding seasons. Weather conditions also influenced both the incidence of double brooding and inter-brood intervals. The relative value of nesting attempts throughout the whole breeding season was assessed which illustrated that later nesting attempts are of lower reproductive value. Assessment of the potential costs of extending the breeding season with late nesting attempts, however, revealed no evidence for any costs of extending the season for individuals. These results suggest that extending the breeding season at the individual level is a low value, low cost strategy. Relative parental investment, in the provisioning of nestlings, was considered as an additional predictor of multi-brooding, but there was no evidence of an effect. Provisioning rates also did not appear to vary substantially across the breeding season or between sexes. The potential for counting singing birds as a method for monitoring breeding season length was assessed by testing the relationship between the number of singing birds and known nests throughout the season and a positive relationship between the number of singing birds and the number of nesting attempts in the early stage of the nesting cycle was found. Season-long censuses of singing birds may therefore offer a low intensity method for estimating breeding season length; a demographic parameter which currently is not well monitored at large spatial scales.

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Chapter 1- General introduction

INTRODUCTION

In seasonally breeding birds, fundamental determinants of reproductive success include brood productivity and the number of broods attempted. Constraints on brood productivity include the size or quality of offspring i.e. egg size (Perrins 1996), the number of offspring per breeding attempt i.e. clutch size (Lack 1968) and the timing of breeding in relation to optimal breeding conditions (Visser *et al.* 2006, Lany *et al.* 2016). For single-brooded species, which only attempt to raise one brood per season, reproductive success may be maximised by increasing clutch size, whereas for multi-brooded species, reproductive success can be additionally improved by producing more than one brood (Crick *et al.* 1993). However, despite the fact that multi-brooding can result in the production of a greater number of offspring (e.g. Ogden and Stutchbury 1996, Weggler 2006), life-history theory dictates that reproduction is costly (Williams 1966, Stearns 1976, Reznick 1985). It follows that there should therefore be additional costs incurred by increasing reproductive effort via attempting to raise multiple broods. The relative costs versus benefits to parent fitness should dictate whether species employ the multi-brooded strategy or not: the reproductive strategy of a given species should reflect evolutionary selection pressures (Martin 1987).

Given the contrasting strategies for maximising reproductive success, it follows that single and multi-brooded species are expected to be under different selection pressures with regard to the timing of breeding. Single-brooded species are predicted to be under selection pressure to time a single breeding attempt with optimal conditions, whilst multi-brooded species are predicted to be under selection pressure to breed as early as possible i.e. before optimal food availability, and to keep breeding until conditions deteriorate (Lack 1968, Crick *et al.* 1993). This is often reflected in clutch sizes, which tend to decrease seasonally in single-brooded species, representing conditions becoming less optimum as the season progresses, but which often peak in the middle of the nesting season in multi-brooded species (Lack 1968, Perrins 1970, Crick *et al.* 1993). These differences in the reproductive ecology of single- and multi-brooded species may result in differing responses/sensitivity to environmental change. Indeed, there is increasing evidence that the breeding season length of some multi-brooded species has extended, while in contrast, the season has shortened in some single-brooded species in association with climate change in recent decades (Møller *et al.* 2010, Halupka and Halupka 2017). These relatively recent changes make the study of multi-brooding and breeding season length ever more important.

The aims of this chapter are to review the causes and consequences of multi-brooding, as well as focusing on the documented changes in breeding timing and season length in multi-brooded species that have occurred in association with climate change and, finally, to introduce the research aims and study system of this project.

MULTI-BROODING DETERMINANTS

Reproductive strategies regarding the number of breeding attempts made within a single breeding season varies between species, populations and individuals. Life-history theory predicts that breeding adults face a compromise between current reproduction and survival for future reproduction in order to maximise fitness (Williams 1966, Linden and Møller 1989). Indeed, a comparative study of birds showed that adult survival is negatively correlated with multiple measures of reproductive effort including the number of broods attempted, as well as clutch size and annual fecundity (Bennett and Owens 2002). Multi-brooding is therefore generally found in species with lower annual survival. Body size was found to be unrelated to the number of broods in British breeding birds (Crick *et al.* 1993), while a study of 25 species in Central Arizona suggested that the number of broods is significantly different between species with different nest types; open nesters have more broods on average than cavity-nesters (Martin and Li 1992). Böhning-Gaese *et al.* (2000) conducted a comparative analysis of the influence of various ecological traits on measures of avian reproduction covering 625 species from North America and Europe. Body size was negatively associated with the number of, while precocial species had fewer broods than altricial species and long-distance migrants had fewer broods than both short-distance and resident species. The number of broods was also found to decrease with increasing latitude. Habitat, diet, nest type, nest location and continent all had no significant influence on the number of broods, and controlling for phylogeny had only a minor influence on all results in the study. It seems that a greater number of broods are generally found in association with the following ecological traits/ life histories: shorter lifespan (Bennett and Owens 2002), smaller body size, altricial development, shorter migratory distance and lower latitudes (Böhning-Gaese *et al.* 2000). However, it must be noted that the results of studies on correlates of reproductive traits do vary considerably based on the diversity of species considered (Böhning-Gaese *et al.* 2000).

Within populations of multi-brooded species, it is generally the case that some, but not all individuals multi-brood within any given season. Most studies of the determinants of double brooding have reported that the timing of the first brood is the most important factor; earlier first broods increasing the likelihood of attempting a second brood (e.g. Geupel and DeSante 1990, Ogden and Stutchbury 1996, Mallord *et al.* 2008, O'Brien and Dawson 2013, Townsend *et al.* 2013, Carro *et al.* 2014, Hoffmann *et al.* 2014, Zając *et al.* 2015, Béziers and Roulin 2016, Jackson and Cresswell 2017) and there are very few exceptions to this finding (Cornell and Williams 2016, Nomi *et al.* 2018). Female age, but not male age, has been found to be important in several species (Geupel and DeSante 1990, Nagy and Holmes 2005b, Weggler 2006, Bulluck *et al.* 2013, Hoffmann *et al.* 2014). The probability of double brooding is commonly found to be negatively correlated with the size of the first brood (Tinbergen 1987, Verboven and Verhulst 1996, Parejo and Danchin 2006), although some studies report no association between first brood size and the incidence of double-brooding (Ogden and Stutchbury 1996, Brinkhof *et al.* 2002), or even a positive correlation (Hoffmann *et al.* 2014). Relatively few studies have found evidence of an effect of food availability, but there is some observational and experimental evidence of a positive association. For example, double-brooded female black-throated blue warblers, *Setophaga caerulescens*, breed in territories with greater food availability than single-brooded females (Nagy and Holmes 2005b) and barn owl, *Tyto* alba, double-brooding is more common in years of high food availability (Jackson and Cresswell 2017). Moreover, food supplementation experiments detected effects of food availability on double-brooding in both mountain bluebirds, *Sialia* currucoides, and black-throated blue warblers (Nagy and Holmes 2005a, O'Brien and Dawson 2013). Food availability should certainly be a limiting constraint on multi-brooding as it constrains other elements of breeding ecology (Martin 1987), while further constraints, in species which are physiologically able to multi-brood, can include high nest predation rates rarely allowing time for multiple successes (Weidinger 2004) and ectoparasites reducing adult condition (Brown *et al.* 2014). Finally, a recent study of Japanese tits, *Parus minor*, found that the relative proportion of provisioning undertaken by males was positively associated with the probability of double brooding, highlighting the role of paternal care, which has generally not been considered in other studies (Nomi *et al.* 2018).

It is important to point out that the incidence or extent of double brooding may also vary considerably between populations of multi-brooded species. As an example, in the UK, both great tits, *Parus major*, and blue tits, *Cyanistes caeruleus*, are considered to be predominantly single-brooded (Crick *et al.* 1993): great tit second broods are rare (Perrins 1970) and blue tit second broods exceptionally so (Perrins 1979), whereas elsewhere in Europe both species are customarily considered to be double-brooded (Visser *et al.* 2003, Parejo and Danchin 2006, Husby *et al.* 2009, Halupka and Halupka 2017). Population-scale variance in double-brooding behaviour is likely to be influenced by factors including latitude, as there is a delayed onset of breeding with increasing latitude (Lack 1968), as well as differences in habitat and therefore food resources between study sites/populations.

COSTS AND BENEFITS OF MULTI-BROODING

The potential benefits of attempting to produce multiple broods in a season are increased annual and lifetime reproductive output, measured by the number of fledglings produced, illustrated extensively in study populations where some individuals are multi-brooded and others are single-brooded within a given breeding season (e.g. Weggler 2006, Bulluck *et al.* 2013, O'Brien and Dawson 2013, Carro *et al.* 2014, Hoffmann *et al.* 2014). Unlike in single-brooded species, whose nest productivity in terms of the production of fledglings tends to decline with later nesting attempts, in multi-brooded species, nest productivity may be similar throughout the season, or can even be greatest for later nesting attempts (Smith and Marquiss 1995, Ogden and Stutchbury 1996, Carro *et al.* 2014, Hoffmann *et al.* 2014). Regardless of the relative contribution of first and second broods, the annual production of fledglings is increased by multi-brooding. However, the likelihood of fledglings ultimately recruiting into the breeding population is generally found to decline seasonally (Hochachka 1990, Barba *et al.* 1995, Smith and Marquiss 1995, Van Noordwijk *et al.* 1995, Verboven and Visser 1998, Cowley 2001, Mallord *et al.* 2008, Brown *et al.* 2014, Hoffmann *et al.* 2014). As selection will act on recruitment rather than nest success, the value of nesting attempts for adult reproductive success can be considered to decline seasonally. In addition to later nesting attempts being found to contribute less to recruitment, there are also potential increased costs to adults for extending the season via multi-brooding.

It is well founded that there can be costs of increased reproductive effort, for example, provisioning larger broods results in lowered adult condition (Martins and Wright 1993, Murphy *et al.* 2000) and larger clutch or brood sizes can ultimately lead to decreased adult survival (Nur 1984, Linden and Møller 1989, Dijkstra *et al.* 1990, Daan *et al.* 1996, Visser and Lessells 2001). Indeed, costs of increased reproductive effort via the raising of multiple broods, or a later end to breeding, have also been documented, including reduced adult survival (e.g. Bryant 1979, Nilsson and Svensson 1996, Verhulst 1998, Brown *et al.* 2014) as well as delayed breeding or reduced breeding productivity in the following season (Nilsson and Svensson 1996, Verhulst 1998, Fayet *et al.* 2016). However, numerous studies have also failed to detect any measurable costs of multi-brooding in terms of reduced adult survival or carry-over effects on the following breeding season (Geupel and DeSante 1990, Smith and Marquiss 1995, Weggler 2006, Carro *et al.* 2014, Cornell and Williams 2016). Costs of extending the breeding season with late nesting attempts are likely mediated by a reduction in time and/or resources allocated to self-maintenance and other life-history events within the annual life cycle such as post-breeding moult and, for migratory species, autumn migration. There certainly is evidence that post-breeding moult is delayed by multi-brooding (Ogden and Stutchbury 1996, Mulvihill *et al.* 2009) and the resultant poorer quality feathers can in turn lead to reduced survival (Nilsson and Svensson 1996). When costs of multi-brooding (or a later end to breeding) are not observed, it is generally assumed that such costs are masked by either territory or individual quality, that is to say that multi-brooding is only attempted by high quality individuals (and/or those occupying high quality territories), that can ‘afford’ the costs of multi-brooding (Geupel and DeSante 1990, Smith and Marquiss 1995, Weggler 2006, Cornell and Williams 2016).

Multi-brooding represents a fitness trade-off between current and potential future reproduction and the overall value of this breeding strategy rests on the relative benefits and costs versus cessation of breeding until the following breeding season. In fact, a study of great tits demonstrated that when the relative costs and benefits of double brooding shifted in association with climate change, the altered profitability of second broods in turn altered the frequency of double brooding in the population (Husby *et al.* 2009). Given that the breeding season has extended in many multi-brooded species in recent decades (Halupka and Halupka 2017), which may allow for a greater number of breeding attempts (Halupka *et al.* 2008), there is considerable potential for climate change to be influencing the frequency and profitability of multi-brooding in many species.

CLIMATE CHANGE AND BREEDING ECOLOGY

Breeding timing

There is extensive evidence of advancing bird breeding phenology in response to climate change, usually from negative correlations with warming spring temperatures with both earlier first and mean egg laying dates in many temperate breeding birds (Crick and Sparks 1999, Both *et al.* 2004, Schaefer *et al.* 2006, Charmantier *et al.* 2008, Halupka *et al.* 2008, Dyrcz and Halupka 2009, Ockendon *et al.* 2013), and this advancement has accelerated recently (Pearce-Higgins and Green 2014). In temperate breeding birds, photoperiod is the principal environmental cue which controls the physiology determining the timing of breeding (i.e. gonadal maturation and regression; Dawson 2008), however, other environmental factors such as temperature, rainfall and food availability additionally influence breeding timing (Jones 1986, Meijer *et al.* 1999, Both *et al.* 2004, Dawson 2008, Pearce-Higgins and Green 2014). In Oxford in the UK, great tits advanced peak egg laying by 14 days from the mid 1970’s to 2007 (Charmantier *et al.* 2008) while pied flycatchers, *Ficedula hypoleuca*, in the Netherlands advanced mean laying dates by 10 days between 1980 and 2000 (Both and Visser 2001). There are countless more examples of climate warming resulting in breeding advancement in the literature, particularly from studies in the Western Palaearctic (Pearce-Higgins and Green 2014).

Although increasing spring temperatures are often found to strongly positively correlate with first egg laying dates, a recent meta-analysis and systematic review confirmed that the majority of climatic responses are mediated via effects on other trophic levels e.g. via food availability or predation, rather than climate directly effecting the focal species (Ockendon *et al.* 2014). Indeed in some avian study systems, invertebrate prey species have advanced their phenology to a greater extent than their avian predators (although it must be noted that these study systems are heavily biased towards woodland breeding passerine species; Visser *et al.* 1998, Nussey *et al.* 2005, Both *et al.* 2006, Laaksonen *et al.* 2006). This is termed phenological mis-match and results in a disjunction between peak food abundance and nestling food demands. For example, in a study of great tits in the Netherlands, where great tit laying dates advanced by 0.18 days a year between 1973 and 2003 (Gienapp *et al.* 2006), their caterpillar prey advanced hatching dates much more rapidly, by 0.74 days a year between 1985 and 2004 (Visser *et al.* 2006). Phenological mis-match has also been found at higher trophic levels, for example, sparrowhawk, *Accipiter nisus*, breeding is mis-timed with that of the main passerine prey species in Denmark (Nielsen and Møller 2006). Such mis-match in the timing of breeding with peak resources can result in reduced breeding success (e.g. Verboven and Visser 1998, Visser *et al.* 1998, Both and Visser 2001, Sanz *et al.* 2003, Visser *et al.* 2006) and there is also evidence that phenological mis-match can ultimately cause population declines (Both *et al.* 2006, Møller *et al.* 2008). Whether or not species are advancing their phenology at a rate matching advancing prey resources may therefore be interpreted as an indication of whether species are responding to climate change at a sufficient rate. Reviewing the literature on the phenomenon, Pearce-Higgins and Green (2014) found evidence for phenological mis-match causing reduced productivity in at least one population of 14 out of 47 species studied.

The incidence and extent of phenological mis-match has been shown to vary both between species (Pearce-Higgins and Green 2014) and between populations of the same species (shown in both great tits and pied flycatchers; Both *et al.* 2006, Visser *et al.* 2006, Charmantier *et al.* 2008). Moreover, species vulnerability to phenological mis-match varies between habitats. For example, European temperate woodland is a highly seasonal habitat with a pronounced spring peak in herbivorous invertebrate abundance (Both *et al.* 2009). In this habitat, the fitness consequences for breeding out of synchrony with the peak in food abundance may be severe. In contrast, temperate wetland habitats are less seasonal, providing a greater spread of food resources throughout the whole breeding season (Bibby and Thomas 1985, Halupka *et al.* 2008, Both *et al.* 2009, Dodson *et al.* 2016). Studies of insectivorous wetland species have failed to find evidence of phenological mis-match (Pearce-Higgins and Green 2014), instead, several wetland species have both advanced breeding in response to climate change and benefited from increased breeding success (Schaefer *et al.* 2006, Halupka *et al.* 2008, Dunn *et al.* 2011). Furthermore, as multi-brooded species do not need to time a single nesting attempt with optimal conditions, they may be less vulnerable to the negative consequences of phenological mis-match.

Breeding season length and multi-brooded species

Much climate change research has been focused on single brooded species and the timing of breeding i.e. the first egg laying dates in response to warming spring temperatures. However, climate change may have a wider range of impacts on multi-brooded species. Multi-brooded species responses to a changing climate could include the timing of first breeding dates, the number of attempts made in a season and the length of intervals between attempts (Møller 2007, Halupka *et al.* 2008).

There is evidence that species having more broods have advanced both arrival timing (Møller *et al.* 2010) and breeding timing (Dunn and Møller 2014) to a greater extent than those having fewer broods. In addition to an advancement in breeding dates, the duration of the breeding season in some multi-brooded species has also extended in line with climate warming (e.g. Møller 2007, Halupka *et al.* 2008, Najmanová and Adamík 2009, Møller *et al.* 2010, Halupka and Halupka 2017). A recent meta-analysis of 65 long-term studies of 54 species from the Northern Hemisphere found that multi-brooded species have generally extended the length of the breeding season, while single-brooded species have shortened the breeding season, by a lower magnitude (Halupka and Halupka 2017). For example, the wood pigeon, *Columba palumbus*, breeding season extended at a rate of almost one day a year between 1970-2007 in Denmark, extending by 36.2 days in total over the study period (Møller *et al.* 2010). Documented extensions in breeding season duration have generally occurred as a result of advancement in the beginning of the breeding season but comparatively little or no change in the end of the breeding season timing (Møller *et al.* 2010). Understanding the mechanisms by which breeding seasons are extending and the effect of longer breeding seasons on population productivity is vital to understanding the impacts of climate change on multi-brooded species.

Potential mechanisms of extended breeding seasons include the production of an additional brood, an increased propensity to double brood, increased failure rates, longer intervals between clutches or broods and increased duration of incubation or nestling periods. Møller (2007) found in barn swallows that the interval between first and second clutches increased when the date of first broods advanced with warming spring temperatures between 1971 and 2005. Furthermore, these longer intervals had fitness benefits: greater annual fecundity and both improved offspring quality and adult survival. A study of woodlark, *Lullula arborea*, reported that earlier breeding gave rise to a greater number of nesting attempts, but this did not increase either fledging success or recruitment rates, as failure rates were high (Mallord *et al.* 2008). In contrast, Neither Halupka *et al.* (2008) or Møller *et al.* (2010) found failure rates to change significantly over their study periods, giving less plausibility to increased failure rates as a mechanism for increased breeding season duration. Halupka *et al.* (2008) stated that the reed warblers, *Acrocephalus scirpaceus*, in their study site now have more nesting attempts as a result of the extended season, with females laying up to four or five clutches in a season. Certainly, there is a need for further studies of breeding season length and particularly to identify the mechanisms driving breeding season extensions in populations, in order to determine whether population impacts of longer breeding seasons may ultimately be positive or negative. It may be particularly key to understand the determinants of multi-brooding at the individual level, as an increased propensity to multi-brood represents one of the key hypotheses for extended seasons.

Weather effects over short study periods

It is important to note that studying the effects of weather on breeding ecology, over relatively short time periods, is not equal to the study of species responses to climate change, which by definition requires long term study. Clearly, weather effects on aspects of breeding ecology over short study periods may not necessarily reflect long term responses or adaptation to climate change. Long term studies are essential for determining potential mechanisms by which aspects of breeding ecology are influenced by changes in climate. Such mechanisms include phenological plasticity, whereby individual genotypes are able to alter their phenotype in response to environmental conditions, and microevolution i.e. selection pressure on traits, causing genetic change within populations over time (Gienapp *et al.* 2008, Visser 2008, Charmantier and Gienapp 2014). Whilst studies of the effects of weather on breeding ecology (studies over relatively short time periods), cannot directly assess the effects of long term climate change, they are still useful for inferring possible effects over longer periods.

REED WARBLERS

The Eurasian reed warbler, *Acrocephalus scirpaceous*, was used as the study species for this project. Reed warblers are a small (around 13 g) member of the Acrocephalidae family which comprises around 50 species within the order Passeriformes (Leisler and Schulze-Hagen 2011). The species has a favourable conservation status both in Europe (Burfield *et al.* 2004) and in the UK, where the long-term population trend has been that of rapid increase in recent decades (Woodward *et al.* 2018). Reed warblers are trans-Saharan long-distance migrants and winter in the Sahel and Sudan zones of Africa, British breeding birds occurring in West Africa from Senegal to Nigeria, as indicated by recoveries and recaptures of ringed birds (Wernham *et al.* 2002, Zwarts and van Horssen 2009). Departure from the wintering grounds begins in March and birds arrive in the UK in April (Wernham *et al.* 2002). Like many species, the beginning of the reed warbler breeding season has advanced in line with spring warming across Europe (Crick and Sparks 1999, Schaefer *et al.* 2006, Halupka *et al.* 2008, Woodward *et al.* 2018) and there is evidence from one study population that the breeding season has extended (Halupka *et al.* 2008).

During the breeding season, reed warblers are strongly associated with marginal wetland reed bed habitat for breeding, and they build open cup nests suspended over water, attached to the stems of common reed, *Phragmites australis*, although other plant species and drier habitats are sometimes used (Catchpole 1974, Dyrcz 1981, Borowiec 1992, Schulze-Hagen *et al.* 1996, Poulin *et al.* 2002). As a consequence of the species' reliance on a patchily distributed habitat, birds often nest in high densities and have been described as breeding in loose colonies (Catchpole 1972). Foraging is undertaken mainly within the reed beds used for nesting but also in surrounding habitats including trees if they are in close proximity to the nest site (Bibby and Thomas 1985, Chernetsov and Manukyan 1999). The species is a generalist insectivore, its diet in the breeding season covering a wide range of invertebrates (Bibby and Thomas 1985).

Within a breeding season, reed warblers can lay up to 5 clutches (Halupka *et al.* 2008) and population level double brooding rates have been observed between 0 and 35 % (Schulze-Hagen *et al.* 1996). Reed warblers are predominantly a socially monogamous species (Leisler and Schulze-Hagen 2011), although low levels of polygyny have been recorded (Halupka *et al.* 2014). Parental care is bi-parental, with incubation and nestling provisioning duties both shared (Leisler and Schulze-Hagen 2011, Klimczuk *et al.* 2015). Mean clutch size in the UK is 3.88 and the vast majority of clutches comprise four eggs (Catchpole 1974, Robinson 2018). Clutches or broods can be lost through predation, starvation, desertion, strong winds and brood parasitism by the common cuckoo, *Cuculus canorus* (Dyrcz 1981).

AIMS AND OUTLINE OF THESIS

The aims of this thesis were to investigate the causes and consequences of multi-brooding in reed warblers as well as seasonal variation in aspects of reproductive ecology. Reed warblers represented an ideal study species for research on multi-brooding and breeding season length, as increased reproductive output has been suggested as a driver of population increase (Woodward *et al.* 2018) and there is evidence that the timing of breeding has advanced (Crick and Sparks 1999) and extended (Halupka *et al.* 2008). I begin by investigating the determinants of double brooding and the interval length between broods (Chapter 3). I then assess the relative value of nesting attempts throughout the breeding season as well as the costs of extending the breeding season via double brooding (Chapter 4). Following this, I examine seasonal variation in parental investment, in terms of nestling provisioning, and the potential for paternal investment to influence double brooding (Chapter 5).

Finally, I consider whether the counting of singing birds could be used as a future method for monitoring breeding season length (Chapter 6), before summarising my findings in the context of the wider literature on multi-brooding and breeding season length and making suggestions for future research on this subject (Chapter 7).

Chapter 2- General methods

STUDY SITE AND PERIOD

The study was carried out at Watermill Broad Nature Reserve, a privately owned, 46 ha site in West Norfolk, UK (TL776957). The site was formerly used for gravel, sand and peat abstraction between 1987 and 2006, after which, the abstraction pits were flooded by the River Wissey. During the study period, the site consisted of a series of ponds and lakes of varying sizes (the flooded pits), fringed with *Phragmites australis* reed bed as well as willow, *Salix sp.*, and alder, *Alnus glutinosa*, scrub, mature woodland and grassland (Figure 2.1). Reed beds were generally very narrow, less than 5 m wide (Figure 2.2) and covered a total area of 7.6 ha. Fieldwork was carried out during April-August inclusive. This study primarily made use of data collected in the 2014-17 seasons. The study population had been previously monitored throughout the breeding season in every year since 2011, using similar protocols for nest searching and monitoring to later years, but lacked other data sets collected from 2014 onwards. The methods hereafter refer primarily to fieldwork carried out in the 2014-17 seasons (use of data from other study years is stated where appropriate in later chapters).

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 2.1. Map of Watermill Broad showing areas of reed bed and woodland (including willow and alder scrub).

Figure 2.2. Typical habitat at Watermill Broad; a reed fringed lake, with surrounding willows.

REED WARBLER BREEDING ECOLOGY

Systematic nest searching specifically for reed warbler nests was carried out each season by cold-searching all reed beds on site, at least once every 7-10 days, throughout mid-April to mid-August (though systematic searching for other species nests within the reed beds begun on the site earlier, from the beginning of March). Nest searching was carried out from regular paths, through the reed bed, in all ponds and lakes, using a stick to part vegetation while searching. All reed bed on site was accessible for nest searching on foot, from within the lakes. Nests were also found throughout the study during the course of other fieldwork activities and 10-figure grid references were recorded for all nests using a Garmin eTrex GPS (+/- 3 m accuracy). Nest searching was effective; very few nesting attempts were found after having fledged (ca. 1 %), whilst a relatively small number (< 11 %) were observed to be fully built but were never observed to contain eggs, and thus were assumed to have failed between nest visits (Table 2.1). Furthermore, the majority of nesting attempts which were observed to contain eggs or nestlings were found early in the nesting cycle (Table 2.2). Some nesting attempts which were abandoned during building were also found but have not been used in any analyses. All active nests were visited at least once every five days until fledging or failure in order to obtain data on first egg dates (the day of laying the first egg of the clutch), clutch size, brood size, the ultimate outcome of the nesting attempt and an estimate of the fledge or fail date.

Table 2.1. The number of fully built nests found, split by those that were observed to contain eggs or nestlings and those which were not.

Year	Contained eggs or nestlings	Not observed to contain eggs or nestlings			Total
		Failed	*Fledged*	*Outcome unknown*	
2014	233	16	4	1	254
2015	278	34	5	2	319
2016	275	39	1	1	316
2017	230	34	2	0	266

Table 2.2. The stage of the nesting cycle which nests were found at (of nests which were ultimately observed to contain eggs or nestlings).

Year	Building	Laying	Incubating	Nestling	Total
2014	106	54	61	12	233
2015	150	53	61	14	278
2016	171	54	48	2	275
2017	153	37	37	3	230

First egg dates (FEDs) were calculated on the basis of individual birds laying one egg per day until clutch completion (Brown and Davies 1949). For nests not observed during the laying period, FEDs were back-calculated on the basis of an incubation duration of 11-13 days from the day the penultimate egg was laid, as 92% of known incubation durations fell within this range (Figure 2.3), and hatch dates estimated by ageing nestlings by appearance. Known incubation durations were taken as nests with exact FEDs (i.e. observed during the laying period), observed clutch size (and therefore the day incubation began) and the nest was observed on the day nestlings hatched. Nestling ageing, required for determining hatch dates, used in FED back-calculations, was considered accurate for day one (hatch day) or day two nestlings, while ageing of older nestlings was assumed to be less accurate and therefore hatch date estimates from older nestlings allowed for plus or minus one day. Ultimately, back-calculations provided potential minimum and maximum FEDs and the median date was taken as the estimated FED (or the upper median when there was an even number of potential dates). FEDs ranged from late April to early August (Table 2.3).

Table 2.3. Summary of the earliest, median and latest first egg dates (FEDs) of nests each season, as well as the mean accuracy of FED calculations for whole season (1 = estimated FED could be plus or minus one day). N = 233 in 2014, 278 in 2015, 275 in 2016 and 230 in 2017.

Year	Earliest FED	Median FED	Latest FED	Mean FED accuracy
2014	27th April	16th June	3rd August	0.7
2015	30th April	10th June	4th August	0.5
2016	11th May	10th June	29th July	0.6
2017	3rd May	9th June	25th July	0.2

Clutch size was taken as the number of eggs observed to be being incubated on at least two separate dates. The modal clutch size in 2014-17 was four (17 clutches of two, 108 clutches of three, 217 clutches of four and 63 clutches of five).

All nestlings were ringed with uniquely coded British Trust for Ornithology (BTO) metal rings between day four and seven (day 1 = hatch day; Figure 2.4). Nests were checked at least once more between nestling ringing and potential fledging, three days after ringing, so on nestling day 7-10. Nests were considered successful if they fledged one or more nestlings and successful nests were determined from the observation of fledglings near or leaving the nest or an intact nest, containing feather scale and/ or covered in droppings on or after nestling day 10. The number of nestlings fledged was calculated from the number observed on the nest visit prior to a fledge check plus any well-feathered nestlings that had been present on a previous visit, as asynchronous fledging is not uncommon and nestlings which die after the age of day 7 are usually left in the nest (pers. obs.).

The end dates of failed nests were taken as the mid-point between the last visit with live nest contents and the concluding visit. The end date of successful nests were also taken as the mid-point between the last visit with nestlings and the fledge check, but the maximum potential fledge date was capped at four days after a penultimate visit when nestlings were already big enough to leave the nest i.e. in cases where a concluding visit to confirm a nest had fledged was late, as nestlings generally fledge at between 10-13 days old (Robinson 2018). Given that the mid-point was taken, fledge dates were therefore often two days after large (well feathered) nestlings were observed.

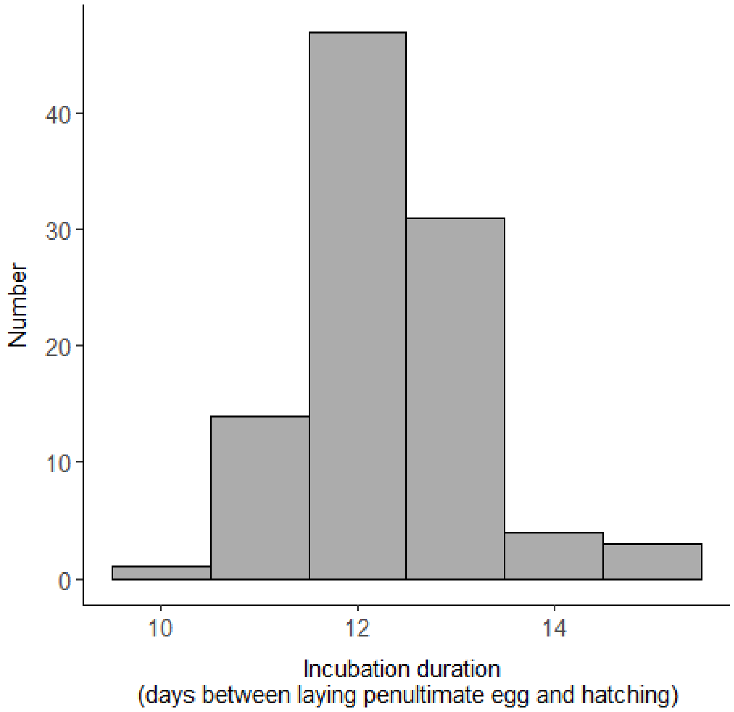

Figure 2.3. Frequency distribution of known incubation durations (number of days between the penultimate egg being laid and the hatch date; n = 100).

Figure 2.4. Reed warbler nest with four, day six nestlings (*Mark Mainwaring*).

NEST OWNERSHIP

Adults were marked with unique two-digit, engraved darvic colour rings when encountered during Constant Effort Site (CES) mist netting April- September each year (since 2013), or via targeted mist netting at nesting territories from 2015 onwards (Figure 2.5). Adults were also sexed, when possible, via the presence of a brood patch (female) or an enlarged cloacal protuberance (male). Nests were video recorded, with a Sony HDR camcorder placed 2-3 m from the nest for approximately one hour in order to identify adults (Figure 2.5). Video recordings were made on the day of nestling ringing in 2014 and from the onset of incubation in 2015-17 and were undertaken at any time of day in fair weather. Video recording nests during rain and strong wind was avoided so as not to cause excessive disturbance, exposing nestlings to adverse weather conditions, when parents may otherwise have been brooding. Additional video attempts were made opportunistically at active nests for which one or both adults remained unidentified, or in territories where a previously un-ringed or metal-only ringed adult had been trapped and colour-ringed.

Figure 2.5. Colour-ringed adult reed warbler (left) and video camera set up on a nest (right).

Many nests still lacked colour-ringed parent identities as a result of both there being un-ringed and metal-only ringed breeding birds in the population as well as high nest failure rates (typical of reed warbler populations; Schulze-Hagen *et al.* 1996) resulting in nests failing before video recording was possible (Table 2.4). A series of decision rules were therefore devised to assign the parental ownership of nests and complete pairs' breeding season histories. A comparison of the distances between nests known to belong to the same pair (identified by colour-ringed birds being video recorded at nests) and the distances between the nearest nests to each other which were active at the same time (and therefore certainly of different pairs), showed that an absolute distance threshold could not be used to identify nesting attempts made by the same pairs (Figure 2.6); breeding territories were small and at high density. Relative proximity of nests to each other was therefore used for assigning the ownership of nests (Figure 2.7).

Table 2.4. Number of nests with colour-ringed adults identified versus those without (of nests which were observed to contain eggs and nestlings).

Year	**Colour-ringed adults identified**		**No colour-ringed adults identified**		Total
	Two c/r adults	*One c/r adult*	*Un- or metal-ringed adults*	*Nest failed before video*	
2014	40	66	58	69	233
2015	60	89	53	76	278
2016	77	81	77	40	275
2017	110	66	40	14	230

The nest assignment process was designed to be run from the perspective of one nest, the 'focal nest' and considered 'candidate nests', for assignment to the same pair identity as the focal nest, following a series of steps. Candidate nests were considered nests within the distance at which 95% of known re-nesting attempts occurred; 38 m (Figure 2.6). Nesting attempts which overlapped temporally were not assigned to the same breeding pair; the FED of the latter nest could not overlap with the end date of an earlier nest, or the five days following this end date, as the minimum observed temporal interval between known re-nesting attempts (end-FED) was five days. The nearest nests to one another that matched these criteria were assigned as belonging to the same breeding pair, as long as neither were more parsimoniously assigned to another pair. For example, the nearest nest which did not temporally overlap with 'nest a', may have been 'nest b', but if 'nests b and c' were closer to one another (and also did not temporally overlap), they would be assigned together instead. Such scenarios were recognised as, once a suitable candidate nest had been identified, the process was run in reverse, i.e. with the selected candidate nest as the focal nest, evaluating if both nests were the best match for each other. The process continued through all candidate nests, for each focal nest, whether or not any had already been successfully assigned to the focal nest, as pairs could have more than two nesting attempts (Halupka *et al.* 2008). Ultimately, this process would lead to nests being assigned together if they did not temporally overlap and were spatially closest to each other, than to other potential nests with which they could have been assigned. Pairs having only one nesting attempt would, therefore, be identified either as a result of there being no candidate nests for assignment (none within 38 m), or all such candidate nests were better assigned to other suitable nests.

The model was tested on data from 2017 as this season had the greatest proportion of colour-ringed individuals (Table 2.4). Polygyny and divorce were very rare in the population (< 1 and 2 % of males and pairs respectively), so nest assignment assumed monogamous pairings. The test data set represented 90 pairs which had one or both parents colour-ringed from the beginning of the breeding season. From these data the earliest known nest of each pair was selected as the focal nest to run through the model of nest assignment. All other nests, observed to contain eggs or nestlings in the 2017 season, were available to be selected as candidate nests, regardless of the status of their parent identity information.

The accuracy of the nest assignment process was evaluated by checking the parent identities of nests assigned to the 90 focal nests. The nest scale accuracy of assignments were considered as: correct, if colour-ringed parents matched between nests, incorrect, if they did not match, or unknown, if the assigned candidate nest had no parent identity, such as nests which had failed before being video recorded. Whether complete pair breeding histories contained any errors or not was also considered (hereafter pair-scale accuracy). An incorrect pair breeding history included either a nest with different parents being assigned to the focal nest, or a nest which was known to truly belong to the focal nest not being successfully assigned. In rare cases of mate switching, nests of the same female were considered to be correctly assigned, if assigned together.

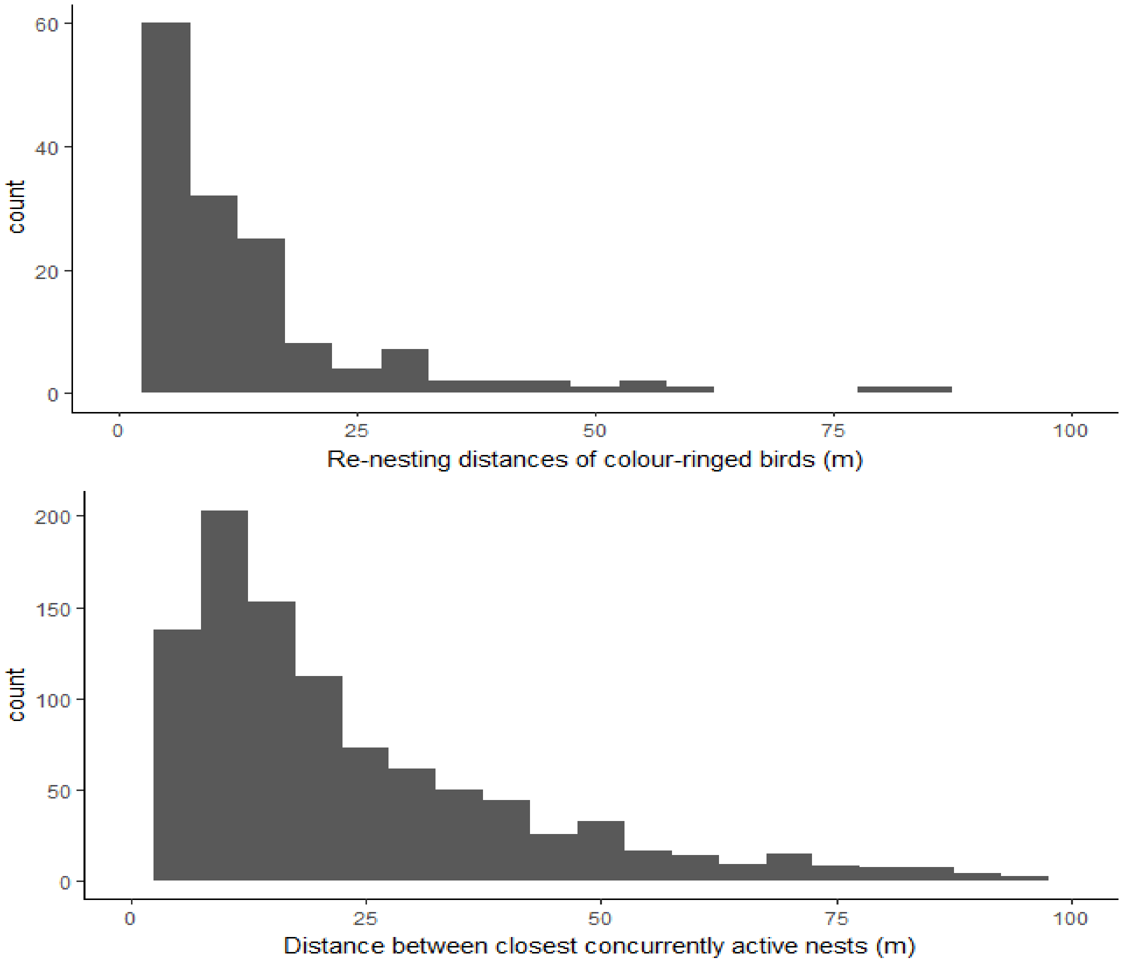

Figure 2.6. Frequency distributions of re-nesting distances between multiple attempts of colour-ringed birds (above; n = 176), and minimum distances between concurrently active nests i.e. not of the same pair (below; n = 1017), shown in 5 m bands. X axis limited to 100 m to remove extreme outliers.

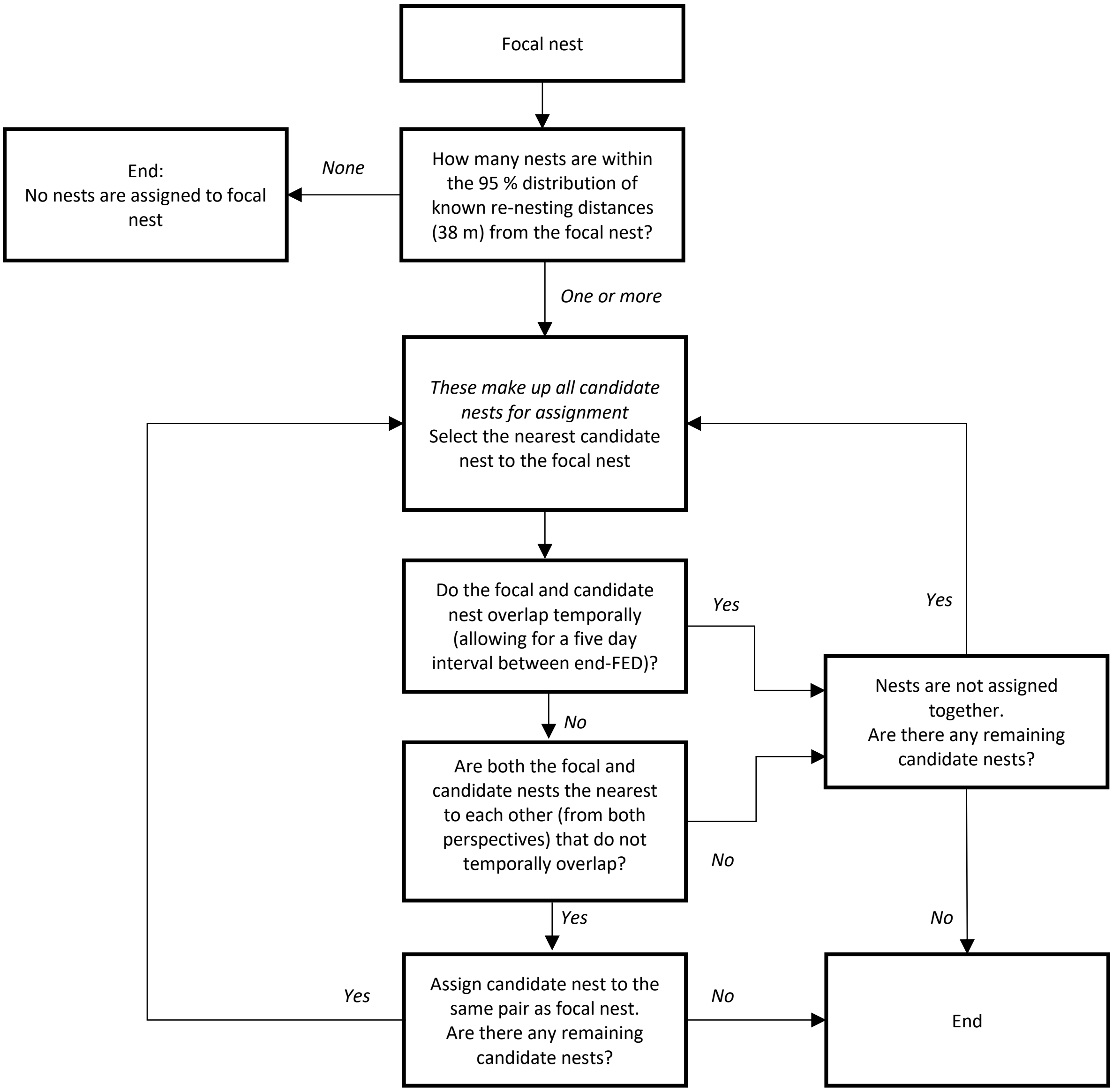

Figure 2.7. Flow chart illustrating the step by step method of considering candidate nests for assignment to the same pair identity as a focal nest.

One hundred nests were assigned as being subsequent nesting attempts of the 90 focal nests. Of nests that were assigned with focal nests, 53 were correct assignments, 17 were incorrect and the remaining 30 were unknown to be correct or incorrect (had incomplete or no parent information). Excluding the unknown ownership nests that were assigned, the proportion of assigned nests that were correct, representing the nest-scale accuracy, was 76 % (n = 70). Twenty two focal nests were identified as being the only attempts for that pair. Of these, three were false negatives i.e. should have been assigned with another nest, while the remainder were correct, as far as was known (it is impossible to be certain of single attempt pairs in the data set as there are nests with unknown ownership in the population). 23 % of focal pairs were associated with an error, making the overall pair-scale accuracy 77 %.

The errors were clearly concentrated in one area of the site, which contained 12 % of all 1016 nests monitored in 2014-17. This area had the highest nest-density; mean number of nests within 38 m of focal nests in this area was 16.6 (n = 15), compared to 7.6 for the whole site (n = 90). Focal nests in this area accounted for a disproportionate amount of the incorrect nest assignment errors (nine out of the 17 errors) and eleven of the twenty one incorrect pair breeding histories. Excluding this part of the study site, the nest-scale and pair-scale accuracy of nest assignment was 89 % (n = 57) and 87 % (n = 75) respectively, with only six nests being incorrectly assigned. This level of accuracy was deemed acceptable; the alternative option of excluding nests lacking confirmed colour-ring adults would have been very likely to result in biased analyses. Firstly, detection of ringed adults is likely biased towards successful breeders and older individuals (Kidd *et al.* 2015), and secondly, leaving nesting attempts unassigned to pair identities would result in underestimation of the number of attempts made by breeding pairs (e.g. Hoffmann *et al.* 2014).

The nest assignment model was therefore used to complete pairs' breeding season histories for 2014-17 data used in the analyses, excluding nests from the area where the model was less accurate. Nest ownership assignment was applied to nests observed to contain eggs or nestlings, as well as fully built nests which were not observed to contain contents e.g. those which were assumed to have failed between nest visits or known to have fledged (Table 2.1), in order to avoid underestimating multi-brooding. An additional step was also included when applying the nest ownership assignment model. As many nests which did not have colour-ringed adults identified, did have some partial adult information such as adults being of un-ringed or metal-ringed status (Table 2.4), when this information provided evidence that a prospective assignment was incorrect, nests were not assigned together. For example, two nests were not assigned if the adults were both un-ringed for nest one and both metal-ringed for nest two. This additional step will have corrected for a small number of the potential errors expected using the nest ownership model. Nest assignment was carried out by generating lists of nests within 38 m of one another (ordered in ascending distance) using ArcGIS and carefully working through the sequence of steps manually.

INVERTEBRATE MONITORING

Invertebrate abundance was primarily measured via the collection of weekly samples, April-August, from water traps placed within reed beds (Figure 2.8). Similar water traps have been used in other studies monitoring invertebrate food availability for breeding birds in wetland habitats (Bibby 1981, Bibby and Thomas 1985) and have been shown to sample similar taxonomic groups to those in the reed warbler diet (Bibby and Thomas 1985). The water traps consisted of yellow 15 cm diameter bowls, mounted on poles approximately 50-100 cm above the water level. Since 2013, three traps were placed at each of seven trapping sites, located in different water bodies, for the purposes of a parallel study assessing the influence of water body edge habitat on invertebrates. In 2015-17, the sample regime was increased to 12 trapping sites (36 individual traps; Figure 2.9). A preliminary analysis showed that using data from one trap was sufficient for measuring invertebrate availability (traps within triplets were highly correlated), so in 2017 the regime was reduced to one trap in each of the 12 trapping sites. Only data from one trap per trapping site were used in data analyses and the following summaries. Water traps contained saline solution and the contents of traps were emptied weekly and stored in 70 % alcohol before being processed in the lab.

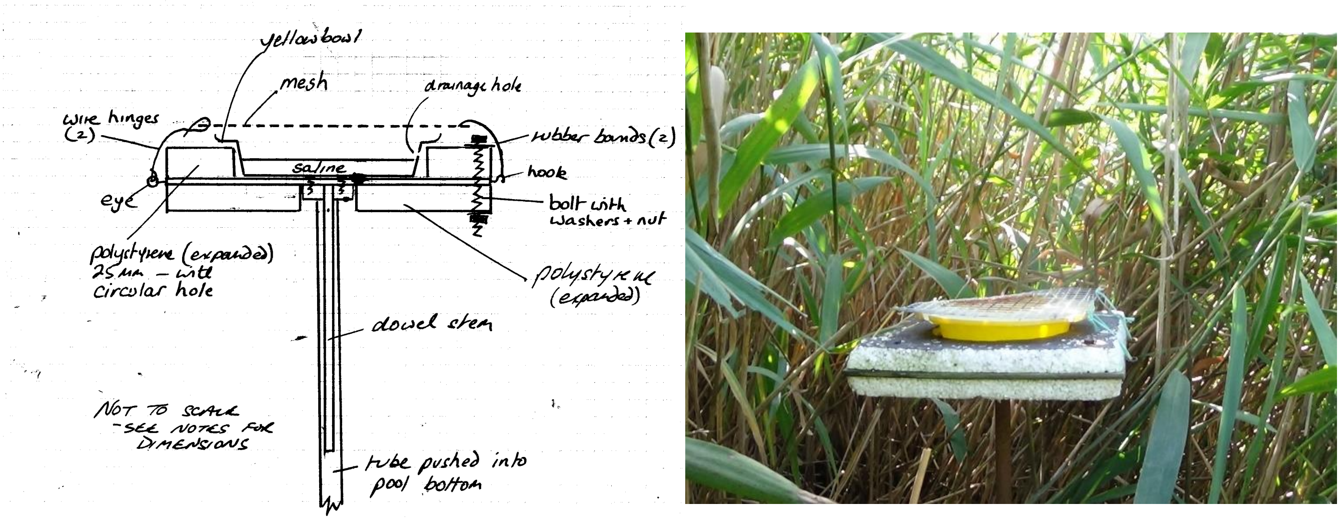

Figure 2.8. Diagram of water trap (left; *Tony Leech*) and photo in situ within the reed bed (right).

Invertebrates were recorded in broad taxonomic groups and 2 mm size categories, consistent with the methods used by Bibby and Thomas (1985). All taxonomic-size category combinations were subsequently assigned a mass value (mg) based on average mass measurements from 2013 and 2014 water trap samples from the study site (when dry mass of all taxa-size categories were measured; Ritchie 2016). This allowed an estimated total mass to be calculated for all weekly water trap samples 2014-17.

The majority of invertebrate mass sampled by water traps were Diptera in all years, which make up the majority of reed warbler diet during the breeding season (Davies and Green 1976, Bibby and Thomas 1985, Evans 1989, Grim and Honza 1996).

Table 2.5. Proportion of invertebrate mass (mg) made up of Diptera across the season in all years (7 water traps in 2014, 12 in 2015-17).

Year	Proportion	SD
2014	0.76	0.14
2015	0.56	0.22
2016	0.60	0.17
2017	0.59	0.18

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 2.9. Map illustrating locations of invertebrate trapping sites; blue= monitored since 2013 sites, pink = additional 2015-17 sites. Reed bed represented by green shading.

Damselflies were not sampled by water traps, as the mesh on top prevented their access (designed to prevent consumption of trapped invertebrates by predators). A few individual damselflies were caught in water traps each year but represented < 0.01 % of trapped items. However, damselflies were observed being frequently fed to nestlings during a pilot study on the site, video recording a sample of nests in 2013. Damselfly abundance was therefore sampled independently from water trapping, throughout the season.

Damselfly abundance was measured by the counting and collection of exuviae at the same sample sites as water traps and samples were collected simultaneously with water trap samples. Exuviae sampling was carried out at all of the water trap sites monitored since 2014 and at two of the additional five sample sites which were added in 2015 (Figure 2.9). At each site, within a search area (1 x 4 m) in the reed bed, all vegetation was 'finger-tip' searched for damselfly exuviae, which were collected and removed.

The total weekly invertebrate mass measured by the seven water trap sample sites monitored since 2013 (Figure 2.9), were significantly correlated with that of the additional five sites monitored in 2015-17, for the period when samples were collected from all sites ($r = 0.82$, $n = 63$ weekly samples, $p < 0.001$). Likewise, total weekly damselfly abundance measured from the sampling sites monitored since 2014 were significantly correlated with the two additional sites monitored since 2015 ($r = 0.68$, $n = 47$ weekly samples, $p < 0.001$). Therefore, measures of site-level invertebrate availability (water trap invertebrate mass and damselfly abundance) used in data analyses were taken from data collected at the seven sample sites monitored in all years (2014-17), in order to derive food availability measures from the same sample regime each season.

CLIMATIC DATA

A weather station was erected on the study site in 2015 but was subsequently flooded in 2016. Mean daily temperatures (◦C) and total daily rainfall (mm) data were therefore subsequently obtained from the nearest Met Office climate station, at Santon Downham, 8.5 km south east of the study site. Data collected from the on-site weather station in 2015 (15th May- 7th August) confirmed strong positive correlations for both mean daily temperature ($r = 0.76$, $n = 85$, $p < 0.001$) and total daily rainfall ($r = 0.77$, $n = 85$, $p < 0.01$) with the Santon Downham data for the equivalent period. Weather data from the Santon Downham MetOffice climate station was thus used in analyses.

Chapter 3- Determinants of double brooding and inter-brood intervals in reed warblers, *Acrocephalus scirpaceus*

INTRODUCTION

In temperate breeding birds, reproductive success is fundamentally determined by the product of brood productivity and the number of broods attempted (Crick *et al.* 1993). One strategy to maximise reproductive success, adopted by some species, is to attempt to successfully raise multiple broods per season. Multi-brooding can substantially increase annual and lifetime reproductive success, measured by the number of nestlings fledged (e.g. Ogden and Stutchbury 1996, Weggler 2006, Hoffmann *et al.* 2014, Cornell and Williams 2016) or the number of offspring recruited into the breeding population (Townsend *et al.* 2013, Hoffmann *et al.* 2014). However, there are also potential costs to being multi-brooded such as reduced parental care for first brood fledglings (Geupel and DeSante 1990, Verhulst and Hut 1996, Naef-Daenzer *et al.* 2011), delayed post breeding moult (Ogden and Stutchbury 1996, Klemp 2000, Morrison *et al.* 2015) and reduced adult survival to the following breeding season (Bryant 1979, Verhulst 1998, Brown *et al.* 2014). In addition, offspring raised from second broods do not necessarily contribute equally to parental fitness as those raised from first broods, as numerous studies have found that offspring raised in later broods are less likely to survive and recruit into the breeding population (e.g. Verboven and Visser 1998, Mallord *et al.* 2008, Hoffmann *et al.* 2014). The decision of whether to multi-brood or not represents a cost-benefit trade-off between current and future reproduction for individuals, in terms of both annual and lifetime fecundity, as predicted by life history theory (Reznick 1985, Stearns 1992). Within multi-brooded populations, there is individual variation in multi-brooding, with some individuals being single-brooded and others multi-brooded in a given breeding season (e.g. Verboven *et al.* 2001, Nagy and Holmes 2005b, Hoffmann *et al.* 2014). Understanding the factors which influence multi-brooding behaviour at the individual level is important, not only because multi-brooding influences population productivity, but also in the context of the extension in the length of the breeding season, which has been observed in some multi-brooded species in response to spring warming in recent decades (Møller *et al.* 2010, Halupka and Halupka 2017).

The timing of the first brood has been found to be the most important determinant of double brooding in most studies across a wide range of species (Geupel and DeSante 1990, Ogden and Stutchbury 1996, Verboven and Verhulst 1996, Brinkhof *et al.* 2002, Weggler 2006, Bulluck *et al.* 2013, O’Brien and Dawson 2013, Townsend *et al.* 2013, Carro *et al.* 2014, Hoffmann *et al.* 2014, Zając *et al.* 2015, Béziers and Roulin

2016, Jackson and Cresswell 2017), although a recent study of European Starlings, *Sturnus vulgaris*, proves an exception (Cornell and Williams 2016). Verboven and Verhulst (1996) dismissed the possibility that earlier breeding individuals are more likely to double-brood as a result of being higher quality, rather than as a direct result of timing, as their experimental manipulations of the timing of breeding in great tits, *Parus major*, confirmed that double brooding probability increased when breeding attempts were advanced and declined when they were delayed. Other potential predictors include parental factors (such as age or effort) or environmental factors (food availability or weather). Several studies have found female age to be important, with older individuals being more likely to double brood, whereas the age of males appears to be unimportant (Geupel and DeSante 1990, Weggler 2006, Bulluck *et al.* 2013, Hoffmann *et al.* 2014). Assessments of the effect of the number of offspring in the first brood on the probability of double brooding, representing a trade-off in parental effort between first and second broods, has revealed conflicting results. Smaller first broods increased the probability of attempting second broods in great tits and blue tits, *Cyanistes caeruleus* (Tinbergen 1987, Verboven and Verhulst 1996, Parejo and Danchin 2006), while the opposite effect was found in Eurasian hoopoes, *Upupa epops* (Hoffmann *et al.* 2014). Furthermore, several other studies have found no effect of first brood size on double brooding probability (Ogden and Stutchbury 1996, Brinkhof *et al.* 2002). Finally, male parental care may also contribute to the decision to double brood as a recent study of Japanese tits, *Parus minor*, found that the likelihood of double brooding increased with the proportion of provisioning undertaken by the male (Nomi *et al.* 2018).

Food availability has been shown to be associated with the probability of double brooding in both observational studies (Nagy and Holmes 2005b, Husby *et al.* 2009, Jackson and Cresswell 2017) and food supplementation experiments (Nagy and Holmes 2005a, O'Brien and Dawson 2013). Weather variables such as temperature and rainfall have also been found to be related to the extent of double brooding in populations and the breeding season length of individuals as a result of their association with the timing of the start of breeding (Carro *et al.* 2014, Jankowiak *et al.* 2014, Jankowiak and Wysocki 2015), although the effect of weather after fledging a first brood on the probability of double brooding does not seem to have been previously assessed. Weather conditions at the time of potential double brooding decisions could, however, be important through potential influences of food availability and post-fledging mortality of fledged first broods.

Further to the 'decision' of whether to attempt an additional brood or not, there is further scope for variation in multi-brooding behaviour in terms of the length of the interval between attempts (hereafter 'inter-brood interval'). A long-term study of barn swallows, *Hirundo rustica*, found that inter-brood intervals between first and second broods have extended as the start of the species' breeding season has advanced, and that longer inter-brood intervals have fitness benefits for adults (Møller 2007). Several studies have shown that inter-brood intervals are longer when first broods are larger, likely because larger first broods require more parental care (Verboven and Verhulst 1996, Møller 2007, Carro *et al.* 2014). Food availability has also been shown to influence inter-brood interval lengths; as food supplementation did not affect the probability of double brooding, but did shorten the inter-brood intervals of double-brooded great tits (Verboven *et al.* 2001). Post-fledging parental care of first broods is often cut short by multi-brooded individuals that attempt second broods and brood overlap may also be employed, whereby care of the first brood continues after the second brood has been initiated (Verhulst *et al.* 1997, Naef-Daenzer *et al.* 2011, Béziers and Roulin 2016, Stępniewski and Halupka 2018). A further fundamental factor determining inter-brood interval lengths is post-fledging mortality, as short intervals may be the result of mortality of part or all of the first brood soon after fledging (Verhulst and Hut 1996).

In this study, the factors influencing the probability of double brooding and the inter-brood interval length between first and second broods was assessed in Eurasian reed warblers, *Acrocephalus scirpaceous* (hereafter 'reed warblers'). Reed warblers are a facultative double-brooded species in which females also frequently replace failed nesting attempts and have been known to lay up to five clutches in a season (Borowiec 1992, Schulze-Hagen *et al.* 1996, Halupka *et al.* 2008). Previous estimates of the frequency of double brooding range between 0-35 % of pairs (Schulze-Hagen *et al.* 1996, Calvert 2005) and the first cases of triple brooding have recently been documented in the population subject to the current study (Batey and Leech 2018/ Appendix 1). The reed warbler is a long-distance migrant whose spring arrival and timing of breeding have advanced significantly with climate change induced spring warming (Crick and Sparks 1999, Schaefer *et al.* 2006, Halupka *et al.* 2008) and there is also evidence of breeding season extension (Halupka *et al.* 2008). Reed warblers are generalist insectivores that breed in wetlands, where invertebrate prey is generally considered to be abundant throughout the breeding season (Bibby and Thomas 1985, Both *et al.* 2009, Dodson *et al.* 2016); however, the timing of first broods has been shown to be influenced by food availability (Vafidis *et al.* 2016).

Further to being an aspect of avian breeding ecology which is relatively understudied, multi-brooding behaviours are of further interest in the context of breeding season extension, which has been observed in some multi-brooded species in recent decades and in association with climate change (Møller *et al.* 2010, Halupka and Halupka 2017). An increased propensity to multi-brood (Halupka *et al.* 2008), increased failure rates resulting in more replacement nesting attempts, or extended intervals between broods

(Møller 2007) are all potential mechanisms behind such breeding season extensions . Reed warblers are a good model species for assessing the potential for increased double brooding to be driving breeding season extension, as increased reproductive output has been suggested as a driver of recent population increase (Woodward *et al.* 2018); therefore increased replacement nesting attempts as a result of increased failure rates is not a likely mechanism of season length extension in reed warblers (Halupka *et al.* 2008, Woodward *et al.* 2018). Instead, it can be postulated that breeding season extension could result from either longer intervals between broods or shorter intervals between broods allowing for an increase in the number of broods attempted. Assessing the relationship between multi-brooding and interval lengths with environmental conditions including wetland food availability, which is likely to have increased with climate warming in recent decades (Vafidis 2014), allows for inference of the likely mechanism.

Using a four year data set from an intensively monitored breeding population, this study investigates the effects of first brood breeding performance (timing and number of nestlings fledged) and environmental factors (food availability, temperature and rainfall) on the decision to double brood and, for double-brooded pairs, the length of the inter-brood interval. This is to my knowledge the first formal analysis of the determinants of double brooding in reed warblers.

METHODS

Data collection

A breeding population of approximately 150 pairs of reed warblers has been monitored at Watermill Broad Nature Reserve, in Norfolk, UK, since 2011. For the purposes of this chapter, breeding ecology data collected in 2014-17 were used as this is the period during which individuals were uniquely marked and videoed at the nest. The site is a 46 ha former abstraction site for gravel, sand and peat (operating 1987-2006) and is characterised by ponds and lakes of varying sizes, fringed predominantly with narrow stretches of *Phragmites australis* reed bed which total 7.6 ha. The lake fringes are also interspersed with willow, *Salix* sp., and alder, *Alnus glutinosa*, scrub as well as mature willows.

Fieldworkers attempted to locate and monitor all reed warbler nests across the whole breeding season each year. Nest finding was carried out via systematic searching of all reed bed on site, at regular intervals (at least once every 7-10 days) during April-August, although nests were also often found in the course of daily fieldwork outside of dedicated systematic searching. Nest searching began in mid-April, when the first birds arrived, and continued until an entire search of the site revealed no new nests, provided this occurred after the 10th August, the date of the latest ever first egg date (FED) recorded on the study site. A total of 1155 nests were found across the study period. These comprised 1016 nests which were observed to contain eggs or nestlings, 12 which were found after nestlings had fledged (missed successful nests) and 127 fully built nests that were never observed to contain eggs or nestlings but which are assumed to have failed during the laying period between nest visits (missed failed nests). Nesting attempts which were abandoned during nest building were also found but were not included in analyses.

Active nests were checked every 3-5 days until failure or fledging and all nestlings were ringed with a single uniquely coded British Trust for Ornithology (BTO) metal ring between day 4-7 (day 1 being the day of hatching). First egg dates (FEDs) for nests monitored during the laying stage were calculated based on one egg being laid per day (Brown and Davies 1949). For nests found after this stage, the potential minimum and maximum first egg dates were back-calculated based on possible incubation lengths of 11-13 days from the day the penultimate egg was laid and estimated hatch dates from nestling ageing (92% of nests with known first egg dates and hatching dates in 2014-17 fell within this range, n = 100; Chapter 2). Accurate ageing was considered possible for day one or day two nestlings, on the basis of size, skin colour and eye opening (pers. obs.), while ageing of older nestlings was assumed to be less accurate, so hatch date estimates allowed for plus or minus one day when the age of older nestlings had been estimated. The median of possible FEDs was then taken (or upper median if there was an even number of possible dates) and used in the analyses. A nest was considered successful if it fledged one or more young and was determined from a nest observed to be empty, intact and containing feather scale or covered in droppings. The number of nestlings fledged was calculated from the number of nestlings in the nest on the penultimate visit, before the visit confirming nest success, plus any well-feathered nestlings that were present on a previous visit, as asynchronous fledging is not uncommon (pers. obs.). The penultimate visit before a fledge check was at least three days after nestling ringing (nestling day 7 at the earliest); nestlings which died older than this age were sometimes found dead in the nest, whereas whenever brood reduction occurred at younger nestling ages, nestlings had been removed from nests (pers. obs.). The end dates of nests (fail or fledge dates) were taken as the mid-point between the last visit with live nest contents and the concluding visit, but fledge dates were capped at two days following the observation of nestlings already large enough to fledge, to avoid cases of late fledge checks extending the estimated date of fledging (Chapter 2).

All adults encountered on site during the breeding season, either during Constant Effort Site (CES) mist netting April- September each year (since 2013), or via targeted mist netting at nesting territories from

2015 onwards, were marked with unique two-digit, engraved darvic rings,. All nests were videoed to identify parents (Chapter 2), from the day of nestling ringing in 2014 and from the first day of incubation in 2015-17. Additional video attempts were made opportunistically at active nests, until both parents were identified.

Food availability was assessed via two methods. Firstly, invertebrates were sampled using seven water traps, placed in different reed beds on the study site (Chapter 2). Water traps were similar to those used to sample invertebrates in reed beds in previous studies (Bibby 1981, Bibby and Thomas 1985) and which have been found to sample a similar range of invertebrates as that found in the diet of reed warblers (Bibby and Thomas 1985). The total mass of invertebrates in each sample (one per trap each week) was calculated by sorting trap contents into taxa-size category combinations which were each assigned a mass value (mg) based on measurements made in previous years on the study site (Ritchie 2016). The second food availability measure involved the systematic collection of damselfly exuviae as a measure of damselfly abundance; reed warblers at this site have regularly been observed feeding both damselfly adults and larvae (presumably gleaned from vegetation prior to metamorphosis) to nestlings but these insects are not represented in water traps samples (Chapter 2). Damsefly exuviae sample sites were located in the same reed bed sites as those where water traps were placed (Chapter 2). Both methods were undertaken weekly, with both samples collected on the same dates, from April-August inclusive.

Mean daily temperature (◦C) and total daily rainfall (mm) data were obtained from the nearest Met Office climate station, Santon Downham, 8.5 km south east of Watermill Broad. Incomplete weather data collected at Watermill Broad indicated that both temperature and rainfall were significantly correlated between the two sites (Chapter 2).

Data analysis

Nests of unknown ownership (including nests of parents with no rings or only metal rings and nests that were found after completion of the nesting attempt, failed or fledged) were assigned ownership in order to complete breeding season histories of pairs. Nest ownership was assigned using a relative proximity method, excluding nests in one reed bed where high nest density means that nests cannot be accurately assigned ownership (Chapter 2). As a result of having unassigned ownership, nests in this part of the site are excluded from all data summaries and analyses hereafter. Outside of the excluded part of the site, the relative proximity nest assignment method is 87 % accurate at completing nest histories at the pair level. Nests within an absolute distance threshold of 38 m were considered for assignment, as this represents the distance within which 95 % of known re-nesting attempts occur (data from re-nesting attempts made by colour-ringed pairs in 2014-17, n = 176)**.** Nests were then grouped together as belonging to the same pair if they were the closest nests to each other that did not overlap temporally (allowing for the minimum temporal re-nesting interval of five days, the minimum time taken to build a new nest; pers. obs.). Finally, any partial parent information available was used to check compatibility of nests belonging to the same pair; for example, a nest with two metal-ringed parents could not be assigned to a subsequent nest with two un-ringed parents. This additional step will have corrected for an unknown amount of errors made by the relative proximity method of assignment, meaning the overall accuracy of nest assignment is greater than 87 % at the pair level.

Statistical analyses were undertaken using R version 3.5.0 (R-Core-Team 2018), using 'dplyr' for data manipulations (Wickham *et al.* 2018) and 'lme4' for modelling (Bates *et al.* 2018). Generalized linear models (GLMs) were used to assess the factors which influenced the probability of double brooding. The data used in this analysis represented all pairs that had a successful first brood (excluding pairs which fledged a cuckoo, *Cuculus canorus* in their first brood), across the four seasons 2014-17. Preliminary analyses of alternative food (invertebrate) and weather predictors were undertaken to identify the most suitable environmental measures for analyses. Two measures of whole-site food (invertebrate) availability at the time of fledging the first brood were considered: (i) the total mass of water trap contents (all sample sites combined) on the sample date on or following the fledge date of nests, and (ii) the total count of damselfly exuviae (all sites combined) on the same sample date. These two measures were highly correlated (r = 0.6), so both were modelled separately and model results were very similar. The models including the water trap measure were used to present the results, as water trap samples were dominated by Diptera, known to be an important part of reed warbler diet from previous studies (Chapter 2). Weather variables (mean daily temperature and total daily rainfall) were assessed across three alternative temporal windows: (i) the 5 days following the fledge date of the first brood (representing the period after fledging a first brood but within the shortest recorded inter-brood interval of 6 days); (ii) from twelve days previous to the fledge date to 5 days following the fledge date (representing the period of nestling provisioning as well as the minimum inter-brood interval; presumably the period over which food demands are greatest); and (iii) the period from the FED to 5 days after the fledge date (representing the minimum period for the whole first brood nesting cycle). All three measures provided similar results (for both temperature and rainfall), but effect sizes were largest using the period 5 days following the first brood fledge date, so this window was used for both weather variables.

Whether or not a pair attempted a second brood after fledging a first brood (1 = yes, 0 = no) was modelled with a binomial error structure and a logit link function. Fixed effects comprised: year, FED of the first brood, number of nestlings fledged from the first brood and three environmental measures; food availability (total mass of all water traps on site from the sample collected on or nearest after the fledge date of nests), temperature (the mean across the five days following fledge date) and rainfall (the total across the five days following fledge date). In addition, the interactions between FED of the first brood and each of the three environmental variables were also included to assess whether any environmental effects varied with the timing of the first brood. Year was fitted as a fixed effect, rather than a random effect, as there were few levels (< 5 years data; Crawley 2002, Bolker *et al.* 2009). All numeric variables were centred and scaled and only observations with complete data for all variables were included in the analysis (n = 348 successful first brood nests). Collinearity between explanatory variables was assessed via pair-wise correlation plots and variance inflation factors (VIFs; as in Zuur *et al.* 2009). All pair-wise correlations had relatively low r values (≤ 0.5) and relatively small VIFs (< 3) and all explanatory variables were retained in the model. The full model was validated, checking for outliers and a reasonable model fit to the raw data, with a binned plot of average residual values against fitted values (Gelman and Su 2018). All possible candidate models were then ranked by their AICc value (sample size adjusted Akaike Information Criterion) and the predictions of the best models (those within 2 AICc units of the best-fitting model) were averaged (Barton, 2015).

The effect of female age on double brooding could not be included in the double brooding probability model due to very small sample sizes of known age females, especially first year breeding birds. Over the four seasons, four known age first year females were double-brooded (n = 26) compared with 28 known age older females (n = 83). The timing (FED) of known age females' first attempts of the season (i.e. regardless of whether the attempt was successful or not) was significantly later for first year females (hatched the previous season) compared to all older females (t = 2.1281, df = 42.326, p < 0.05; excludes repeated measures of individuals). Mean FED was 7th June for first year females (sd = 17.6 days, n = 24) and 28th May for females aged two years and above (sd = 15.6 days, n = 45). Given this difference in breeding timing, the influence of female age may be accounted for, to some extent, by the FED term in the double brooding model.

For pairs which were double-brooded, a linear model was used to assess the factors influencing the interval length between first broods and second brood attempts (number of days between the fledge date of the first brood and the FED of the second brood). Data from 2016 were excluded from this analysis as a flood in the middle of this season resulted in a week when no new nests could be initiated on the study site due to temporary loss of nesting habitat under water. All double-brooded pairs in this season had successful first broods before the flood event and therefore had unusually long inter-brood intervals, which if included in analyses may have reduced their ability to assess the role of the main factors of interest in determining interval length. As with the double brooding model, pairs which fledged a cuckoo in their first brood were also excluded. Explanatory variables comprised: year, FED of the first brood, number of nestlings fledged from the first brood, and the same measures of food availability, temperature and rainfall as those used in the double brooding analysis. Numeric variables were centered and scaled and only observations with complete data were included in analyses (n = 99 interval lengths between first and second broods). Of the explanatory variables, year had a very high VIF (14), so year was removed from the model. Once year had been removed, all remaining explanatory variables had VIF values < 2 and pair-wise correlations with r values ≤ 0.7. Three interaction terms were included in the global model; between FED of the first brood and each of the environmental variables. The full model was validated, checking for outliers and a reasonable model fit, after plotting the distribution of residuals and the residuals versus fitted values. All candidate models were ranked and the best models averaged using the same methods as for the double brooding model.

For the models of both double brooding and interval length, some individuals contributed to multiple years of data, as illustrated by the age structure of colour-ringed birds (Table 3.1). However, this level of pseudoreplication cannot be fully accounted for due to the number of birds with no rings (Table 3.2). While it is reasonable to expect that the probability of double brooding (or interval length) may vary between individuals (e.g. as suggested to influence double brooding in starlings by Cornell and Williams 2016), it seems unlikely that individual effects could either drive or mask external effects such as those of timing, first brood performance, or weather conditions. It was therefore deemed preferable to include the full sample of pairs for analyses in the study rather than analyse the very small sample of colour-ringed birds only. All figures were produced using ‘ggplot2’ (Wickham 2009) and ‘gridExtra’ (Auguie 2017).

RESULTS

A total of 1021 nests were found in the study site across the four seasons (excluding the area where nests could not accurately be assigned ownership). Of these, 507 nests had at least one identified colour-ringed adult; the remaining 514 had either no colour-ringed adults or no parent identity information and were assigned nest ownership. Of known age birds, individuals aged between 1 and 4 represent the majority and the proportion of colour-ringed birds in the population has increased during the study (Tables 3.1 and 3.2). The estimated number of pairs nesting in each season was 121 in 2014, 154 in 2015, 139 in 2016 and 105 in 2017. Pairs had 1-4 nesting attempts in a season (except in one extreme case of a pair having seven unsuccessful first brood attempts, five of which were observed to contain eggs).

The percentage of pairs that attempted a second brood after previously fledging a first brood (including those that were triple-brooded but excluding pairs which hatched a cuckoo in any of their nesting attempts) ranged between 13-47 % annually and was 26 % across the four years (Table 3.3). There were 12 cases of pairs having second attempts at failed second broods and there were also three cases of triple brooding (two of which are described in Batey and Leech 2018/ Appendix 1). Excluding second attempts at second broods, the mean FED of second broods was 6th July (sd = 11.2 days, n = 127) while the earliest and latest were 6th June and 1st August respectively. The mean FED of successful first broods for pairs that were ultimately double-brooded was 24th May (sd = 10.0 days, n = 123); for pairs that were single-brooded it was 21st June (sd = 18.4 days, n = 225; Figure 3.1). Of pairs that were double-brooded, the latest first brood had a FED of 21st June. The distributions of successful first brood FEDs of single and double-brooded pairs overlap considerably but have different means, and the variance in first brood FEDs of single-brooded pairs is greater than that for double-brooded pairs (Figure 3.1). Single-brooded pairs produced on average 3.0 fledglings (sd = 1.0, range = 1-5, n = 214), compared to an average of 5.6 (sd = 1.8, range = 1-9, n = 119) for all double-brooded pairs and 6.1 (sd = 1.6, range = 2-9, n = 96) for double-brooded pairs which were successful with both broods.

The median interval between first and second broods (days between first brood fledging and second brood FED) was 17 days (sd = 5.3 days, n = 99) and ranged from 6-28 days (Figure 3.2). Typical intervals between failed nests and replacements were much shorter (median = 7 days, 75 % of intervals $\leq$ 10 days, n = 236).

Food availability was generally higher in 2014 than in the other three seasons (Figure 3.3). Furthermore, food availability is relatively low at the start of the season (except in 2014), is greatest in the middle of the season before declining to low levels once more at the end of the season (shown for 2014-16 as sampling ceased earlier in 2017). There was no clear peak in food availability in any season except 2016, where a peak in invertebrate mass occurred the week following an extensive flood.

Determinants of double brooding

Double brooding was predicted by the first egg date (FED) of first broods, food availability, rainfall (in the 5 days following the fledging of the first brood) and the interaction between FED and food availability, with all these terms present in all best-fitting models (Table 3.4). The model-averaged probability of double brooding declined with FED of the first brood (Figure 4). Greater food availability was predicted to increase the likelihood of double brooding (Figure 3.5), but this effect varied with the timing of the first brood; the likelihood of double brooding if the first brood was very early was relatively high regardless of food availability, whereas for later first broods, food availability appeared to have a strong positive effect (Figure 3.6). The probability of double brooding declined with increasing rainfall, but the effect of the interaction with FED appeared to be relatively weak (Figure 3.7). There was evidence of a small negative effect of the number of fledglings produced by first broods and a small positive effect of temperature (during the 5 days following the fledging of the first brood) on the propensity to double brood, however these terms had relatively small effect sizes (Table 3.4). In addition, the interaction between FED and temperature was present in one of the best-fitting models, although the effect size was also relatively small (Table 3.4).

Determinants of inter-brood intervals

The number of fledglings produced by the first brood, food availability and both rainfall and temperature in the five days following the fledging of the first brood were the most important predictors of the length of the interval between first and second broods, being present in all best-fitting models (Table 3.5). Interval length was predicted to increase with the number of nestlings fledged in the first brood (Figure 3.8). Both increased rainfall and temperature were also predicted to extend interval length (Figure 3.9, Figure 3.10), while greater food availability had a negative influence on interval length (Figure 3.11). Although FED of first broods was present in two of the best-fitting models, this effect appeared negligible as the effect size was small (Table 3.5) and confidence intervals of predictions based on different FEDs completely overlapped (Figure 3.9, Figure 3.10). Likewise, the interaction terms between FED and both rainfall and temperature, although present in best-fitting models, appeared to be unimportant (Figure 3.9, Figure 3.10).

DISCUSSION

The annual proportion of pairs that were double-brooded in the study population varied considerably between the four years from 13 to 47 % of breeding pairs (Table 3.3). The average figure over the course of the study of 26 % of pairs being double-brooded falls within, but is at the upper end of, the range of previously published estimates of double brooding rates in other European reed warbler populations (0-35 %; Dyrcz 1981, Schulze-Hagen *et al.* 1996, Calvert 2005, Halupka *et al.* 2008). It was also found that double-brooded pairs produced more fledglings annually than single-brooded pairs, as has been found in other studies of double brooding across a wide range of species (e.g. Ogden and Stutchbury 1996, Weggler 2006, Hoffmann *et al.* 2014, Cornell and Williams 2016).

Determinants of double brooding

A key predictor of double brooding was the timing of the first successful brood; an earlier first brood increased the probability of attempting a second brood. This is in agreement with the vast majority of previous studies which have assessed the determinants of double brooding in other species (Geupel and DeSante 1990, Ogden and Stutchbury 1996, Verboven and Verhulst 1996, Brinkhof *et al.* 2002, Weggler 2006, Bulluck *et al.* 2013, O'Brien and Dawson 2013, Townsend *et al.* 2013, Carro *et al.* 2014, Hoffmann *et al.* 2014, Zając *et al.* 2015, Béziers and Roulin 2016, Jackson and Cresswell 2017). It is of course logical that the time remaining in the breeding season should be a considerable constraint on double brooding, as conditions suitable for breeding deteriorate and there are other demands on the adult annual life cycle beyond breeding. Other demands on breeding adults at the end of the breeding season include post-breeding moult, which may conflict with later breeding (Ogden and Stutchbury 1996, Klemp 2000) and post-breeding migration for long-distance migrants, as is the case with reed warblers. Although timing is the most important factor for double brooding, the frequency distributions of the timing of first broods of single- and double-brooded pairs overlap substantially, indicating that other factors contribute to the 'decision' to double brood. Moreover, the 'decision' not to double brood is unlikely to simply be a result of adult mortality, as adult reed warbler survival is high during the breeding season (Wierucka *et al.* 2016).

Food availability had a positive influence on the probability of double brooding. While the influence of food resources on the timing of breeding at the start of the season are well founded (Pearce-Higgins and Green 2014), including in reed warblers (Vafidis *et al.* 2016), few studies have reported evidence of an effect of food availability on multi-brooding. Increased food availability has previously been found to positively influence the probability of individuals' double brooding via experimental food supplementation in both black-throated blue warblers, *Setophaga caerulescens*, and mountain bluebirds, *Sialia currucoides* (Nagy and Holmes 2005a, O'Brien and Dawson 2013). Food resources are likely to be a limiting factor on double brooding as abundant food may be needed both for the successful raising of nestlings and for self-maintenance of breeding adults. Interestingly, there appeared to be a minimal effect of food availability on double brooding probability when first broods were very early; the earliest breeders appear not be limited by food availability but this may be because food resources are still increasing at this point in the breeding season. Evidence suggests that food availability in wetland habitats is relatively abundant throughout the whole breeding season for generalist insectivores such as reed warblers (Bibby and Thomas 1985, Halupka *et al.* 2008). This is because *Phragmites* reed continues to grow throughout the summer (Dykyjova *et al.* 1970), insects continue to emerge from the water all season (Dodson *et al.* 2016) and there are sequential peaks of different taxonomic groups of invertebrates (Both *et al.* 2009, Vafidis 2014). Furthermore, there is experimental evidence that invertebrate availability in reed bed habitat is related to climate: Vafidis (2014) found that warmer temperatures resulted in faster reed growth as well as earlier and longer peaks of invertebrate abundance. Moreover, the effects of increased temperature on invertebrate availability appeared to be long-lasting, that is to say that warmer temperatures did not just result in an earlier peak but consistently greater food availability for a duration of at least two months (the duration of data collection; Vafidis 2014). These results, in combination with the observed effect of food availability on double brooding in the present study, suggest that increased invertebrate abundance facilitating greater rates of double brooding could be the mechanism driving extended breeding season lengths in some multi-brooded species (Møller *et al.* 2010, Halupka and Halupka 2017). Certainly, climate is known to influence invertebrate availability for breeding birds in other study systems such as woodland habitats, where spring warming has advanced the peak in caterpillars, the staple prey species for many woodland passerines (e.g. Visser *et al.* 2006, Mallord *et al.* 2017). Therefore, the potential for increased food availability to facilitate greater rates of double brooding may depend on the invertebrate resources of varying habitats as well as the dietary strategies of multi-brooded species. Husby *et al.* (2009) found that earlier peaks in caterpillar prey resulted in a reduced frequency of double brooding in great tits, which may be a result of the fact that woodland habitats are characterised by a short peak in invertebrate prey, in contrast to wetland habitats (Both *et al.* 2009). Furthermore, dietary flexibility of multi-brooded species may influence potential effects of changing invertebrate phenology on birds (Mallord *et al.* 2017).

Environmental conditions at the time of fledging first broods were also found to influence the probability of double brooding. Pairs were more likely to double brood when rainfall was lower at this time. Weather effects on the timing of breeding in birds are well documented (Pearce-Higgins and Green 2014). It has been widely found that favourable early season conditions, such as warmer springs (Halupka *et al.* 2008, Møller *et al.* 2010) or wetter springs (Carro *et al.* 2014) advance the timing of breeding, while earlier breeding in turn leads to an increased propensity to double brood (e.g. Weggler 2006, Carro *et al.* 2014, Hoffmann *et al.* 2014). The effect of weather conditions on double brooding, through their effect on the timing of breeding at the beginning of the season (i.e. influencing breeding phenology) is therefore well founded. However, this study potentially provides the first evidence of weather conditions following the first brood having a direct influence on double brooding decisions, after controlling for the effect of timing of breeding.

It must be noted that weather conditions are not likely to exert a direct effect on double brooding decisions, but instead probably act indirectly through, for example, effects on food availability (invertebrate abundance or activity), or the post-fledging survival of first brood offspring, either of which may potentially influence parental investment in an additional brood, or self-maintenance. A meta-analysis on the effects of climate on natural populations reported that climate effects are more likely mediated via indirect biotic mechanisms such as food availability than via direct abiotic mechanisms such as weather (Ockendon *et al.* 2014). Although a measure of food availability was included in analyses, it is possible that the observed weather effects are a result of the weather variables capturing some aspect of food availability not captured by the water trap data. For example, increased rainfall may make foraging more difficult, irrespective of invertebrate abundance, resulting in greater effort being required for the provisioning of fledglings as well as for self-maintenance. Post-fledging care and mortality is an understudied aspect of breeding ecology (Streby *et al.* 2014) and future research should be aimed at addressing the extent to which environmental conditions effect levels of post-fledging care as well as mortality. Furthermore the potential role of post-fledging mortality in double brooding decisions requires attention.

Although a small effect, the number of nestlings fledged in the first brood was inversely related to double brooding probability. The same effect has been found in several studies of other species (Tinbergen 1987, Verboven and Verhulst 1996, Parejo and Danchin 2006) although not in others (Ogden and Stutchbury 1996, Brinkhof *et al.* 2002, Hoffmann *et al.* 2014). Double brooding may be more likely following smaller first broods as larger broods may impose greater costs on parents via increased parental care (Verhulst and Hut 1996). Furthermore, it may be more profitable for females to reduce effort for smaller broods and instead invest in an additional brood (Grüebler and Naef-Daenzer 2008). An alternative hypothesis could be that if smaller broods are less likely to result in any fledglings being reared to independence, parents may be more likely to invest in a second brood, which in terms of rearing offspring to independence could actually be considered the replacement of the previous brood. There is a need for future studies to investigate the role of post-fledging care and mortality in double brooding and the extent to which second broods actually replace post-fledging failures.

Although female age has been found to influence double brooding in several studies (Geupel and DeSante 1990, Weggler 2006, Bulluck *et al.* 2013, Hoffmann *et al.* 2014), the current study lacked the data to test for the effect of female age on double brooding while accounting for other predictors. However, first year individuals breed later than older individuals, so simply as a function of the effect of timing on the probability of double brooding, first year birds are presumably less likely to double brood. Indeed, there were few cases of known first year females attempting to double brood and, from the raw data alone, these birds appear approximately half as likely to double brood as older females. Interestingly, the timing of breeding of first year females in this study approximately represents the timing at which the reed warbler breeding season used to begin in the UK before the breeding timing advancement apparent since the 1970s (i.e. beginning in late May; Beddall Smith 1919, Brown and Davies 1949, Lack 1963). Assuming first year individuals bred relatively later than older individuals before the advancement of breeding observed during climate change (Crick and Sparks 1999), it may well be the case that first year individuals did not previously have the opportunity to double-brood.

Determinants of inter-brood intervals

There was a great amount of variation in inter-brood intervals between first and second broods, ranging from 6-28 days. The most important predictor explaining this variation was the number of nestlings fledged in the first brood. Longer intervals followed larger first broods, which is in agreement with several previous studies (Tinbergen 1987, Verboven and Verhulst 1996, Møller 2007, Carro *et al.* 2014). One possible explanation for interval length being dependent on the number of fledglings from the first brood is that interval length could be driven largely by post-fledging mortality. For example, if the rate of post-fledging mortality is independent of the number of fledglings, then when fewer offspring fledged, it would be more likely that all fledglings would die before reaching independence. This would clearly reduce the time spent on post-fledging parental care and consequently the interval length. However, if post-fledging mortality was the main driver of interval length, the shortest intervals would be expected to represent whole broods not surviving to independence, as has been observed to be the case in a study of great tits (Verhulst and Hut 1996). This does not seem to be the case in the present study, as cases of fledglings from first broods having survived to independence (determined through capture later in the season during constant effort mist netting) when there was an inter-brood interval of just six days, the minimum observed interval, have been observed during the study. It therefore seems that the effect of the number of nestlings fledged in the first brood on the inter-brood interval length cannot simply be the result of post-fledging mortality, but instead could reflect reduced parental effort required to raise smaller broods to independence, as has previously been suggested by Tinbergen (1987) and Carro *et al.* (2014). Shorter inter-brood intervals following smaller first broods are likely to occur as a result of brood overlap, whereby the male cares for the offspring of the earlier brood while the female initiates the next brood, as has been reported in numerous other multi-brooded species (e.g. Haftorn 1978, Tinbergen 1987, Béziers and Roulin 2016, Stępniewski and Halupka 2018). For example, substantial brood overlap occurs in bearded tits (*Panurus biarmicus*), which have been observed initiating the next nesting attempt while the nestlings of the previous brood are as young as three days old (Stępniewski and Halupka 2018). This raises the question of whether male parental care could be an important factor influencing interval length between broods, as male effort has already been linked to the incidence of second broods (Nomi *et al.* 2018).

Food availability was found to be negatively associated with interval length, greater food resources predicting a shorter interval between broods. This supports the findings of previous research, where experimental food supplementation shortened the inter-brood interval of great tits (Verboven *et al.* 2001). Presumably, greater food availability either shortens the period of post-fledging care before offspring become independent, or provides greater opportunity for brood division/ brood overlap, whereby males undertake the majority of post-fledging care, allowing the female to initiate the subsequent brood. As food availability has been shown to be positively related to temperature in reed bed habitats (Vafidis 2014), increased intervals between nesting attempts as a potential mechanism behind climate change induced breeding season extension, is not supported by the current study (cf. Møller 2007).

Environmental conditions in the days following the fledging of first broods were also found to affect the inter-brood interval; both increased rainfall and temperature resulted in longer intervals. This is in contrast to a previous study which found no effect of mid-season conditions on interval lengths between barn swallow clutches (Møller 2007). Variation in weather may control an aspect of food availability not captured by the water trap data. Alternative explanations for the effect of rain could be the fact that in poorer conditions, second brood laying is delayed or nest building is either delayed or extended, as reed warbler nests are exposed to the prevailing weather. In addition, costs of first brood fledgling care or self-care may be increased in conditions with more rainfall. A possible explanation for the positive effect of temperature on interval length could be that warmer temperatures may result in invertebrates being more active and therefore more difficult to catch, resulting in increased time devoted to foraging. Ultimately, the effects of both environmental measures presumably result in conditions which either make foraging more difficult, thereby increasing the costs of parental care, or increase post-fledging survival, also resulting in increased care requirements for fledglings. Informative future work could assess the length of the period of post-fledging care in relation to weather conditions.

Interestingly, the timing of the first brood was not an important predictor of interval length. While Verboven and Verhulst (1996) also found no such effect of timing in great tits, other studies have found that interval lengths shorten as the season progresses, for example in barn swallows (Møller 2007) and black redstarts (*Phoenicurus ochruros*; Weggler 2006). Furthermore, Møller (2007) found that the earlier breeding individuals which employed longer inter-brood intervals gained fitness benefits from doing so. In contrast it seems that in the reed warbler population studied here, either there is no benefit of shortening the inter-brood interval later in the season, or breeding adults are already at maximal reproductive effort and are therefore unable to shorten the interval. While the timing within the season appears to be the most important factor influencing the decision of whether or not to double brood, it does not appear to influence the length of the interval between broods, for birds which do double brood.

CONCLUSION

As with most previous studies, the timing of the first brood was found to be the primary factor influencing the decision to double brood in reed warblers. Food availability was also important and, as food resources are associated with climate, increased double brooding rates is a plausible mechanism for the extension of breeding season length that has been observed in some multi-brooded species (Møller *et al.* 2010, Halupka and Halupka 2017). Inter brood intervals were longer when more nestlings were fledged from first broods and under conditions with reduced food availability. As temperature is positively related to invertebrate availability (Vafidis 2014), spring warming should result in shorter intervals between broods.

Therefore, longer intervals between broods was not supported as a mechanism driving breeding season extension by this study. Weather conditions were additionally found to influence both the probability of double brooding and interval length (controlling for the effect of food availability). Greater levels of rainfall following the first brood both reduced the probability of double brooding and for pairs which did double brood, increased the inter-brood interval. While there is a wealth of evidence for the effect of food availability and spring weather conditions on the timing of breeding at the beginning of the breeding season (Pearce-Higgins and Green 2014), this study adds to the relatively small number of studies which have found effects of mid-season weather conditions and food resources on the incidence and timing of multi-brooding. Further analyses should assess the relative costs and benefits of double brooding to reed warblers.

Table 3.1. Matrix of all known-age breeding reed warblers in 2014-17. Age 1 = 1st year i.e. born in previous year. Numbers split by sex in parentheses (female, male). The first season during which the entire cohort of nestlings were ringed (i.e. the birds 'available' to be subsequently re-sighted as known age breeders) was 2011.

Year	Age							Total known age birds	Number of pairs
	1	2	3	4	5	6	7		
2014	10 (5,5)	9 (2,7)	4 (2,2)	2 (0,2)	NA	NA	NA	25 (9,16)	121
2015	7 (5,2)	15 (7,8)	9 (3,6)	3 (1,2)	3 (1,2)	NA	NA	37 (17,20)	154
2016	25 (13,12)	17 (11,6)	22 (10,12)	10 (4,6)	3 (1,2)	4 (2,2)	NA	81 (41,40)	139
2017	7 (3,4)	28 (17,11)	15 (8,7)	18 (9,9)	9 (4,5)	1 (0,1)	3 (1,2)	81 (42,39)	104

Table 3.2. Proportion of breeding reed warblers that were colour-ringed, out of all individuals observed on nest videos in 2014-17.

Year	Proportion of individuals colour-ringed
2014	0.24
2015	0.44
2016	0.62
2017	0.81

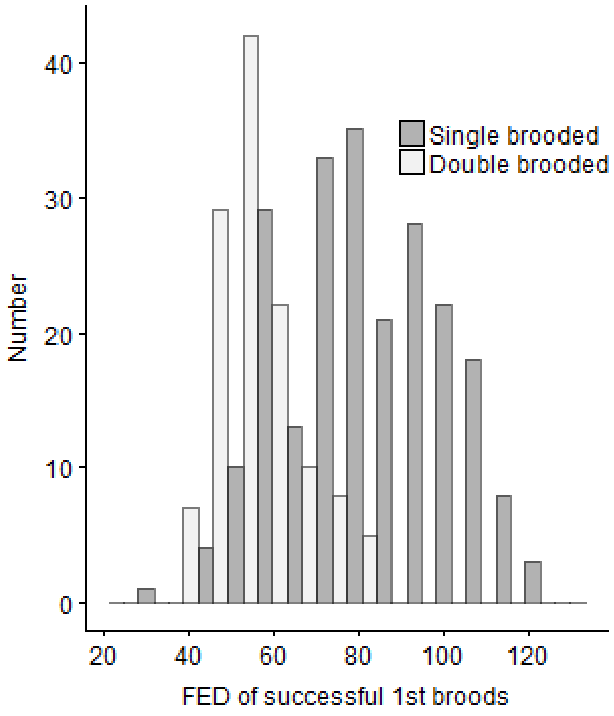

Figure 3.1. Frequency distribution of first egg dates (FED; 1 = 1st April) of successful 1st broods 2014-17 for pairs that did (pale grey) and did not (dark grey) attempt second broods (n= 348).

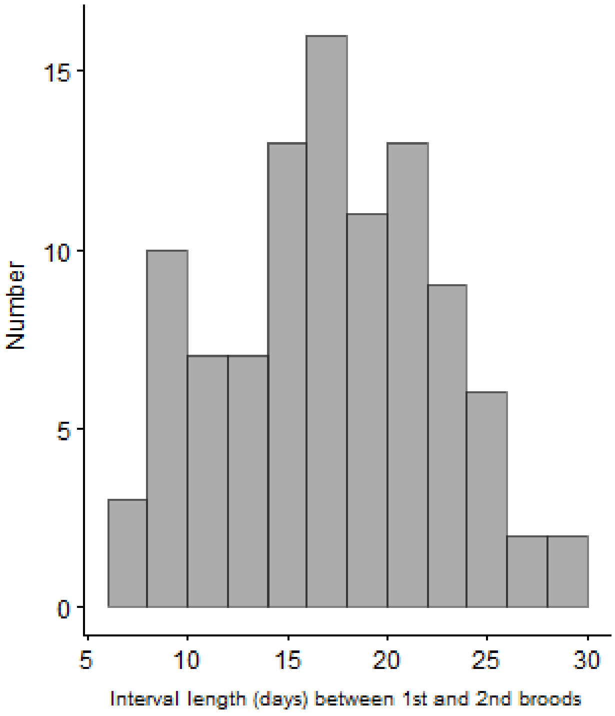

Figure 3.2. Frequency distribution of interval lengths between 1st and 2nd broods (days between 1st brood fledge date and 2nd brood first egg date) in 2014, 2015 and 2017 (n = 99).

Table 3.3. Matrix of the number of pairs that were single, double-, or triple-brooded during 2014-17, split also by the ultimate success of their final brood. Pairs which hatched a cuckoo egg in any of their nesting attempts within the season have been excluded (16 in 2014, 14 in 2015, 8 in 2016 and 9 in 2017). Two further pairs have been excluded (one each from 2015 and 2016) as the outcome of one nesting attempt each was unknown.

Year	Single-brooded		Double-brooded		Triple-brooded		Percentage double (or triple) brooded	Total
	Failed	Successful	Failed	Successful	Failed	Successful		
2014	10	46	6	42	1	0	47	105
2015	27	69	7	34	1	1	31	139
2016	72	41	8	9	0	0	13	130
2017	22	58	2	13	0	0	16	95
Total	131	214	23	98	2	1	26	469

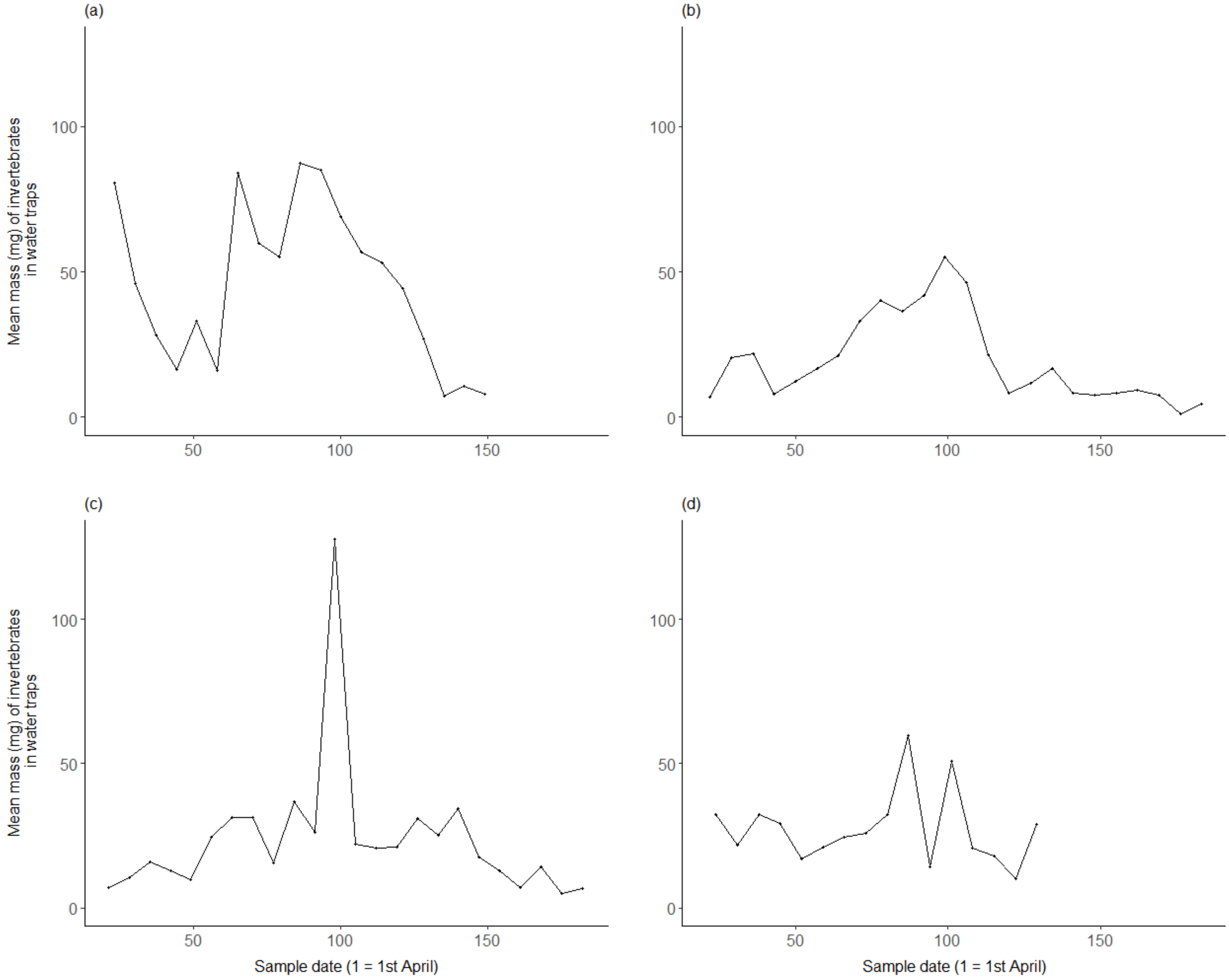

Figure 3.3. Seasonal food availability in (a) 2014, (b) 2015, (c) 2016 and (d) 2017 represented by the weekly mean mass (mg) of invertebrates in water traps. 2014 data taken from the mean of 7 water traps, 2015-17 data from the mean of 12 water traps. Weekly sample dates range from 23rd April- 27th August in 2014, 22nd April- 30th September in 2015, 21st April- 29th September in 2016 and 24th April- 7th August in 2017.

Table 3.4. Parameter estimates for the best-fitting generalized linear models of the probability of attempting a second brood after a successful first brood (2014-2017; n = 348). Null model also included. "+" indicates presence of categorical variable. See Appendix 2 for average model coefficient plot.

Intercept	Year	FED 1st brood	Number of fledglings	Food availability	Rainfall	Temperature	FED 1st brood X Food availability	FED 1st brood X Rainfall	FED 1st brood X Temperature	AICc	Delta AICc	Weight
-2.428		-3.604	-0.248	1.636	-0.784		1.486			217.7	0.00	0.148
-2.470		-3.570		1.640	-0.795		1.530			217.8	0.03	0.146
-2.619		-3.835		1.672	-0.816	0.232	1.515			218.7	0.96	0.091
-2.568		-3.849	-0.235	1.665	-0.805	0.212	1.474			218.9	1.17	0.082
-2.497		-3.630	-0.250	1.588	-1.214		1.367	-0.454		219.1	1.38	0.074
-2.537		-3.593		1.594	-1.221		1.415	-0.447		219.2	1.43	0.072
-2.495		-3.793		1.695	-0.859	0.032	1.583		-0.359	219.7	1.93	0.056
-0.604										454.1	228.46	0.000

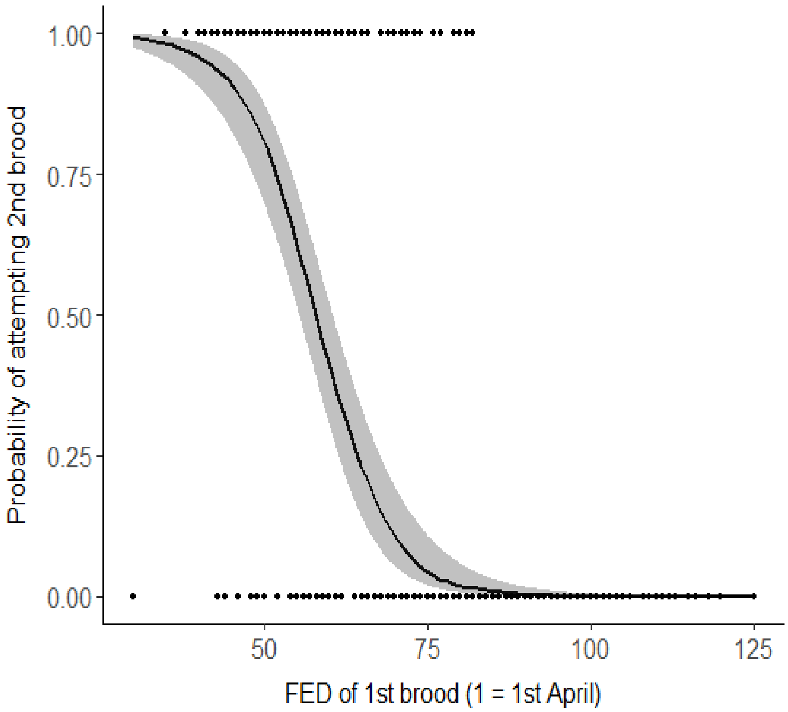

Figure 3.4. Model-averaged, predicted relationship between the probability of double brooding and the first egg date (FED) of 1st broods (n = 348). The shaded area represent the 95 % confidence intervals. Raw data indicated by points.

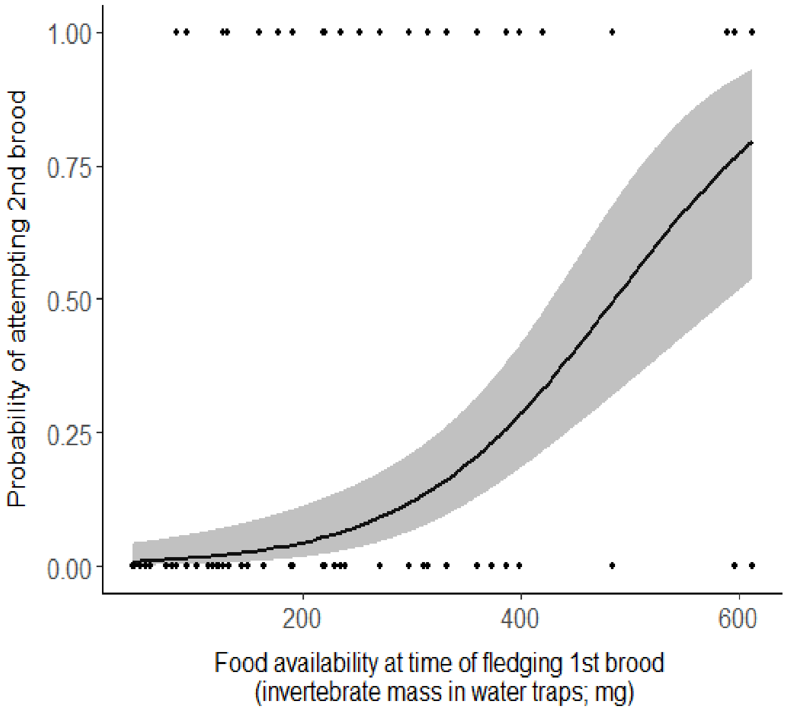

Figure 3.5. Model-averaged, predicted relationship between the probability of double brooding and food availability at the time of the 1st brood fledging (n = 348). The shaded area represents the 95 % confidence intervals. Raw data indicated by points

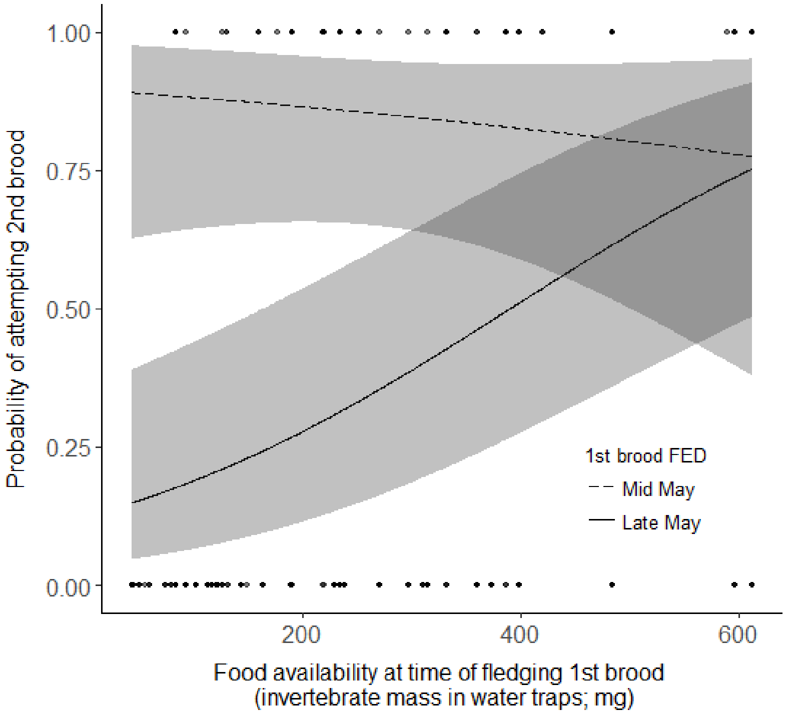

Figure 3.6. Model-averaged, predicted relationship between the probability of double brooding and food availability for relatively early (May 16th) and late (May 30th) 1st broods (n = 348). Shaded areas represent the 95 % confidence intervals. Raw data indicated by points.

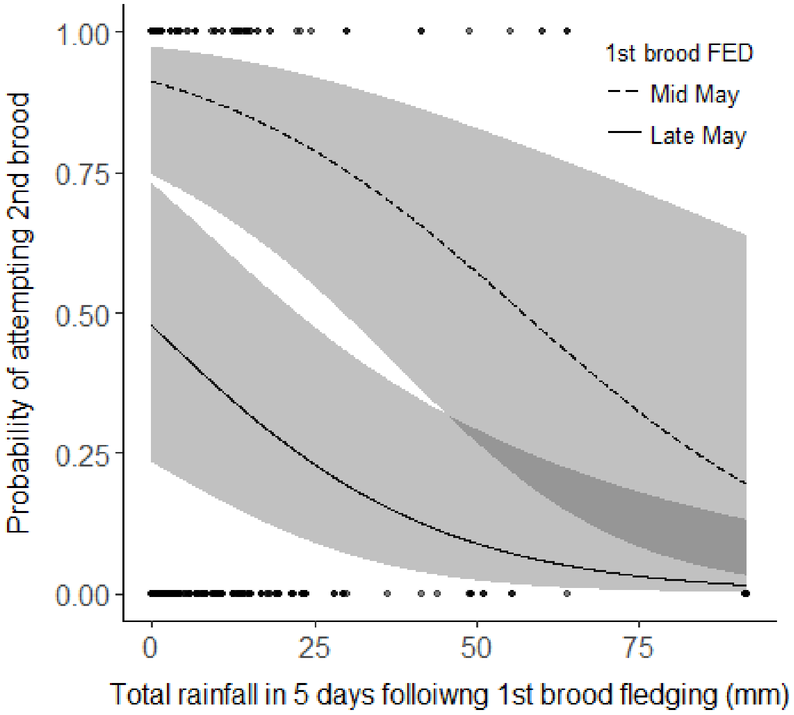

Figure 3.7. Model-averaged, predicted relationship between the probability of double brooding and rainfall in the 5 days following the 1st brood fledging for relatively early (May 16th) and late (May 30th) 1st broods (n = 348). Shaded areas represent the 95 % confidence intervals. Raw data indicated by points.

Table 3.5. Parameter estimates for the best-fitting linear models explaining interval length between 1st and 2nd broods in 2014, 2015 and 2017 (n = 99). Null model also included. See Appendix 2 for average model coefficient plot.

Intercept	FED 1st brood	Number of fledglings	Food availability	Rainfall	Temperature	FED 1st brood X Food availability	FED 1st brood X Rainfall	FED 1st brood X Temperature	AICc	Delta AICc	Weight
17.08	0.462	2.766	-1.953	2.314	0.686			-0.999	591.1	0.00	0.209
16.66		2.619	-1.625	2.259	0.930				591.6	0.48	0.164
16.82	0.1553	2.751	-1.971	2.495	0.850		0.683	-0.887	592.0	0.90	0.133
16.66									616.0	24.38	0.000

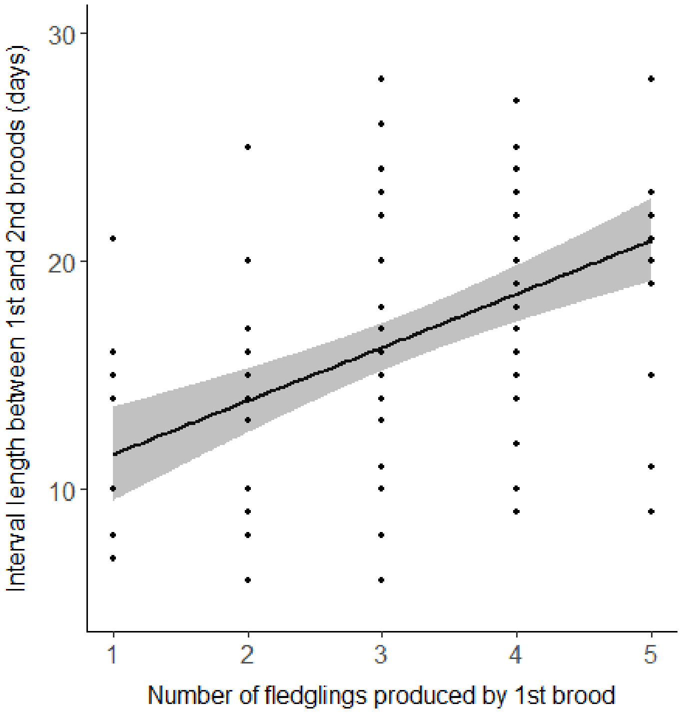

Figure 3.8. Model-averaged, predicted relationship between the interval length between 1st and 2nd broods (days from 1st brood fledge date to 2nd brood first egg date) and the number of fledglings produced from 1st broods (n = 99). The shaded area represents the 95 % confidence interval. Raw data indicated by points.

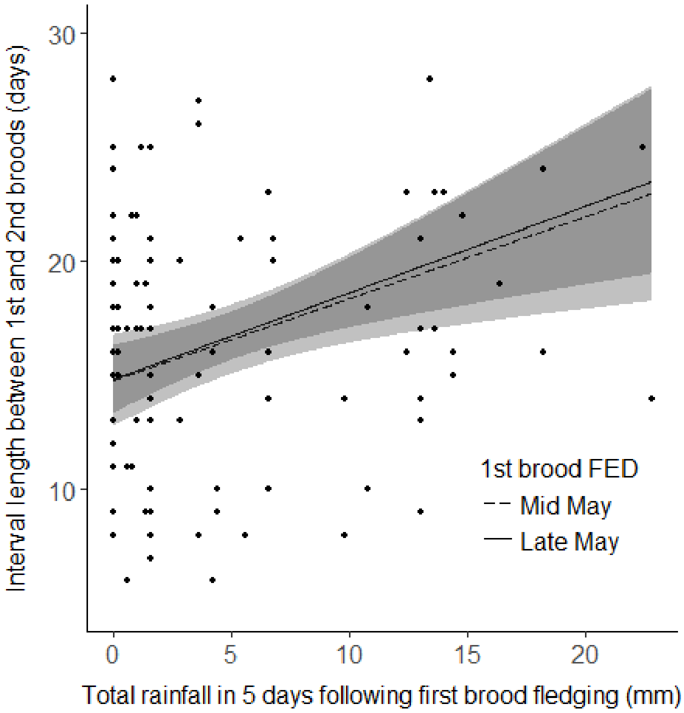

Figure 3.9. Model-averaged, predicted relationship between the interval length between 1st and 2nd broods (days from 1st brood fledge date to 2nd brood first egg date) and rainfall following the 1st brood, for relatively early (May 16th) and late (May 30th) 1st broods (n = 99). Shaded areas represent the 95 % confidence intervals. Raw data indicated by points.

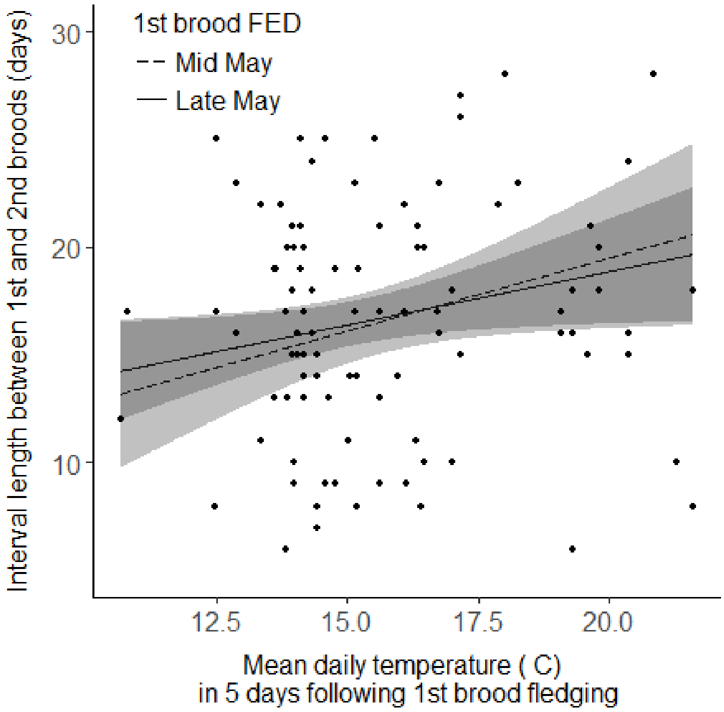

Figure 3.10. Model-averaged, predicted relationship between the interval length between 1st and 2nd broods (days from 1st brood fledge date to 2nd brood first egg date) and mean temperature the 5 days following the 1st brood, for relatively early (May 16th) and late (May 30th) 1st broods (n =99). Shaded areas represent the 95 % confidence intervals. Raw data indicated by points.

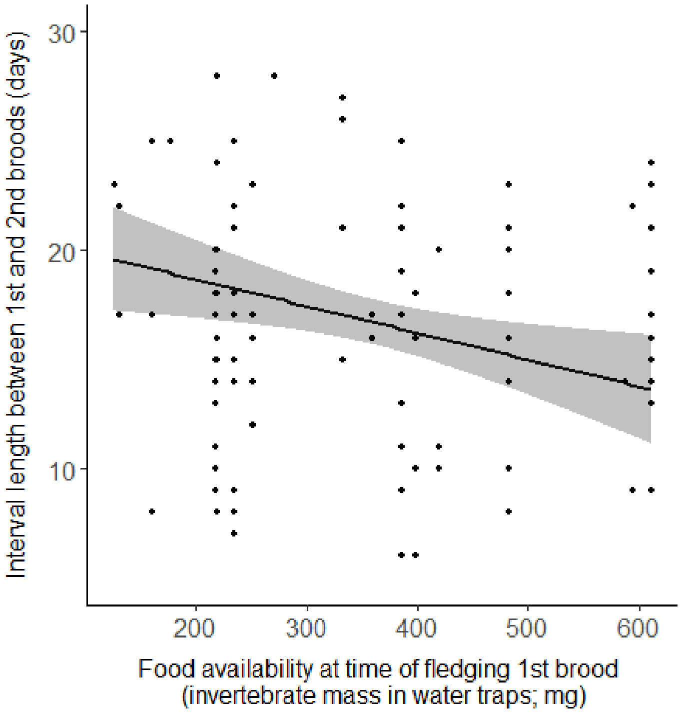

Figure 3.11. Model-averaged, predicted relationship between the interval length between 1st and 2nd broods (days from 1st brood fledge date to 2nd brood first egg date) and food availability at the time of fledging the 1st brood (n = 99). The shaded area represents the 95 % confidence interval. Raw data indicated by points.

Chapter 4- An assessment of the seasonal value of nesting attempts and the individual-level costs of extending the breeding season for reed warblers, *Acrocephalus scirpaceus*

INTRODUCTION

Multi-brooded species are able to increase their annual reproductive productivity by both the replacement of failed nesting attempts, reducing the chance of complete failure (in common with some single-brooded species), as well as by double brooding, the attempt to raise a second brood after successfully fledging a first brood. In contrast to most species which are typically single-brooded, the success of individual nests does not necessarily decline seasonally in multi-brooded species (Hochachka 1990, Mallord *et al.* 2008) and may even increase seasonally (Geupel and DeSante 1990, Soler *et al.* 1995, Weggler 2006). The number of fledglings produced by successful nests, however, is generally found either to be similar between first and second broods (Mallord *et al.* 2008, Carro *et al.* 2014, Hoffmann *et al.* 2014) or to be greater for first brood nests (Smith and Marquiss 1995, Ogden and Stutchbury 1996, O'Brien and Dawson 2013). Regardless of differing contributions of first and second broods to the production of fledglings, double brooding can increase annual productivity; double-brooded individuals produce a greater number of fledglings annually than single-brooded individuals in a wide range of species (Chapter 3, Geupel and DeSante 1990, Ogden and Stutchbury 1996, Weggler 2006, O'Brien and Dawson 2013, Townsend *et al.* 2013, Carro *et al.* 2014, Hoffmann *et al.* 2014, Béziers and Roulin 2016, Cornell and Williams 2016).

While late nesting attempts of multi-brooded populations, comprising both replacement attempts and second (or third) broods, can increase the number of fledglings produced, selection acts on recruitment of offspring into the breeding population, making recruitment a more useful measure of breeding success. Many studies of multi-brooded populations have shown a seasonal decline in the probability of nesting attempts producing fledglings which ultimately recruit into the breeding population (Hochachka 1990, Barba *et al.* 1995, Smith and Marquiss 1995, Van Noordwijk *et al.* 1995, Verboven and Visser 1998, Cowley 2001, Mallord *et al.* 2008, Brown *et al.* 2014, Hoffmann *et al.* 2014). This raises the question of why late nests are attempted at all. In fact, a 50-year study of great tits, *Parus major*, found that as breeding became increasingly out of synchrony with food resources, second broods were attempted less often as their relative value in terms of recruitment potential declined (Husby *et al.* 2009). It follows that, in populations where the recruitment potential of late nesting attempts is low, it should be expected that the relative costs associated with late nesting attempts should also be low for these nesting attempts to remain potentially profitable.

Life history theory predicts that reproduction is costly, leading to a trade-off between investment in current and future reproductive attempts (Williams 1966, Stearns 1976, Reznick 1985). Indeed, studies of breeding birds have found reduced adult condition and survival resulting from increased reproductive effort (e.g. Bryant 1979, Martins and Wright 1993, Daan *et al.* 1996, Nilsson and Svensson 1996, Murphy *et al.* 2000). Potential costs to adults with seasonally late nests include reduced probability of survival to the following breeding season and/or carry-over effects into the following season for surviving adults such as delayed timing of breeding or reduced productivity (Bryant 1979, Nilsson and Svensson 1996, Verhulst 1998, Brown *et al.* 2014, Fayet *et al.* 2016). If breeding ends later, costs may occur as a result of the delay in other important life history events such as post-breeding moult and migration. Post-breeding moult has been found to be delayed in double-brooded individuals (Ogden and Stutchbury 1996, Mulvihill *et al.* 2009) and although this can be compensated for by completing the moult more quickly (Morrison *et al.* 2015), this may still be costly, as quicker moult results in the production of poorer quality feathers with inferior flight and thermoregulation performance (Nilsson and Svensson 1996, Dawson *et al.* 2000). For example, in blue tits, *Parus caeruleus*, individuals whose breeding attempts ended later, delaying moult, expended more energy in thermoregulation and experienced higher mortality rates during winter (Nilsson and Svensson 1996). Other studies of the potential costs of late nesting have reported mixed results. Reduced survival to the following season of double-brooded females, but not males, has been found in studies of both great tits and house martins, *Delichon urbicum* (Bryant 1979, Verhulst 1998), while a study of sand martins, *Riparia riparia*, found reduced survival of late nesting individuals of both sexes (Brown *et al.* 2014). However, other studies have reported no measurable effect on survival of double brooding by females (Weggler 2006, Carro *et al.* 2014) or both sexes (Smith and Marquiss 1995) or even the reverse effect, with double-brooded females (Cornell and Williams 2016) or both sexes (Geupel and DeSante 1990) having an increased probability of survival to the following breeding season. Furthermore, in European starlings, *Sturnus vulgaris*, there was no effect of double brooding on the timing of breeding in the following season (Cornell and Williams 2016). In contrast, experimentally increasing the length of the breeding season has been found to delay the start to breeding, as well as reduce reproductive success, in the following season is several studies (Nilsson and Svensson 1996, Fayet *et al.* 2016). In addition, Verhulst (1998) found in great tits, that curtailing the effort invested in second broods (via the removal of second clutches) resulted in a greater number of fledglings being produced in first broods the next season, compared to control birds, further illustrating carry-over effects on the subsequent season as a result of increased breeding effort. Other studies of passerines, however, have found no such effect of double brooding on productivity in the following season (Smith and Marquiss 1995, Weggler 2006).

Understanding the relative value and costs associated with extending the breeding season with late nesting attempts is particularly important given that breeding season extension has been observed in some multi-brooded species, and is associated with climate change (Møller *et al.* 2010, Halupka *et al.* 2017). Without the need to time a single breeding attempt with optimal conditions, breeding advancement and season extension could be beneficial for multi-brooded populations, for example through increased opportunities for additional or replacement nesting attempts (Halupka *et al.* 2008) or via fitness benefits associated with increased intervals between nesting attempts (Møller 2007). However, season extension may not necessarily increase population productivity or individual fitness if relatively late nesting attempts are less valuable or incur substantial fitness costs.

In this study, the relative value of nesting attempts throughout the breeding season and the potential costs of late nesting and double brooding were assessed in the Eurasian reed warbler, *Acrocephalus scirpaceus* (hereafter 'reed warbler'). The reed warbler is a long-distance migratory, multi-brooded species. Double brooding is facultative and failed nests are often replaced throughout the breeding season such that females generally lay between one and four clutches within a single season (ca. 95 % lay between 1-3 clutches; Chapter 3). The timing of the beginning of the breeding season of reed warblers has become earlier across Europe in recent decades in line with climate change induced spring advancement (Crick and Sparks 1999, Schaefer *et al.* 2006, Halupka *et al.* 2008). However, contrasting trends in the timing of the end of the breeding season have been reported; remaining constant in a study population in south-western Poland, resulting in an overall extended breeding season (Halupka *et al.* 2008), but ending earlier in northern Bavaria, Germany, resulting in no significant change in season length (Schaefer *et al.* 2006). Previous studies of reed warblers have found that nest success increases seasonally due to high egg stage failure rates early in the season (Bibby 1978, Davies and Brooke 1988, Schulze-Hagen *et al.* 1996). In this study, the relative reproductive values of reed warbler nesting attempts throughout the breeding season are assessed in terms of the probability of nest success (producing at least one fledgling), the number of fledglings produced and the probability of producing fledglings which recruit into the local breeding population. Adult survival to the following breeding season and timing of breeding in the following season were assessed as potential costs of late nesting and double brooding. This study is the first analysis of the consequences of late nesting and double brooding in reed warblers.

METHODS

Data collection

Reed warbler nests were monitored from 2012-17 at Watermill Broad Nature Reserve in Norfolk, UK. The study site consisted of a series of ponds and lakes of varying sizes, fringed with *Phragmites australis* reed beds which total 7.6 ha. All reed beds were systematically searched for nests at regular intervals (usually every 7- 10 days) between mid-April and mid-August each year. Nests were checked regularly in order to record first egg dates, ring nestlings and to record whether nests were successful or not. First egg dates (FEDs) for nests observed during the laying period were calculated on the basis of one egg being laid per day (pers. obs.). For nests found after the laying period, the FED was taken as the median of the minimum and maximum possible FEDs (the upper median was taken when there was an even number of dates) as a result of back-calculations from observations during the incubation and the nestling stages. Back-calculations were made on the basis of a potential incubation period of 11-13 days (from the day the penultimate egg was laid to the hatch date; Chapter 2) and it being possible to age day one or day two nestlings (day one = hatch day; pers. obs.). Few nests were found at a more advanced stage than day two nestlings and in these cases back-calculations were also based on nestling ageing but calculations allowed for inaccuracy in ageing of plus or minus one day. Nestlings were ringed between day four and seven with a single uniquely numbered metal ring. A nest was considered successful if it fledged one or more young and a fledged nest was determined from observing an intact nest containing feather scale and or covered in droppings. The number of fledglings was derived from the number of nestlings present in the nest visit prior to successfully fledging plus any well-feathered nestlings that were present and capable of fledging on a previous visit (as nestlings can fledge asynchronously; pers. obs.).

Adults were colour-ringed with a single, two-digit, darvic plastic ring during constant effort mist netting sessions run once every 10 days between April-September during the seasons 2013-2017 and additionally via targeted mist netting in nesting territories in 2015-2017. Nests were video recorded to identify parents when nestlings were at ringing age in 2014 and from the first day of incubation in 2015-2017 (Chapter 2).

Data analysis

For 2014-17, nests of unknown adult ownership such as nests with adults which were not colour ringed, or nests which failed before adults could be identified, were assigned pair ownership based on a relative proximity method to complete breeding season histories of pairs (Chapter 2). This process was not applied to one part of the study site, where high nesting density prevents accurate assignment of nest ownership (Chapter 2). This area accounted for a mean of 11.6 % of nests per season during the study period and was excluded from all analyses in this study. For the rest of the site, nest assignment included all nests which were observed to contain eggs or nestlings as well as fully built nests which were never observed to contain eggs (which were assumed to have failed at the egg stage between nest visits) so as not to underestimate attempted double brooding. All nests (2014-17) were then categorised as a brood type i.e. first broods, replacement first broods second broods, replacement second broods or third broods. Second brood nests were defined as nest attempts of the same social pair following a successful first brood, while nests following failed nest attempts were considered replacement attempts of that brood, such that pairs may have multiple attempts at their first and or second brood (Chapter 3). Replacement second brood and third nests were not used in analyses considering brood type due to small sample sizes. Once all nests from 2014-17 had been assigned to pairs, all pairs were given a unique identity code. Double-brooded pairs were defined as those which attempted a second brood, regardless of the outcome.

Nests from 2012 and 2013 were additionally used in order to assess effects over a greater number of study years, although nests from these years were not assigned pair ownership as videoing to identify parents of nests across the site had not taken place. Nest monitoring data collected in 2012 and 2013 followed the same methods as described for 2014-17 (Chapter 2) and derived variables were calculated in the same way for these data. Several analyses were therefore repeated separately for the datasets comprising the study periods of 2012-17 and 2014-17, with the 2014-17 data containing additional information on parent identities and brood types of nests.

All statistical analyses were conducted using R version 3.5.0 (R-Core-Team 2018). Data manipulations were performed using ‘dplyr’ (Wickham *et al.* 2018) and linear models were conducted with ‘lme4’ (Bates *et al.* 2018). Year was fitted as a random effect in analyses which contained more than five years data (i.e. those using the 2012-17 data set) but as a fixed effect in analyses containing less than five years data (i.e. those using the 2014-17 data set; as per Crawley 2002, Bolker *et al.* 2009). Throughout, numeric variables were centred and scaled and collinearity between explanatory variables (fixed effects) was checked with pair-wise correlations and variance inflation factors (VIFs; Zuur *et al.* 2009).

Seasonal variation in the reproductive value of nesting attempts

Generalised linear mixed effect models (GLMMs) were used to assess the effect of nest timing on nest success, linear mixed effects models (LMMs) were used to assess the effect of nest timing on the number of fledglings produced by successful nests and GLMMs were used to assess the probability of successful nests producing a recruit into the breeding population. Nests in which a cuckoo, *Cuculus canorus*, nestling hatched were excluded from these analyses.

The effect of nest timing on nest success for the 2012-17 data was modelled using a GLMM. Whether a nest was successful or not (1= successful, 0= unsuccessful), was modelled with a binomial error structure and a logit link function. Unknown outcome nests were very rare (21 out of 1541 nests), but were excluded from analyses of nest success. Year was included as a random effect to control for annual variance in nest success and the FED of nests was included as the only fixed effect in this model. The sample size for this analysis was 1435 nests.

To assess the effect of brood type on nest success, a GLMM was used on the 2014-17 data. Whether nests were successful was modelled with a binomial error structure and a logit link function, with pair identity as a random effect, to account for multiple nests within the same seasons belonging to the same pairs. Fixed effects comprised the FED of nests, the brood type of nests and year. FED of nests and brood type were collinear ($r = 0.6$) but VIFs were relatively low (< 2), and therefore both were retained in the model so that the effect of brood type could be assessed while accounting for the effect of nest timing. Other pair-wise correlations were relatively low (r values < 0.2) and VIFs low (< 1.3). The sample size for this analysis was 833 nests.

In order to assess the effect of female and male age on nest success, a GLMM was run on subsets of the 2014-17 data for which the age of females or males were known i.e. separate analyses for each sex. Nest success was modelled with a binomial error structure and a logit link function, with the nested random effects of individual identity within pair identity, to account for both within-season and between-season pseudoreplication. Fixed effects included the FED of nests, year (which had too few levels to be fitted as a random effect) and the age of females or males dependent on the analysis. Age was assessed as a categorical variable with two levels: first year individuals (born in the previous season) and older than first year individuals. Brood type was excluded from both the female and male analyses due to high collinearity between FED of nests and brood type in these data sets ($r = 0.6$ and 0.7 in the female and male data sets respectively). All other pairwise correlations were relatively low (r values < 0.5 and 0.3 and VIFs < 1.4 and 1.3 respectively for the female and male data sets). Sample sizes were 206 nests from 70 individuals and 210 nests from 68 individuals for females and males, respectively.

Egg stage success (i.e. hatching success) was also modelled following the same methods as for the analyses of the effect of overall nest success. Egg stage success models were structured identically to the nest success models which assessed the effect of brood type and of female and male age (i.e. three models). Nests which were known to fail but at unknown stage (egg or nestlings) were excluded from the analysis (62 out of 834 and these were not biased seasonally). Nestling stage success (i.e. fledging success of nests which had hatched) was not modelled as, unlike egg stage success rates, nestling stage success rates showed no seasonal trend (see results).

The effect of nest timing and brood type on the number of fledglings produced from successful nests was modelled with a LMM using 2014-17 nest data. Pair identity was included as a random effect to account for multiple nests within seasons belonging to the same pair. The FED of nests, brood type, and year were included as fixed effects. Although FED of nests and brood type were collinear (r = 0.6), all VIFs were relatively low (< 2) and both predictors were retained so that any different effects between brood types could be assessed after accounting for the effect of nest timing. The sample size for this analysis was 447 nests.

To assess the effect of female and male age on the number of fledglings produced by successful nests, separate LMMs were run on subsets of the 2014-17 data with female or male known age. Individual identity nested within pair identity were included as random effects for both the female and male models, to control for individuals having multiple nests both within and between seasons. For the female model, all pair-wise correlations ($r \leq 0.5$) and VIFs were relatively low (all VIFs < 2) and all predictors were retained in the model. For the male model, brood type was removed due to high collinearity with FED of nests (r = 0.7 and VIFs > 2.5), while other fixed effects had low pairwise correlations (all r values < 0.3) and low VIFs (all < 1.4). Sample sizes were 110 nests of 62 individuals and 121 nests of 60 individuals for the female and male analyses, respectively.

Seasonal variation in the recruitment probability of successful nesting attempts

The effect of nest timing on the probability of producing a recruit was modelled using a GLMM for 2012-16 data. Whether or not a successful nest produced a recruit (1= produced one or more recruits, 0= produced no known recruits) was modelled with a binomial error structure and a logit link function. A recruit represented an individual which was videoed at a nest (i.e. proven breeding) on the study site in any subsequent year (i.e. in 2013-2017) after the year it had been ringed as a nestling. The binary measure of producing a recruit or not was used as cases of successful nests producing more than one known recruit were very rare. Year was included as a random effect to control for annual variation in recruitment probability. As recruits were not necessarily detected in their first year of breeding, any effect of year in models would not necessarily represent a true difference in recruitment probability and therefore is not interpreted as such. Fixed effects included the FED of nests as well as the number of fledglings, to control for the effect of brood size on the probability of producing a recruit. The sample size in this analysis was 618 successful nests.

To assess the effect of brood type on recruitment probability, a GLMM was used on 2014-16 data. Whether successful nests produced a recruit was modelled, with a binomial error structure and a logit link function, with pair identity included as a random effect in order to control for multiple nests belong to the same pair within seasons. The FED of nests, the number of fledglings, brood type and year were included as fixed effects as well as the interaction between the FED of nests and the number of fledglings. The FED of nests and brood type were collinear (r = 0.6) but had low VIFs (< 2.1) so both were retained in the model in order to assess if brood type influenced the probability of recruitment after accounting for the effect of nest timing (FED). All other pair-wise correlations and VIFs were low (all r values < 0.4 and VIFs < 1.2). The sample contained 335 successful nests. The effect of female or male age on recruitment probability could not be modelled due to very small sample sizes (see results).

Costs of late nesting and double brooding

The effect of late nesting on adult survival to the following season and breeding timing in the following season was assessed with generalised linear models (GLMs) and LMMs, respectively. Both analyses were carried out separately for females and males, on colour-ringed individuals only and with data from the 2014-17 seasons, excluding individuals that were triple brooded or that hatched a cuckoo in any of their nests.

Whether or not individuals were re-sighted in the following season was used as a proximate measure of survival. Survival to the following breeding season was assessed using a GLM with a binomial error structure and a logit link function. Re-sighting was determined by whether or not individuals were videoed at a nest in the following season. The legitimacy of using re-sighting in the following season as a measure of survival was assessed by calculating an observation probability of colour-ringed individuals which were known to be alive. By taking all the cases for which an individual was observed in year one and year three, the observation probability was taken as the proportion of those individuals that were also observed in year two and this equalled 88.6 % (n = 44) for all individuals and was similar for known sex individuals (females; 88.2 %, n = 17, males; 83.3 %, n = 24). Fixed effects included the date of the end of individuals' breeding seasons, whether individuals were single- or double-brooded and year. The date of the end of individuals' breeding seasons was taken as the end date of the final nesting attempt (i.e. the fledging date or date of failure). Individuals were only included in the analysis once, retaining the data from the first year in which individuals were observed breeding. Pair-wise correlations between variables had relatively low r values (=< 0.5) in both the female and male analyses and VIFs were relatively low in both analyses (< 1.6 and 1.7 in the female and male analyses, respectively). The female analysis comprised data on 92 individuals and the male analysis 74 individuals.

To assess whether age influenced survival probability, the survival analyses were repeated for the subset of data where the age of individuals was known. Age was determined for breeding birds that had been ringed as nestlings. Fixed effects comprised: the date of the end of individuals' breeding seasons, whether individuals were single- or double-brooded, year and age (first year of breeding or older). For the female analysis, all pairwise correlations of variables were relatively low (r values < 0.4) and VIFs were also low (< 1.5) so all explanatory variables were retained in the model. For the male analysis, whether individuals were single- or double-brooded was highly correlated with year (r = 0.6) and the number of broods attempted had a relatively high VIF (2.7), so year was removed from the model. After the removal of this variable, all other pairwise correlations ($r \leq 0.5$) and VIFs (< 1.7) were relatively low. However, a binned residual plot revealed that many points were outside of two standard error bounds, so the end date of individuals breeding seasons was also removed from the model, after which there were no further issues. Sample sizes for models were 49 and 31 for female and male analyses respectively.

To assess if there was a carry-over effect of late nesting on the following season, the effect of late nesting on the timing of breeding in the following season was modelled with LMMs. The first egg date of individuals’ first breeding attempts in the subsequent season represented the breeding timing response variable; individuals whose first nesting attempt in the subsequent season had an unknown first egg date (i.e. a fully built nest that failed before eggs were observed) were excluded from the analysis. Individual identity was included as a random effect in both the female and male analyses, to account for individuals breeding in multiple seasons. The end date of individuals’ breeding seasons, whether they were single- or double-brooded and year were included as fixed effects. In addition, the FED of the first attempt of the season was included as a fixed effect, in case the start timing of breeding between seasons were related. All pairwise correlations of variables were relatively low ($r \leq 0.4$ for both analyses), as were the VIFs (< 1.8 and < 1.3 for the female and male analyses, respectively). Model validations highlighted two outliers in the male model. The model was therefore run both with and without the outliers and it was found that both the predicted effects of explanatory variables was similar and the best-fitting model was the same using both data sets. The model including outliers is presented in the results. Sample sizes were 53 observations of 39 individuals for the female analysis and 82 observations of 61 individuals for the male analysis (model including outliers). The effect of age on the timing of breeding in the following season could not be assessed for either sex due to small samples.

All LMMs were validated by plotting the distribution of the residuals as well as the residuals versus the fitted values. GLMs and GLMMs were validated using a binned plot of average residuals versus fitted values. In all analyses, all candidate models were fitted and ranked by their AICc value (sample size adjusted Akaike Information Criterion) using 'MuMIn' (Bartoń 2018). Figures of modelled effects represent average predictions from the best-fitting models (those within 2 AICc of the top model; Bartoń 2018), or represent the best-fitting model only, in analyses where no additional candidate models were within 2 AICc of the best-fitting model. Figures were produced using 'ggplot2' (Wickham 2009) and 'gridExtra' (Auguie 2017).

RESULTS

A total of 1719 nests were monitored during the study period 2012-17, including 1541 which were observed to contain eggs or nestlings and 178 fully built nests that were never observed to contain eggs but are assumed to have failed early in the egg stage between nest visits.

Seasonal variation in the reproductive value of nesting attempts

Overall, 53.3 % of nesting attempts with eggs or nestlings were classed as 'successful' in fledging at least one nestling (excluding those which hatched a cuckoo; n = 1434, data for 2012-2017). However, this is an overestimate of the success rate of nesting attempts as 178 fully built nests were never observed to contain eggs but are assumed to have been laid in and to have subsequently failed between nest visits. Including these nests lowers the estimated success rate to 47.4 % (n = 1612, data for 2012-2017). Nest success rate at the egg stage (i.e. hatching) was low earlier in the season and increased seasonally, while, in contrast, the nestling stage success rate of nests varied relatively little and remained high throughout the season (Figure 4.1).

Overall, the probability of nest success was positively related with the FED of nests (Table 4.1, Figure 4.2). Brood type was present in the best-fitting model (Table 4.2) and although second brood nests were predicted to be more successful than first and replacement first brood nests, the confidence intervals of model predictions for all three brood types overlapped so this effect was negligible (Figure 4.3).

In addition to the FED of nests, male age and the interaction between male age and the FED of nests were strong predictors of nest success, as these terms were all present in the best-fitting model (and no other candidate models were within 2 AICc; Table 4.2). The probability of nest success was higher for older males and, for older males, success increased gradually as the season progressed (Figure 4.4). In contrast, nest success of first year males was very low at the beginning of the season and was predicted to increase more rapidly throughout the season (Figure 4.4). The predicted probability of nest success of first year males increased beyond that of older males at the end of the season, but the confidence intervals of both predictions overlap. In contrast to the effect of male age, there was no apparent effect of female age on nest success (Table 4.2).

The results of models of the probability of egg stage success (Table 4.3) mirrored those of overall nest success (Table 4.2). Egg stage success increased seasonally for all brood types (Figure 4.5) and male age, but not female age, had a positive effect on success (Figure 4.6).The fact that the egg stage success model effects reflect those of the overall nest success analyses illustrates that variation in egg stage success rates drive overall seasonality in nest success (Figure 4.1).

The best-fitting model for the number of fledglings produced by successful nests contained the FED of nests and brood type. The number of fledglings declined with later FEDs for all brood types but the effect of brood type was weak, as the confidence intervals of each brood type overlapped considerably (Figure 4.7). Age and the interaction between FED and age were also present in the best-fitting models for females only (Table 4.4), however both of these effects appeared very weak as confidence intervals overlapped (Figure 4.8).

Seasonal variation in the recruitment probability of successful nesting attempts

From the seasons 2012-16, 16.9 % of successful nests were known to produce a recruit (113 out of 668). Only 19 successful nests which had FEDs in July were known to have produced a recruit, the latest of which had a FED of the 19th July. For successful nests of known brood type (i.e. 2014-16 data), the mean recruitment probability was 22.2 % for first broods (n = 171), decreasing to 15.0 % for replacement first broods (n = 107) and 9.5 % for second brood nests (n = 84). No successful replacement second brood or third brood nests produced any known recruits (n = 11 and 1 respectively). Of the 113 nests known to have produced a recruit, a total of 131 recruits were recorded, suggesting that a minority of nests produced more than one recruit. The sex ratio of recruits was remarkably even with 62 females and 63 males of 125 recruits for which sex was confirmed. Of successful nests with known age females, only one first year female was known to produce a recruit from 2014-16 (4.3 %; out of 23), compared with 13.3 % of successful nests for older females producing a recruit (six out of 45). For successful nests with known age males, none with first year age males produced a recruit (out of 15) in contrast to 20.9 % of successful nests of older males producing a recruit (14 out of 67).

The FED of nests was an important predictor of recruitment probability, having the largest effect in the best-fitting models (Table 4.5, Table 4.6). The probability of nests producing a recruit declined seasonally (Figure 4.9, Figure 4.10). Recruitment probability also increased when a greater number of fledglings were produced (Table 4.5, Table 4.6). The interaction between the FED of nests and the number of fledglings, although present in one of the best-fitting models, was relatively unimportant as the effect size was very small (Table 4.6). Brood type was also apparently unimportant, being absent from the best-fitting model (Table 4.6).

Costs of late nesting and double brooding

There was considerable variance and overlap in the end date of the breeding seasons of single- and double-brooded pairs in 2014-17 (Figure 4.11), such that late nesting attempts were a mixture of first, replacement first and second broods (Figure 4.12). On average, the last nesting attempt of single-brooded pairs ended on the 11th July (range = 24th May- 25th August, sd = 18.8 days, n = 306) compared with the 31st July for double-brooded pairs (range = 24th June- 26th August, sd = 11.7 days, n = 116).

The number of broods attempted, end date of the breeding season and age were all relatively unimportant predictors of the probability of survival to the following year, in both males and females, as the null model was included within the set of best-fitting models in all analyses (Table 4.7). According to the raw data, the proportion of double-brooded individuals which were re-sighted the following breeding season was actually greater than for single-brooded individuals (Table 4.8).

The end timing of the breeding season and the number of broods attempted did not predict the timing of breeding in the following season for females or males (Table 4.9). In females, the timing of the first breeding attempt predicted the timing of breeding in the following year; breeding early in year one predicted breeding early in year two (Figure 4.13). The analysis for males identified the null model as the best-fitting model, suggesting that none of the explanatory variables had strong effects on timing of breeding in the subsequent season (Table 4.9).

DISCUSSION

Seasonal variation in the reproductive value of nesting attempts

At Watermill Broad, the success of individual reed warbler nesting attempts increased over the breeding season. This complies with several other studies of multi-brooded species which found that nest success increased seasonally (Geupel and DeSante 1990, Soler *et al.* 1995, Weggler 2006). The seasonal trend in nest success was the same for all brood types, illustrating that the seasonal incline in the probability of nest success was primarily a function of timing rather than whether a nesting attempt was a first or second brood attempt of a breeding pair *per se* (Figure 4.3).

The seasonal increase in nest success was a result of higher egg stage failure rates in the earlier part of the breeding season (Figure 4.1). High failure rates, specifically during the egg stage of nesting attempts, have been reported in other studies of reed warblers (Bibby 1978, Davies and Brooke 1988, Schulze-Hagen *et al.* 1996 and references within). The majority of nest failures were apparently due to predation where eggs were removed from undamaged nests (unpublished data). In previous studies, cuckoos have been responsible for high egg stage nest failures of reed warblers as they are known to predate complete clutches, leading to replacement clutches being available for parisitism. Reed warbler populations free of cuckoo parasitism can achieve higher breeding success (Schulze-Hagen *et al.* 1996) and cuckoos are also documented as being responsible for the majority of observed predation events in the UK (Wyllie 1975, Bibby 1978). Further evidence for this hypothesis is provided by Davies and Brooke (1988) who found that already parasitized nests were less likely to be predated than un-parasitized reed warbler nests during the egg stage. On the current study site, only four predation events have been observed, all at the egg stage (three via video recording and one observed in person) with cuckoo and Eurasian jay, *Garrulus glandarius*, being responsible for two cases each. While the true proportion of nest predation attributable to either predator (or any others) is clearly unknown, it seems likely that high egg stage failure in the early part of the season, compared to later failure rates, may be due to the additional contribution of predation by cuckoos. Unlike other nest predators, cuckoos almost exclusively predate nests at the egg stage (Wyllie 1975, Bibby 1978) and predation by cuckoos is also seasonally biased as breeders leave the study site to begin their autumn migration before the reed warbler breeding season ends, explaining the decline in nest failures later in the season. A further factor which may contribute to greater nest failure rates earlier in the season is the visibility of nests, as nesting begins while new reed growth is still relatively undeveloped. Nest exposure to predators would be difficult to quantify in this study system, as the foraging strategies of nest predators are unknown.

Male age was an important predictor of nest success, with different seasonal trends apparent between first year and older males (Figure 4.4). While nest success did increase seasonally for older males, nest success of first year males began extremely low and rose more rapidly through the season. The high failure rates observed in the early part of the season are therefore exaggerated in first year males. The same effect of male age was found in a previous study of wrentits, *Chamaea fasciata* (Geupel and DeSante (1990), whereby first year males experienced significantly higher egg stage failure than older males, though they offered no hypothesis for why this was the case. Possible mechanisms behind this age related difference in nest success could be: (i) age effects; first year birds are of lower quality (and improve after their first year) and/ or pair with lower quality females, (ii) territory effects; first year birds occupy lower quality territories which are more vulnerable to predation, or (iii) selection; that first year birds provide a broader subset of the population, where the poor quality individuals do not survive after their first year, so the average quality of individuals is increased for second year birds. Male quality, taken as reproductive effort, has indeed been shown to influence reproductive productivity (e.g. Bart 1990, Nomi *et al.* 2018); however, the territory quality hypothesis is supported by the fact that male age was an important predictor of nest success in the current study, while female age was not. If individual quality was the underlying mechanism influencing nest success, it would be expected that female age would also be an important predictor, especially as female age is known to influence productivity in other ways such as the propensity to double-brood (e.g. Weggler 2006). In reed warblers, territory selection is attributable to males as they defend territories and then attract a female to pair with them (Brown and Davies 1949, Calvert 2005) and aspects of territory habitat have also been shown to influence nest success via predation (Honza *et al.* 1998) as well as the probability of cuckoo parasitism (Alvarez 1993). It follows that as first-year males arrive later than older individuals (Calvert 2005), they may occupy poorer quality territories, leading to their observed higher vulnerability to nest failure than older males. Attributes of territories which may be related to later arriving first year males and therefore increase predation risk could be breeding density or habitat structure. Certainly some, but not all, late occupied territories seem to be in less dense reed bed and isolated from other breeding territories (pers. obs.). However, aspects of territory habitat and predation risk requires further investigation in the study population.

Seasonal variation in the recruitment probability of successful nesting attempts

The probability of successful nests producing a recruit declined seasonally (Figure 4.9, Figure 4.10), which is in agreement with other studies of multi-brooded populations (Hochachka 1990, Barba *et al.* 1995, Smith and Marquiss 1995, Van Noordwijk *et al.* 1995, Verboven and Visser 1998, Cowley 2001, Mallord *et al.* 2008, Brown *et al.* 2014, Hoffmann *et al.* 2014). The brood type of nests appeared to have no additional influence on the probability of producing a recruit, indicating that the seasonal effect was an effect of timing alone rather than a particular difference between first and second broods. Although the number of fledglings produced by successful nests also declined seasonally (Figure 4.7), this did not drive the decline in recruitment probability as it was accounted for in the models of recruitment. A common issue of analyses of the seasonal probability of recruitment is the unknown extent of non-local recruitment (i.e. recruitment outside of the study area) and whether or not this is related to fledging date, thereby compromising inferences based on local recruitment data. In snow buntings, *Plectrophenax nivalis*, first and second brood fledglings moved similar distances between their natal site to their first breeding site (Smith and Marquiss 1995). In contrast, a study of marsh tits, *Parus palustris*, found that later hatched males dispersed further than earlier hatched individuals (Nilsson 1989). Verboven and Visser (1998) also found evidence of non-local recruitment being biased towards later great tit fledglings (in males only), although even after accounting for non-local recruits, the authors still found recruitment to be negatively related to fledging date. For the current study population, no non-local recruitment has been reported, although systematic searching has not been undertaken for this purpose, so this reflects a spatial bias in effort. However, a preliminary analysis of UK-wide reed warbler ringing data do not support fledging date as a covariate of dispersal distance (J.G. Davies pers. comm.).

Potential reasons for a seasonal decline in recruitment probability could be a decrease in fledgling survival either during the period of post-fledging care and/or after independence from parents. In addition, for migratory species, reduced juvenile survival could be the result of later autumn migration. Reduced care in the form of desertion of nestlings by parents has in fact been found to be biased towards later nesting attempts in red-necked grebes, *Podiceps grisegena* and seemed to be the result of a trade-off between parental duties and post-breeding moult (Kloskowski 2003). This reduction in parental care for later fledglings as a result of a trade-off with post-breeding moult was also hypothesised to be the mechanism underpinning the lower recruitment probability of late woodlark, *Lullula arborea*, fledglings (Mallord *et al.* 2008). Conversely, Hochachka (1990) found no seasonal variation in offspring survival of song sparrows, *Melospiza melodia*, while in the care of parents. Food shortages have been implicated in reduced survival of later fledged young in several studies as fledgling weight has been found to be negatively related to recruitment probability (Barba *et al.* 1995, Verboven and Visser 1998). While the mass of fledglings was not measured in the current study, food availability is generally considered to be high throughout the breeding season in wetland habitats (Bibby and Thomas 1985, Halupka *et al.* 2008, Both *et al.* 2009, Dodson *et al.* 2016) and this is supported by data from the study site (Chapter 3). Competition with earlier fledged young could alternatively be responsible for increased fledgling mortality throughout the season as it has been found in great tits that establishment and dominance in winter flocks is influenced by age and also that second brood fledglings survival is negatively related with the density of first brood fledglings (Verboven and Visser 1998 and references within). Aspects of post-fledging mortality, both during and after the period of post-fledging parental care, should clearly be a high priority for future research. This could be achieved by radio tracking of fledglings. Confirmation of a negative relationship between fledging date and post-fledging survival would also improve confidence that seasonal recruitment probability was a result of survival rather than natal dispersal.

Costs of late nesting and double brooding

This study found no evidence of reduced survival of adults whose breeding season ended later or for double-brooded compared to single-brooded adults. There have been very mixed results from similar analyses in the literature, with some studies finding evidence of reduced survival for double-brooded (Bryant 1979, Verhulst 1998) or late nesting individuals (Nilsson and Svensson 1996, Brown *et al.* 2014), while others have reported there to be no effect of attempting a second brood on survival (Smith and Marquiss 1995, Weggler 2006, Carro *et al.* 2014) or that the probability of survival is greater for double-brooded individuals (Geupel and DeSante 1990, Cornell and Williams 2016). There is certainly evidence that survival costs of late nesting can be mediated via quicker post-breeding moult, which results in the production of poorer quality feathers, reducing flight and thermoregulatory performance (Nilsson and Svensson 1996, Dawson *et al.* 2000). Observational studies have further hypothesised that survival costs may be mediated by delayed migration for migratory species, as well as reduced body condition, which occurs during nesting attempts, accumulating to a greater extent, with additional nesting attempts (Bryant 1979, Brown *et al.* 2014). In studies which find no evidence of a cost of double brooding in terms of adult survival, it is generally accepted that this is likely due to any costs being counteracted by individual or territory quality, such that only individuals which can manage the costs of extending the breeding season, attempt to do so (Geupel and DeSante 1990, Smith and Marquiss 1995, Weggler 2006, Cornell and Williams 2016). This could well be the case for reed warblers, although, it is worth noting that Verhulst (1998) found that survival costs resulting from double brooding were only apparent in seasons that were followed by winters with poor food availability. Potential survival costs of late nesting and double-brooding should therefore also be assessed over long-term studies, given that costs may vary between years. Also, it is possible that some single-brooded individuals which do not survive to the following breeding season actually suffer mortality during the breeding season and this could, to a small extent, mask reduced survival of double-brooded individuals versus surviving single-brooded individual i.e. those birds that have survived until the end of the breeding season when they may pay a cost resulting from their reproductive effort. However, breeding season mortality has been shown to be low in another reed warbler population (Wierucka *et al.* 2016) so this may not be an important issue.

There was no evidence for sub-lethal carry-over effects of double brooding or a later end to nesting on the timing of breeding in the following season for either sex. This finding is in support of a study of starlings, where double-brooding had no impact on the following season timing of breeding in females and may indicate that individuals which extend their nesting season are higher quality than those which do not (Cornell and Williams 2016). In contrast, a later end to breeding has been shown to delay breeding the following year in other studies (Nilsson and Svensson 1996, Fayet *et al.* 2016), the mechanism behind the effect being poorer quality post-breeding moult in blue tits (Nilsson and Svensson 1996). It would therefore be interesting to investigate the timing and extent of moult in reed warblers in relation to the cessation of breeding. Generally, reed warblers only undertake a partial moult post-breeding and pre- autumn migration, but anecdotal evidence suggests that individual variation in the extent of moult prior to migration can occur (Nissardi and Zucca 2001), so variation in moult timing or extent post-breeding may still be worthy of future study. Furthermore, given reed warblers undertake long-distance migration in autumn, it would be interesting to assess the variance in timing of individual migration and whether this also relates to the cessation of breeding. As this study has highlighted great variation in the timing of breeding cessation, there is potential for considerable differences in the timing of other life history events. It is possible that any costs of reproduction, in terms of reduced body condition, may be compensated for between the cessation of breeding and the beginning of autumn migration. This is an extension of the idea that only individuals which are in greater condition and can therefore afford the costs of extending the breeding season, attempt to do so, as also concluded by several other studies which found there to be no costs of double-brooding and/ or a later end to breeding (Geupel and DeSante 1990, Smith and Marquiss 1995, Weggler 2006, Cornell and Williams 2016). The future direction of research into the costs of the length of the breeding season, should certainly focus further on the links between the cessation of breeding, post-breeding moult and autumn migration timing. Attaching geolocators to breeding birds is therefore required to monitor migration, as well as multiple adult captures, during the later stages of the breeding season to assess moult progression.

How a continued trend for earlier breeding seasons (Pearce-Higgins and Green 2014) will affect reed warblers in the future, will depend on the mechanisms underlying the current seasonal trends in nest success and recruitment probability and the extent to which these track changes in the timing of reed warbler breeding. As cuckoos are likely responsible for high nest failure rates at the beginning of the season (Wyllie 1975, Bibby 1978, Davies and Brooke 1988, Schulze-Hagen *et al.* 1996), a lot will depend on the relative change in phenology between the two species. In fact, several studies show that cuckoos have become increasingly out of synchrony with short-distant migrants, but have tracked advancing phenology in long-distance migrants, such as reed warblers (Saino *et al.* 2009, Douglas *et al.* 2010), which are consequently exposed to relatively greater parasitism (and therefore also predation) levels than other hosts (Møller *et al.* 2011). However, this is further complicated by the decline of cuckoo populations (Douglas *et al.* 2010). It is difficult to speculate on how seasonality in recruitment potential may change with earlier starting and/ or longer breeding seasons, as the mechanisms underpinning recruitment probability are not understood. Whether the greater probability of recruitment from earlier nests is a result of a longer period of independence prior to autumn migration, and/ or either fledgling density- or food-related post-fledging mortality must be determined.

Regardless of the beginning of the breeding season getting earlier across many species (Pearce-Higgins and Green 2014), it could be expected that the end timing of the breeding season should remain relatively unchanged in multi-brooded species in which there appear to be no costs associated with late nesting. Curiously, this was indeed found to be the case in one long-term reed warbler study, where the start of the season advanced but the end did not (Halupka *et al.* 2008), but not in another where the entire season shifted earlier (Schaefer *et al.* 2006). More widely, for other multi-brooded species, all possible trends in the end timing of the season have been reported, either earlier (Husby *et al.* 2009), relatively unchanged (Møller *et al.* 2010) or later (Weatherhead 2005, Najmanová and Adamík 2009) cessation of breeding. The relative value and costs of late nesting attempts certainly deserves further study, in the context of breeding season length, in order to ascertain whether extended seasons in multi-brooded populations (Møller *et al.* 2010, Halupka and Halupka 2017) will have positive impacts on populations. Long-term studies of the value of extending the breeding season (e.g. the value of second broods) would be the most informative. To date, such a study has been carried out in great tits, where it was documented that a long term decline in the profitability of second broods had resulted in a decline in double brooding frequency (Husby *et al.* 2009), but similar analyses of other multi-brooded species are needed.

CONCLUSION

Despite the fact that the probability of nest success increased seasonally in the studied reed warbler population, the probability of successful nests producing recruits declined over the season. Consequently, selection should favour early nesting when recruitment potential is greatest, while subsequent nesting attempts, regardless of whether they follow failed or successful nests (i.e. replacement or second broods) are of decreasing value as the season progresses. While the value of late nesting attempts is low, there were also no measurable costs, in terms of survival or delayed breeding in the following season, of either a later end to nesting or double brooding. However, It could still be the case that the cost of a later end to nesting varies between years (Verhulst 1998), as although a year effect was not apparent, the number of study years was still relatively small. Moreover, late nesting attempts may in some years be more valuable, if for example the seasonal decline in recruitment potential is related to fledgling density from earlier nests (Verboven and Visser 1998) or food availability, then variability in such parameters could potentially result in late nests being more profitable in some years (Smith and Marquiss 1995). The low costs associated with late nesting documented here are in contrast to life history theory which predicts substantial costs of reproductive effort (Stearns 1976, Reznick 1985) but are supported by several other multi-brooded study populations (e.g. Smith and Marquiss 1995, Weggler 2006, Cornell and Williams 2016). Extending the breeding season with a later end to nesting therefore appears to be a low value: low cost endeavour (Smith and Marquiss 1995) and may not be selected against despite its limited benefits (cf. Husby *et al.* 2009). As a result of late nesting attempts being of low value, breeding season extension of multi-brooded species, including reed warblers (Halupka *et al.* 2008, Møller *et al.* 2010, Halupka and Halupka 2017) may not be as beneficial as previously assumed.

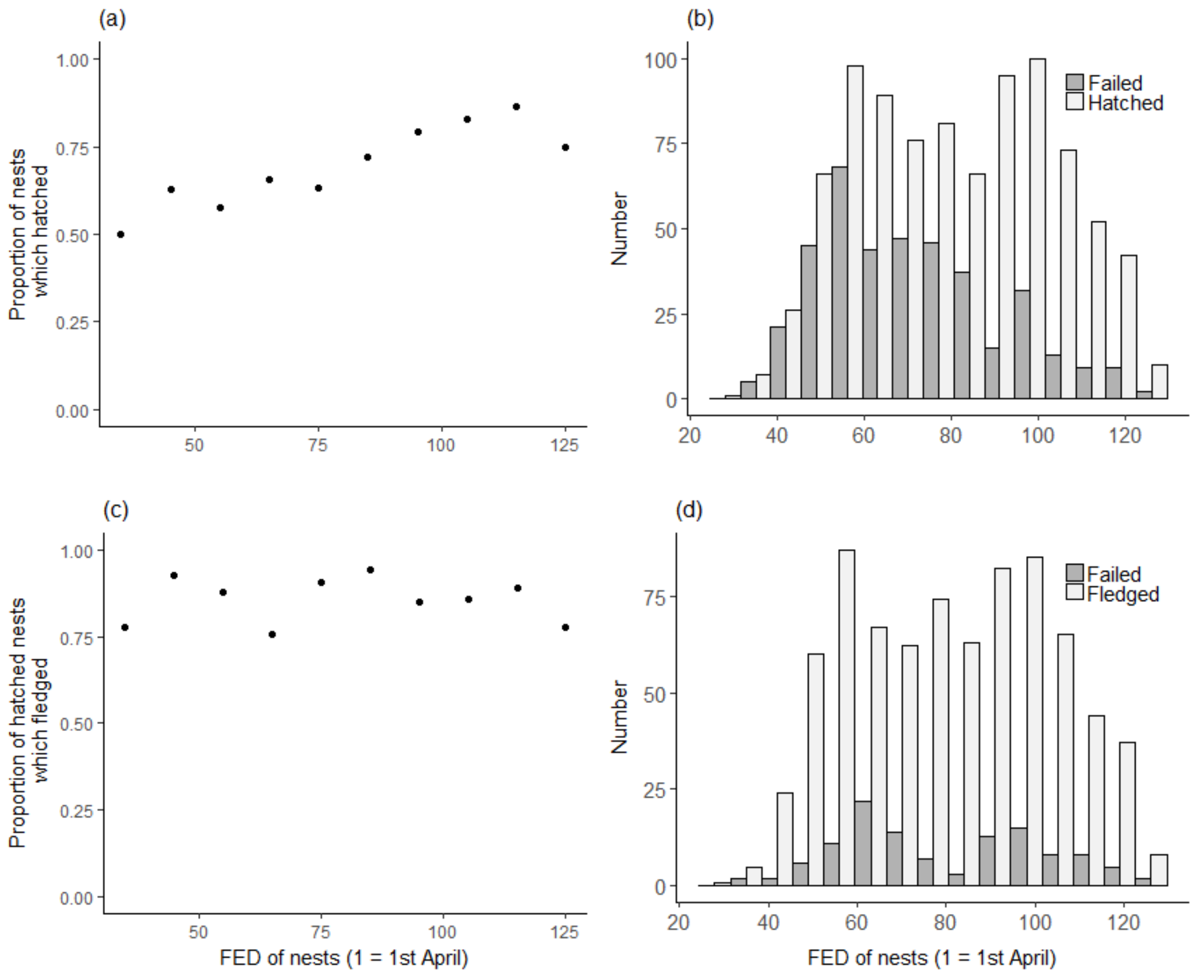

Figure 4.1. Seasonality of egg stage success (i.e. hatching; a, b) and nestling stage success (i.e. fledging; c, d). Plots illustrate the proportion of nests observed to contain eggs which hatched (a) and the proportion of hatched nests which fledged (c) in ten day intervals. Frequency distributions reflect sample sizes of 1275 and 882 for the egg stage (b) and nestling stage (d) data sets, respectively (2012-17).

Table 4.1. Parameter estimates for the generalised linear mixed effects model of the probability of nest success, 2012-2017 (n = 1434). See Appendix 3 for coefficient plot.

Intercept	First egg date (FED)	AICc	Delta AICc	Weight
0.178	0.360	1859.2	0.00	1
0.175		1896.8	37.55	0

Table 4.2. Parameter estimates for the best-fitting generalised linear mixed effects models of the probability of nest success for models including brood type (n = 833), female age (n = 206) and male age (n = 210) in 2014-2017. Null models are also included. “NA” = term not included in global model, “+” = presence of categorical predictor. See Appendix 3 for average model coefficient plots.

Intercept	Year	First egg date	Brood type	Age	FED X Age	AICc	Delta AICc	Weight
Brood type								
1.037	+	0.444		NA	NA	1033.5	0.00	0.556
0.925	+	0.366	+	NA	NA	1034.0	0.46	0.443
0.170				NA	NA	1153.0	119.51	0.000
Female age								
0.726	+	0.441	NA			277.7	0.00	0.567
0.140			NA			290.7	13.06	0.001
Male age								
0.128	+	1.587	NA	+	+	270.4	0.00	0.883
0.307			NA			292.3	21.91	0.000

Table 4.3. Parameter estimates for the best-fitting generalised linear mixed effects models of the probability of egg stage success (i.e. hatching) for models including brood type (n = 772), female age (n = 191) and male age (n = 199) in 2014-2017. Null models are also included. “NA” = term not included in global model, “+” = presence of categorical predictor. See Appendix 3 for average model coefficient plots.

Intercept	Year	First egg date	Brood type	Age	FED X Age	AICc	Delta AICc	Weight
Brood type								
1.414	+	0.400	+	NA	NA	900.6	0.00	0.718
1.601	+	0.495		NA	NA	902.5	1.88	0.280
0.794				NA	NA	980.3	79.67	0.000
Female age								
0.855		0.401	NA		NA	239.8	0.00	0.507
0.820			NA		NA	243.7	3.86	0.073
Male age								
0.112		2.028	NA	+	+	220.6	0.00	0.525
0.721	+	2.058	NA	+	+	220.9	0.23	0.467
1.021			NA			236.8	16.15	0.000

Table 4.4. Parameter estimates for the best-fitting linear mixed effects models of the number of fledglings produced by successful nests for models including brood type (n = 447), female age (n = 110) and male age (n = 121) in 2014-2017. Null models are also included. “NA” = term not included in global model, “+” = presence of categorical predictor. See Appendix 3 for average model coefficient plots.

Intercept	Year	First egg date	Brood type	Age	FED X Age	AICc	Delta AICc	Weight
Brood type								
3.320	+	-0.317	+	NA	NA	1246.9	0.00	0.564
3.306	+	-0.321		NA	NA	1248.2	1.25	0.303
3.306				NA	NA	1295.1	52.99	0.000
Female age								
2.892		-0.211				309.8	0.00	0.331
3.050		-0.595		+	+	310.2	0.38	0.273
2.896						312.8	2.98	0.074
Male age								
2.909		-0.444	NA			349.2	0.00	0.522
2.909			NA			369.4	20.17	0.000

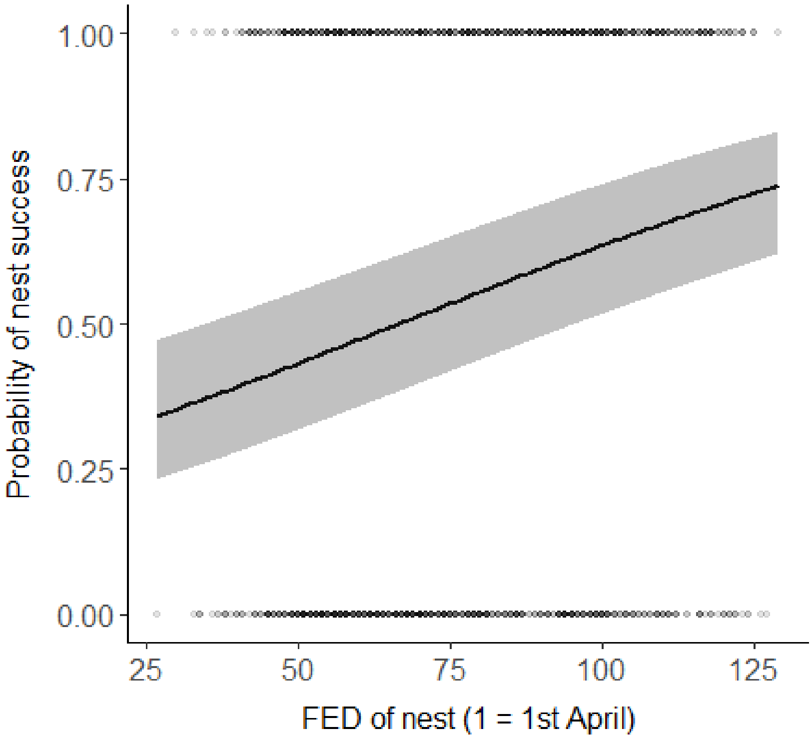

Figure 4.2. Predicted relationship between the probability of nest success and the timing of nesting attempts (first egg date; FED), 2012-17 (n = 1434). The shaded area represents the 95 % confidence interval. Raw data indicated by points.

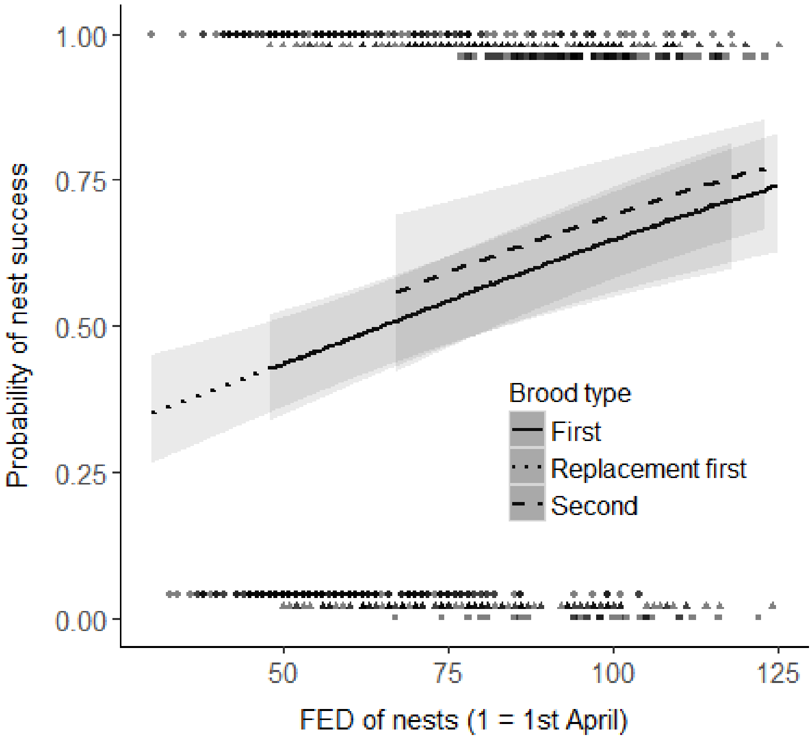

Figure 4.3. Model-averaged, predicted relationship between the probability of nest success and the timing of nesting attempts (first egg date; FED) for different brood types, 2014-17 (n = 833). Lines for brood types have been truncated by minimum and maximum observed FEDs. Shaded areas represent 95 % confidence intervals. Raw data indicated by points (seperated by brood types: first = circles, replacement first = triangles, second = squares).

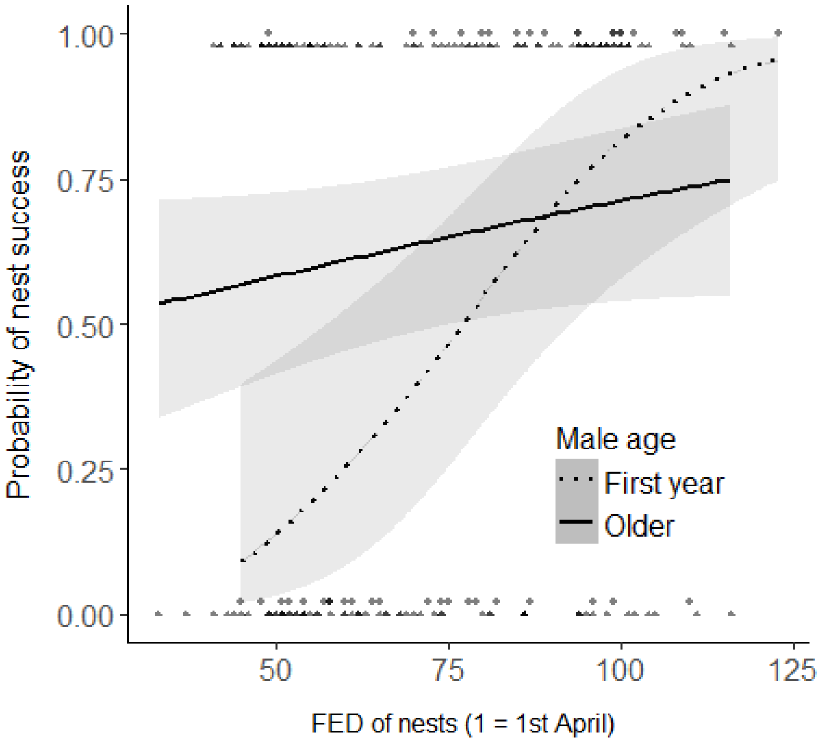

Figure 4.4. Model-averaged, predicted relationship between the probability of nest success and the timing of nesting attempts (first egg date; FED) for first year and older males, 2014-17 (n = 210). Lines for male age categories have been truncated by minimum and maximum observed FEDs. Shaded areas represent 95 % confidence intervals. Raw data indicated by points (separated by male age: first year = circles, older = triangles).

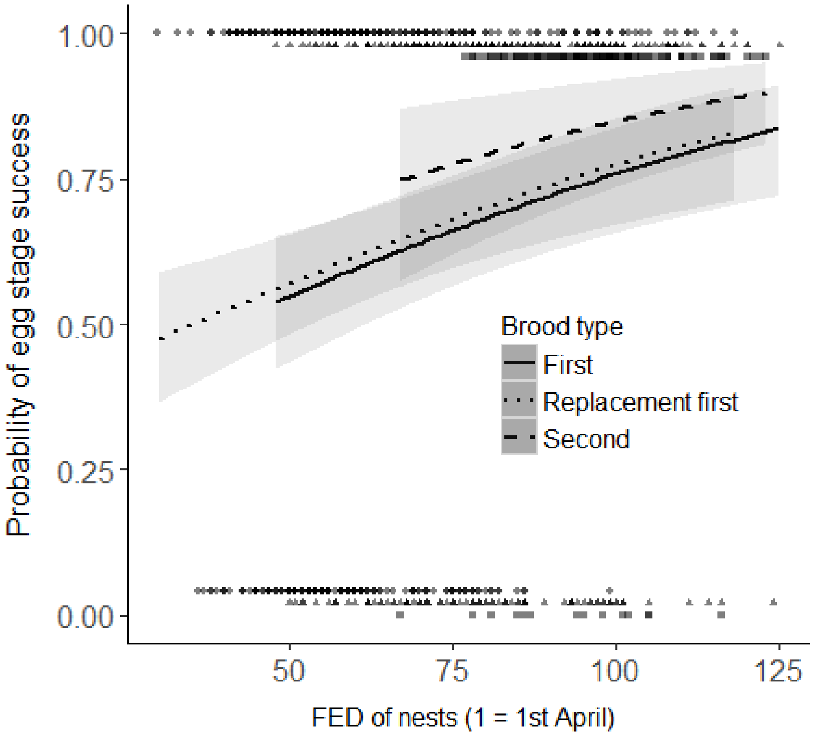

Figure 4.5. Model-averaged, predicted relationship between the probability of egg stage success (i.e. hatching) and the timing of nesting attempts (first egg date; FED) for different brood types, 2014-17 (n = 772). Lines for brood types have been truncated by minimum and maximum observed FEDs. Shaded areas represent 95 % confidence intervals. Raw data indicated by points (separated by brood type: first = circles, replacement first = triangles, second = squares).

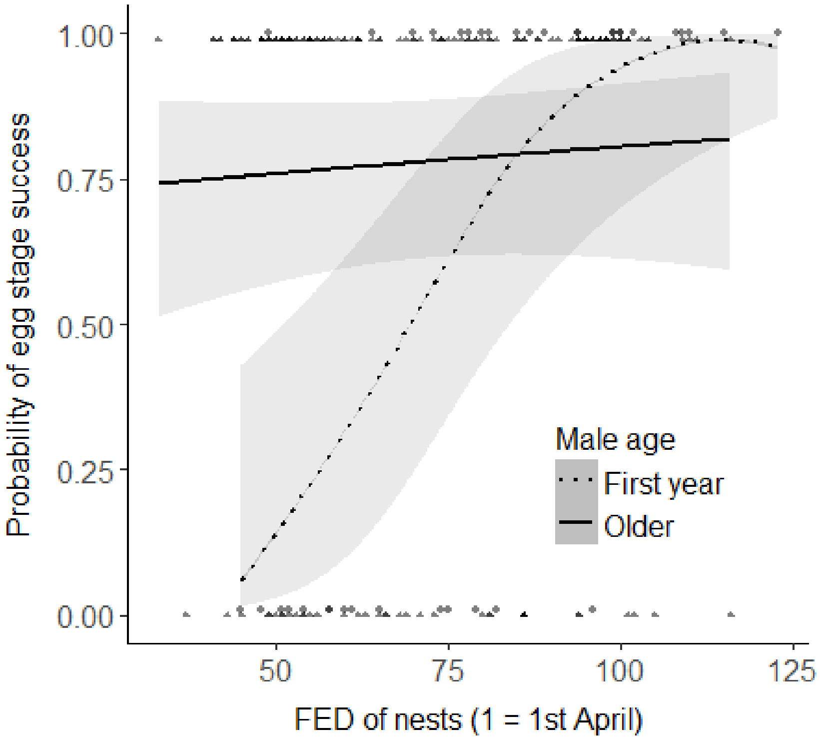

Figure 4.6. Model-averaged, predicted relationship between the probability of egg stage success (i.e. hatching) and the timing of nesting attempts (first egg date; FED) for first year and older males, 2014-17 (n = 199). Lines for male age categories have been truncated by minimum and maximum observed FEDs. Shaded areas represent 95 % confidence intervals. Raw data indicated by points (separated by male age: first year = circles, older = triangles).

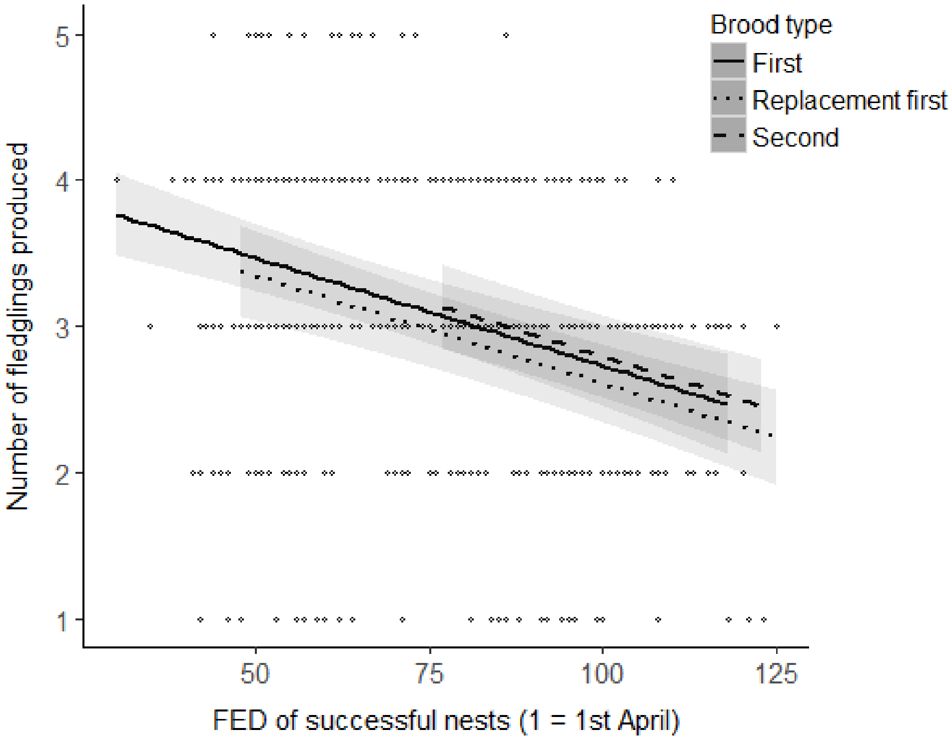

Figure 4.7. Predicted relationship between the number of fledglings produced by successful nests and the timing of nesting attempts (first egg date; FED) for different brood types, 2014-17 (n = 447). Lines for brood types have been truncated by minimum and maximum observed FEDs. Shaded areas represent 95 % confidence intervals. Raw data indicated by points.

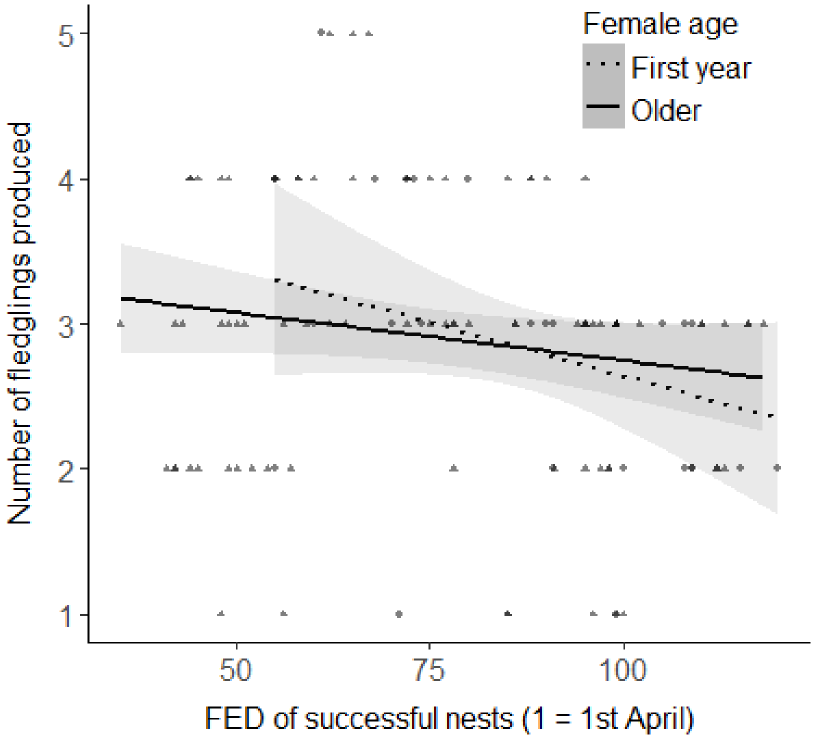

Figure 4.8. Model-averaged, predicted relationship between the number of fledglings produced by successful nests and the timing of nesting attempts (first egg date; FED) for first year and older females, 2014-17 (n = 110). Lines for female age categories have been truncated by minimum and maximum observed FEDs. Shaded areas represent 95 % confidence intervals. Raw data indicated by points (separated by female age: first year = circles, older = triangles).

Table 4.5. Parameter estimates for the generalised linear mixed effects model, with the first egg date (FED) of nests versus the null model, of the probability of successful nests producing a recruit, 2012-2016 (n = 649). See Appendix 3 for coefficient plot.

Intercept	First egg date (FED)	Number of fledglings	AICc	Delta AICc	Weight
-1.919	-0.682	0.396	542.4	0.00	0.984
-1.657			594.9	52.47	0.000

Table 4.6. Parameter estimates for the best-fitting generalised linear mixed effects models of the probability of successful nests producing a recruit, for models including brood type (n = 335) in 2014-2016. Null model also included. "NA" = term not included in global model, "+" = presence of categorical predictor. See Appendix 3 for average model coefficient plot.

Intercept	Year	First egg date (FED)	Number of fledglings	Brood type	FED X Number of fledglings	AICc	Delta AICc	Weight
-2.067	+	-0.626	0.417			304.2	0.00	0.500
-2.086	+	-0.619	0.366		-0.107	305.9	1.77	0.207
-1.553						332.9	28.74	0.000

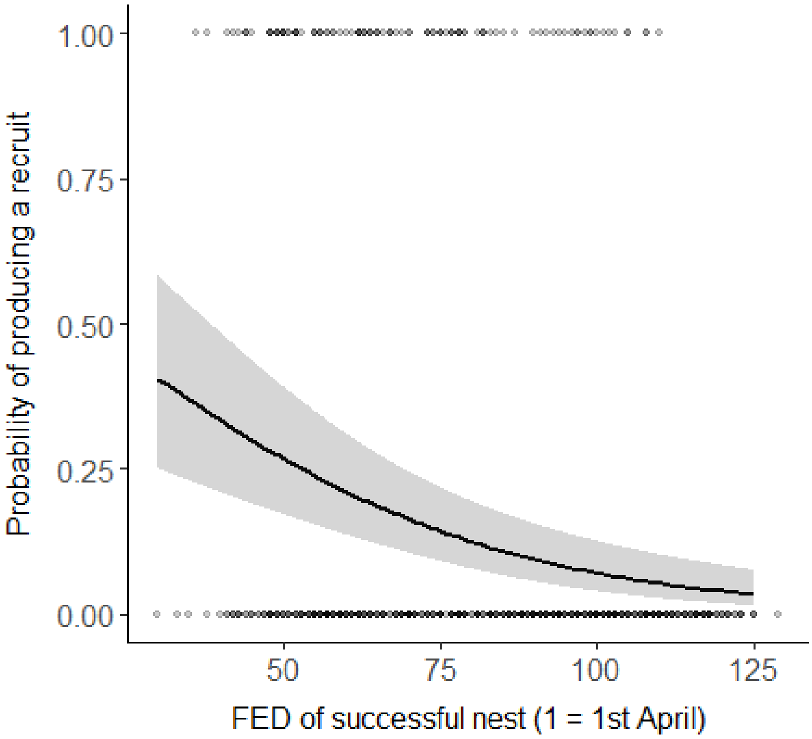

Figure 4.9. Predicted relationship between the probability of successful nests producing a recruit and the timing of nesting attempts (first egg date; FED), 2012-17 (n = 649). Shaded area represents 95 % confidence interval. Raw data indicated by points.

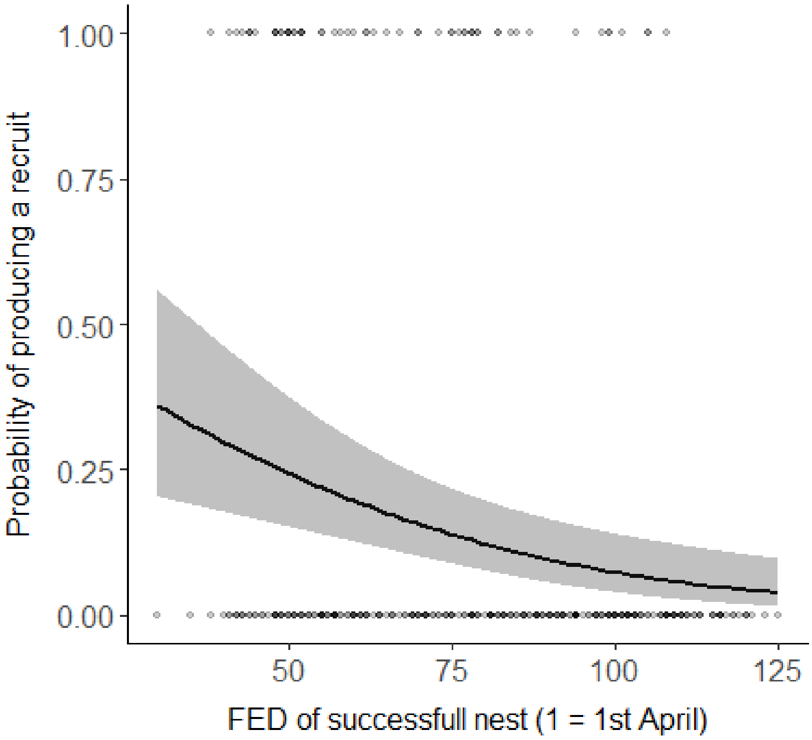

Figure 4.10. Model-averaged, predicted relationship between the probability of successful nests producing a recruit and the timing of nesting attempts (first egg date; FED), 2014-17 (n = 335). Shaded area represents 95 % confidence interval. Raw data indicated by points.

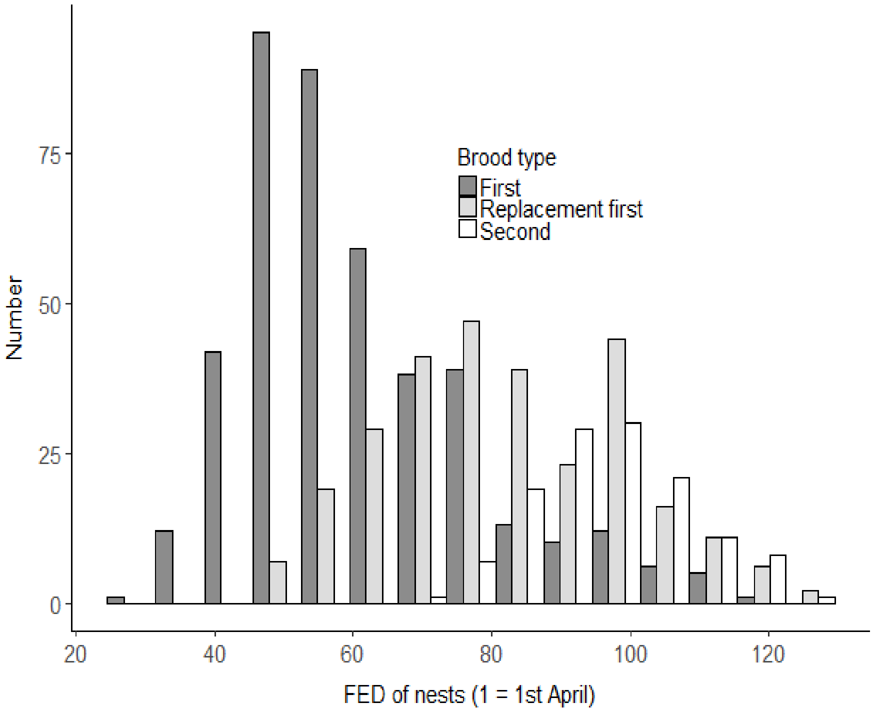

Figure 4.11. Frequency distribution of first egg dates (FED) of first, replacement first and second brood nests (n = 833, 2014-17).

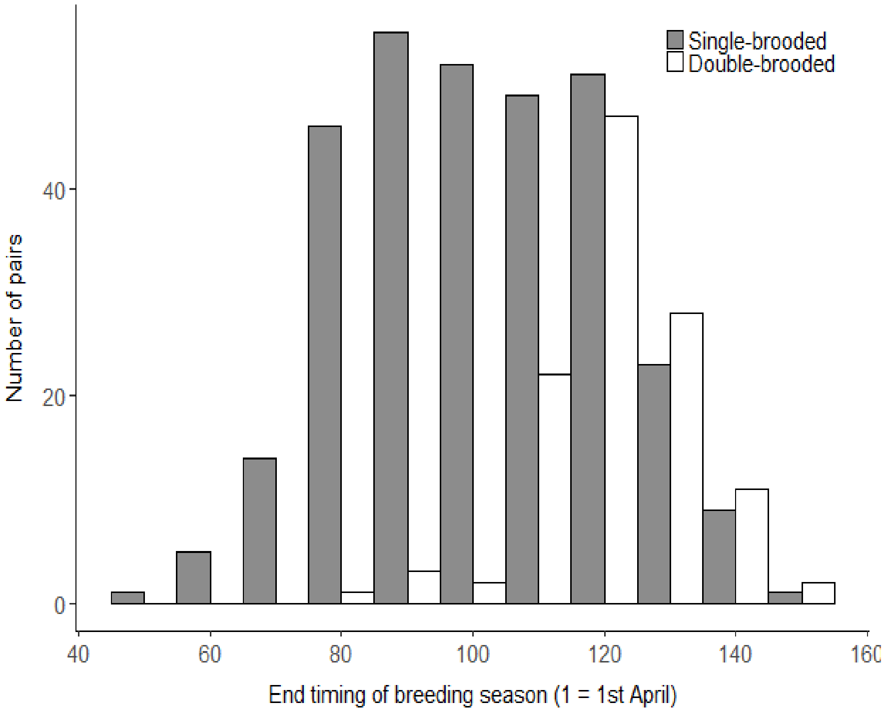

Figure 4.12. Frequency distribution of the end date of pairs' breeding seasons (date of failure or fledging of final nesting attempt), grouped by single- and double-brooded pairs (n = 422, 2014-17).

Table 4.7. Parameter estimates for the best-fitting generalised linear models of the probability of breeding adults being re-sighted in the following season, for females (n = 92), females of known age (n = 49), males (n = 74) and males of known age (n = 31) in 2014-2017. “NA” = term not included in global model, “+” = presence of categorical predictor. See Appendix 3 for average model coefficient plots.

Intercept	Year	Single- or double-brooded	End date of breeding season	Age	AICc	Delta AICc	Weight
Females							
-0.307				NA	127.4	0.00	0.342
-0.501		+		NA	128.4	0.98	0.209
-1.078			0.007	NA	129.2	1.73	0.144
Females of known age							
0.041					70.0	0.00	0.240
0.045			-0.410		70.2	0.25	0.212
Males							
-1.813	+	+		NA	103.5	0.00	0.282
-0.163				NA	104.2	0.62	0.208
-0.427		+		NA	104.9	1.41	0.140
Males of known age							
-0.194	NA		NA		44.8	0.00	0.429
-0.693	NA	+	NA		45.5	0.63	0.313

Table 4.8. Number and proportion of single- and double-brooded individuals of known sex which were re-sighted in the following breeding season, for the seasons 2014-16 combined (i.e. re-sighted in 2015-17).

Sex		Re-sighted	Not re-sighted	Proportion re-sighted
Female	Single-brooded	20	33	38
	Double-brooded	19	20	49
Male	Single-brooded	15	23	39
	Double-brooded	19	17	53

Table 4.9. Parameter estimates for the best-fitting linear mixed effects models of the timing of breeding in the following season (first egg date of the first nesting attempt), for females (n = 53), and males (n = 82). Null models also included. “NA” = term not included in global model, “+” = presence of categorical predictor. See Appendix 3 for coefficient plots.

Intercept	Year	Single- or double-brooded	End date of last nest	FED of first nest	AICc	Delta AICc	Weight
Females							
52.15				7.554	398.4	0.00	0.526
52.27					419.6	21.20	0.000
Males							
52.77					647.2	0.00	0.205
52.79			1.655		647.4	0.27	0.179
52.90			1.795	-1.532	648.2	1.08	0.119
52.86				-1318	648.3	1.19	0.113
52.20		+			649.1	1.98	0.076

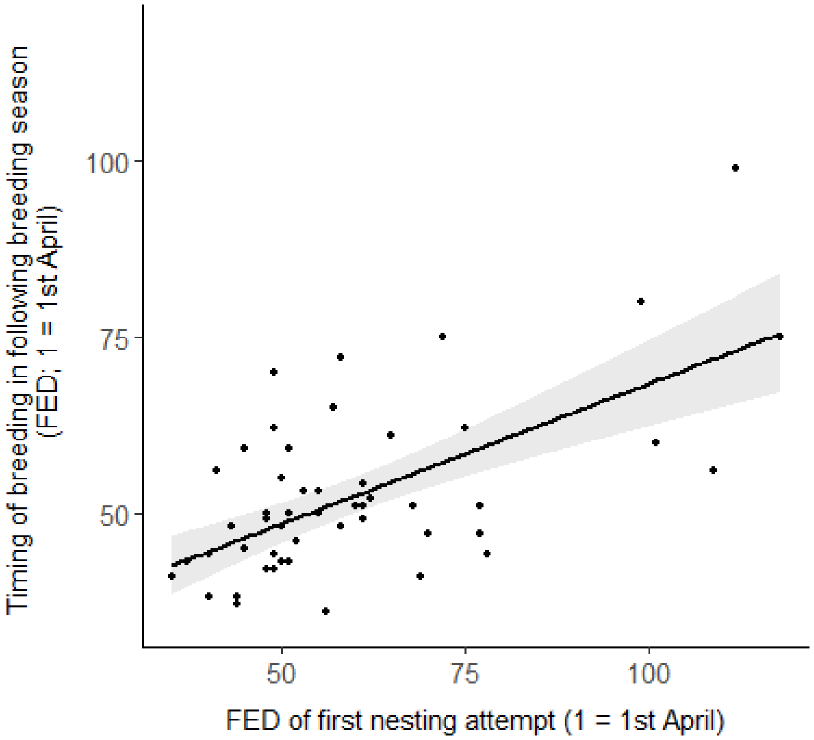

Figure 4.13. Model-averaged, predicted relationship between the start timing of breeding between breeding seasons (first egg date; FED of first nesting attempt), 2014-17 (n = 53). Shaded areas represent 95 % confidence intervals. Raw data indicated by points.

Chapter 5- Parental investment in the provisioning of nestlings in reed warblers, *Acrocephalus scirpaceus*

INTRODUCTION

Bi-parental care is the most common form of parental care in birds (Cockburn 2006). The extent of bi-parental care at different stages of breeding e.g. nest building, incubation and the provisioning of nestlings, varies between species, but both parents often contribute to nestling provisioning in altrical species. The combined provisioning efforts of both parents has been found to be related to brood mass and the fledging success of nesting attempts in several studies (Whittingham *et al.* 1994, Sánchez *et al.* 2018). However, successful reproduction has also been widely found to be possible for single female parents via male removal experiments, as well as in polygynous study systems, where some females receive no 'help' from male partners (Bart and Tornes 1989, Duckworth 1992, Gowaty 1996, Sejberg *et al.* 2000). This raises the question of the value and function of paternal care. It has been theorised that paternal care may only be necessary for successful reproduction in relatively poor conditions and that the costs to males of additionally providing care in good conditions may be so low that there has not been strong selection for condition-dependent care (Bart and Tornes 1989, Duckworth 1992). In addition, paternal care may increase the likelihood of having future broods, either by maintaining the females' condition which may improve the probability of her laying an additional clutch or by increasing the females' willingness to remain with the male for an additional clutch, rather than mate-switching (Bart and Tornes 1989).

There is a growing amount of research on multi-brooded species, showing that multi-brooding individuals can achieve greater annual and lifetime reproductive success (e.g. Chapter 3, Weggler 2006, Townsend *et al.* 2013, Hoffmann *et al.* 2014, Cornell and Williams 2016). The vast majority of studies of the determinants of multi-brooding have reported that the timing of the first brood is the most important factor (e.g. Chapter 3, Weggler 2006, Townsend *et al.* 2013, Hoffmann *et al.* 2014). Several studies have additionally reported that female traits influence the probability of multi-brooding (Geupel and DeSante 1990, Weggler 2006, Bulluck *et al.* 2013, Hoffmann *et al.* 2014), while very few studies have even considered the potential influence of male traits. A recent study of Japanese tits, *Parus minor*, found that the contribution of males to overall first brood nest provisioning rates was positively associated with the probability of double brooding (Nomi *et al.* 2018). However, previous work on dark eyed juncos, *Junco hyemalis*, found male provisioning care (presence versus absence of male care) had no effect on the probability of females double brooding (Wolf *et al.* 1991).

In this study, the extent of male reed warbler, *Acrocephalus scirpaceus*, contributions to the provisioning of nestlings on the probability of double brooding was assessed. The influence of paternal investment on the current breeding attempt was not assessed, as within breeding attempts, reduced provisioning effort by one parent can be compensated for by the other (e.g. Whittingham *et al.* 1994, Sandell *et al.* 1996, Sejberg *et al.* 2000). Instead, the prediction was that increased male parental investment during first broods, would reduce the workload of females, resulting in improved female condition and consequently an increased propensity to attempt a second brood. The difference in parental investment by males and females across the breeding season was also assessed. Other studies report that male and female parental investment during nestling provisioning may differ as a result of different foraging techniques, with for example, the sexes bringing different sized prey deliveries to nestlings (Sejberg *et al.* 2000, Krupa 2004, Krystofkova *et al.* 2006, Falconer *et al.* 2008, García-Navas *et al.* 2013), but it is not clear whether this is consistent seasonally. Hypothetically, if a function of male care is ultimately to increase the probability of the female initiating another clutch, male provisioning effort may decline during the breeding season relative to female effort, as the probability of double-brooding is known to decline seasonally (Chapter 3).

METHODS

Reed warblers are multi-brooded (Chapter 3), predominantly monogamous (Halupka *et al.* 2014) and exhibit bi-parental care throughout the nesting cycle. Both sexes contribute to incubation and nestling provisioning duties (Brown and Davies 1949, Klimczuk *et al.* 2015). The species is a generalist insectivore that breeds in wetland habitats, which are generally considered to provide abundant insect food resources throughout the breeding season, as there are sequential peaks in the population sizes of different insect taxa (Halupka *et al.* 2008, Both *et al.* 2009).

Data collection

All nests were systematically found and monitored in a population of approximately 150 pairs of reed warblers at Watermill Broad Nature Reserve, Norfolk, UK, throughout the 2014-15 seasons. Nests were checked regularly to determine first egg dates (FEDs), brood size and ultimate success (taken as the fledging of one or more nestling) or failure (Chapter 2). Adults were colour-ringed and also sexed, whenever possible (Chapter 2). Parent identities at nests were ascertained by setting up a video camera, on a tripod, approximately 2-3 metres away from nests. Reeds obscuring the view of the nest from cameras were tied back with wire and videos lasted approximately one hour. Nest videos were undertaken only at the nestling stage in 2014, but from the incubation stage in 2015 and subsequent video attempts were made at active nests until both parents were identified. All videos were carried out in fair weather.

Provisioning data were collected from nests of pairs where both parents were known to be colour-ringed (although this sample was supplemented for one analysis, see data analysis) and which were video recorded when nestlings were day 4-6 (day 1 = hatch day). Most nestling stage videos were carried out at this time as nestlings were ringed at this age and video recording was often undertaken immediately prior to nestling ringing. Some individuals showed avoidance behaviour after the camera was set up, before resuming provisioning. A preliminary analysis (ANOVA) of the rate of provisioning visits in 10 minute time blocks showed that provisioning rates of both sexes were lowest in the first 10 minutes, significantly so for males only ($p = 0.018$, $n = 10$) and there were no significant differences between any other 10 minute time blocks (50 minutes of 10 nests were assessed). Data were therefore collected from 10 minutes after the camera was set up, for both sexes. Data were collected for up to 50 minutes thereafter (or until the end of the video if it was shorter than 60 minutes in total) and an hourly provisioning rate was then calculated. Provisioning rates of both parents were calculated by recording the time and parent identity, identified from rings, of each food delivery. Identification of adults based on differences in facial plumage markings was additionally necessary for a very small minority of visits e.g. in cases where a reed stem completely obscured the view of the colour-ring. Ultimately, all provisioning visits were successfully assigned to individuals. The bolus size of food deliveries was also classified as small or large (taken as being less than or greater than the size of the parents' bill) and prey was identified in rare cases where this was possible (see Appendix 4). The extent of brooding behaviour of both sexes was also recorded.

Weather data from the nearest Met Office climate station at Santon Downham, 8.5 km southeast of the study site was used in analyses (as in Chapter 3). Food availability (invertebrate abundance) was measured via the weekly collection of invertebrate samples from seven water traps placed within reed beds during both study years, following the methods used by Bibby and Thomas (1985, see Chapter 2).

Data analysis

Statistical analyses were performed in R version 3.5.0 (R-Core-Team 2018), using 'dplyr' for data manipulations and 'ggplot2' for the production of figures (Wickham 2009, Wickham *et al.* 2018). In all analyses, numeric variables were centred and scaled and collinearity between explanatory variables (fixed effects) was assessed via pair-wise correlations and variance inflation factors (VIFs; Zuur *et al.* 2009). Data from nests in which one or both parents still exhibited camera shyness/nest avoidance during the data collection period (i.e. after the first 10 minutes of videos) were excluded from all analyses. Camera shyness/nest avoidance behaviour was considered present for an individual fulfilling one or more of the following criteria: an individual with zero provisioning visits during the video; an individual which persistently alarmed for long periods of the video; or an individual that was observed carrying food but not delivering it to the nest (either as a result of not approaching the nest or approaching and retreating). Data from 16 nests were excluded on this basis (camera shyness being classified for the male only, female only or both parents for nine, five and two nests respectively).

To assess the potential for male effort to influence the likelihood of double brooding, the probability of attempting a second brood after fledging a first brood (1 = yes, 0 = no) was modelled with a general linear model, with a binomial error structure and a logit link function. This analysis was therefore carried out on successful first brood nests only. The number of first brood nests with both parents colour-ringed available for this analysis was insufficient, so the sample was supplemented with nests from 2014, for which only one parent was colour-ringed (and the sex was known) and the other parents' status was also known i.e. whether it was not ringed or had a metal ring only. This increased the sample size from 25 to 38 nests, however, one of these was excluded as it was part of a case of divorce, whereby the female did re-nest after the first brood, but with another male. The sample used in the analysis was therefore 37 first broods. Identification of nests as first brood attempts as well as whether or not pairs were ultimately double-brooded or not, was determined from complete breeding histories of all pairs, completed using a relative proximity method of nest ownership assignment (Chapter 2). Individual identity could not be fitted as a random effect as a result of the presence of individuals which were not colour-ringed in the sample. However, pseudo-replication, as a result of individuals being present in the sample more than once, was considered to be minimal, as only four males were known to be present in both study years in the sample used in this analysis (n = 37 colour-ringed male observations), and of these, three were double-brooded in one but not the other year. It therefore seems unlikely that individual effects would have a large influence on the probability of double brooding in this sample. The main fixed effect was the proportion of total nest provisioning contributed by the male. While the absolute rate of provisioning would be influenced by factors, which were not consistent between video recordings, such conditions would be experienced equally by both parents, so the proportion of visits by the male was taken as representative of his level of investment. Other fixed effects comprised explanatory variables already known to influence the probability of double brooding in the study population: first egg date, food availability, temperature, rainfall and brood size (Chapter 3). Temperature (^{o}C) and rainfall (mm) were taken as the mean and total for the five days following the fledge date of nests, as in Chapter 3. Food availability was taken as the total invertebrate mass of the seven water traps, in the weekly sample collected on the date on or following the fledge date of nests (as in analyses in Chapter 3). First egg date and temperature were collinear (r

= 0.7), however, both were retained in the model, in order to be able to account for all explanatory variables previously identified as important predictors of double brooding. All other pair-wise correlations between fixed effects were relatively low (r values ≤ 0.5, VIFs < 2.7) and the model was validated with a binned plot of the average residuals and fitted values.

The effect of nest timing on the provisioning rate of individuals (the number of deliveries per hour) was modelled using a linear mixed effects model. This analysis was undertaken only on nests for which both individuals were colour-ringed (n = 80 individuals from 40 nests). Individual identity, nested within pair identity was included as a random effect, to control for the occurrence of multiple nests of the same pair within a season. Fixed effects included date and sex as well as year (there being insufficient levels to fit year as a random effect; Bolker *et al.* 2009), brood size (on the day of video recording), time of day and a measure of food availability as well as the interaction between sex and date, in order to assess if seasonal trends in provisioning rates differed between females and males. Time of day was included as a categorical variable, with early, mid or late timing of videos determined from whether the nearest hour to the start of data collection period (i.e. 10 minutes after the start of the video) was earlier than 11 am, between 11 am and 2 pm or after this period. Food availability was included in order to control for the influence of resources on provisioning rates and was taken as the total invertebrate mass of all water traps on site, collected on the sample date equal to, or the nearest following, the video recording date. All pair-wise correlations were relatively low; r values ≤ 0.5 and VIFs < 1.7. Plotting the model residuals against the fitted values, identified one outlier, so the model was run both with and without the outlier (without the data from either individual from the nest). There were no fundamental differences between the predictions from either model and the model including the outlier is reported in the results.

The effect of nest timing on the size of food deliveries of individuals, was modelled using a generalised linear mixed effects model. The proportion of deliveries which were classified as large bolus sizes, out of all deliveries to the nest by each individual, was modelled with a binomial error structure and a logit link function. The same sample and model structure was used as for the provisioning rate analysis. The model was validated with a binned plot of average residuals versus fitted values.

For all analyses, models were run using 'lme4' (Bates *et al.* 2018) and 'MuMIn' was used to fit and rank all candidate models by AICc values (sample size adjusted Akaike Information Criterion; Bartoń 2018). The best-fitting models, those within 2 AICc units of the top model, were averaged for figures of modelled effects.

RESULTS

Provisioning rates (feeds per hour) were similar between females (mean = 9.0, sd = 4.5) and males (mean = 10.4, sd = 4.8, n = 40 colour-ringed pairs) and showed no clear seasonal trend in either sex according to the raw data (Figure 5.1). Of the 40 colour-ringed pairs, 24 females brooded the nestlings during videos (range = 1-39 minutes), while seven of the males undertook any brooding (range = 4-32 minutes per hour). The proportion of first brood provisioning undertaken by males varied from 22-93 % (mean = 53%).

Of the 37 first broods included in the model of double brooding probability, 20 were of pairs which were ultimately double-brooded. The model identified similar predictor effects as documented previously in Chapter 3, although the previously documented effects of food availability and temperature were both absent in the best-fitting models (Table 5.1). The effect of temperature was likely absent as a result of being collinear with FED, the most important predictor of double brooding, while the effect of food availability could have been absent as a result of the study years used in the analysis representing the seasons with most abundant food resources (compared to additional seasons used in analyses in Chapter 3). The probability of double brooding was predicted to increase with an increasing proportion of the total nest provisioning contributed by the male (Table 5.1), however, this effect was small and the prediction confidence intervals were very wide, so there is insufficient evidence for a real effect (Figure 5.2).

The best-fitting models of provisioning rate, contained sex, date and the interaction between these terms (Table 5.2): all three of these effects were weak (Figure 5.3). Seasonal variation was minimal and although there was a suggestion that males may provision at greater rates, the confidence intervals for the predicted rate for females and males overlapped. Sex was an important predictor of the proportion of large bolus deliveries, males providing a greater proportion (Table 5.3, Figure 5.4). As with provisioning rates, the proportion of large bolus deliveries did not change seasonally. Date was present in one of the best-fitting models but had a very small effect size, while the sex-date interaction was absent. Food availability had a small effect on the proportion of large deliveries but only a negligible effect on provisioning rates (Table 5.2, Table 5.3).

DISCUSSION

The hypothesis that male contribution to nestling provisioning would influence the probability of double brooding, was not supported by this study. Very few preceding studies have assessed the role of paternal care in influencing the likelihood of multi-brooding. In agreement with the current study, Wolf *et al.* (1991) reported, in dark-eyed juncos, that male contribution to provisioning had no influence on double brooding, as females whose partners were experimentally removed, were just as likely to initiate additional clutches as control females. However, Nomi *et al.* (2018) recently reported that in Japanese tits, male contribution to provisioning did have a positive influence on double brooding. The differences between the results of these studies could be related to the fact that Japanese tits have larger brood sizes, which may mean there is greater scope for costs of reproduction to females whose partners contribute less to care (Nomi *et al.* 2018). In reed warblers, male contribution to provisioning may not be necessary for successful reproduction and therefore variance in male effort may not impact upon female condition to the extent that influences her likelihood of laying another clutch. Indeed, it has been found in reed warblers that successful reproduction, the fledging of nestlings, is achievable for single females, though male care may be necessary at the end of the season when conditions may be poorer (Duckworth 1992). However, many studies have found a cost of reproduction (references within Chapter 4) and in fact, even in Wolf *et al.*'s (1991) study, despite single females being just as likely to initiate second broods, they did suffer a cost in body condition. Females paired with males which contribute less may therefore still suffer a cost, alternative to a reduced likelihood of multi-brooding.

The current study likely had a relatively low ability to detect a small effect of male contribution, as a result of controlling for several other factors already known to influence double brooding in the study population. Nomi *et al.*'s (2018) model of double brooding was comparatively simple, however, the effect of male contribution which they found in Japanese tits is quite remarkable, as its effect was considerably more important than the timing of hatching. This is interesting, as in the vast majority of study systems, for which multi-brooding determinants have been investigated, the timing of the first brood has been found to be the most important factor (e.g. Geupel and DeSante 1990, Ogden and Stutchbury 1996, Verboven and Verhulst 1996, Brinkhof *et al.* 2002, Weggler 2006, Bulluck *et al.* 2013, O'Brien and Dawson 2013, Townsend *et al.* 2013, Carro *et al.* 2014, Hoffmann *et al.* 2014, Zając *et al.* 2015, Béziers and Roulin 2016, Jackson and Cresswell 2017).

It is necessary to acknowledge that a possible limiting aspect of the current study to detect an effect of paternal care, could have been the age of nestlings at the time of video recordings. Previous studies have found that the roles of parents can change during the course of the nestling stage (García-Navas *et al.* 2012) and therefore it may be that male contribution at later stages may be more important for lightening the load of parental investment on females. At the relatively young age of nestlings in this study, variance in male effort may not impact on female condition. Future work could therefore look at potential effects when nestlings are older, or even during post-fledging care. In fact, a study of house wrens, *Troglodytes aedon*, found that male provisioning care of fledglings actually reduced their probability of having a second brood, as a result of losing the nesting territory during care (Bart 1990). But there appear to be no other analyses of this nature, no doubt due to the fact that studying the period of post-fledging care is challenging. Given that the majority of care during the post-fledging period of dependence of young may be carried out by males (Verhulst and Hut 1996) and that brood division and/ or brood overlap, whereby males care for fledglings (Stępniewski and Halupka 2018) while females initiate the next brood, are strategies often used by multi-brooded species, there is potential for paternal effort during this stage of the nesting cycle to affect multi-brooding.

Clearly, a greater number of studies on other species, of the potential role of male care in multi-brooding are required, as it has so rarely been considered. Future studies could use experimental approaches, such as handicapping males, to reduce their provisioning rate e.g. via cutting tail feathers (Whittingham *et al.* 1994) or attaching weights to tail feathers (Wright and Cuthill 1990) and comparing their likelihood of double brooding with control pairs.

Parental investment was found not to change seasonally for either sex (in terms of provisioning rates or bolus sizes). This contradicted the hypothesis that male parental investment would decline relative to female investment, if a primary function of male care had been to increase the probability of females initiating an additional brood. Instead, parental investment of both sexes appears to be relatively stable throughout the season. This is in agreement with a study of starlings, where provisioning effort by females was independent of date, as well as being consistent between first and second broods, suggesting that female parental care was fixed at a certain level throughout the season (Fowler and Williams 2015). In breeding females, providing care to their nestlings is the only way to improve fitness, whereas for males, fitness can be increased via several other means including seeking extra pair copulations. The fact that male parental investment during nestling provisioning is similar to females throughout the season, therefore suggests that at this stage of the nesting cycle, males may be dedicated to nestling provisioning. A trade-off between paternal care effort and seeking extra pair copulations has been shown previously, during the incubation stage of nesting attempts (Magrath and Elgar 1997). Extra pair paternity is not uncommon in reed warblers (Davies *et al.* 2003) so there may be greater potential for sexual conflict during the incubation stage, when male reed warblers undertake a smaller share of incubation duties (Klimczuk *et al.* 2015). It would be interesting if future work explored whether paternal investment between the incubation and provisioning stages correlate.

Interestingly, the inclusion of food availability as a covariate in analyses of whole season parental investment in provisioning, revealed that food availability had a negligible effect on provisioning rate, but did have a small effect on the size of deliveries. Food availability in wetland habitats is generally considered to be high throughout the nesting season (Bibby and Thomas 1985, Halupka *et al.* 2008, Both *et al.* 2009, Dodson *et al.* 2016), but these results suggest there may be some food limitation within the season. However, effects of food availability and provisioning on nestling mass and survival would require further study to assess food limitation. Additionally, the fact that males provided larger deliveries than females aligns with several other studies of passerines (Sejberg *et al.* 2000, Krupa 2004, Krystofkova *et al.* 2006, Falconer *et al.* 2008, García-Navas *et al.* 2013), in as much as it suggests that male and female reed warblers may have differing foraging techniques.

CONCLUSION

The level of paternal investment during nestling provisioning was not found to influence the probability of double brooding. Similarly, there was no seasonal variation in the level of parental care in either sex. There have been very few analyses of multi-brooding which have considered the role of males in bi-parental species and this should be addressed in future studies, especially as a recent study found a strong effect of male provisioning investment (Nomi *et al.* 2018). Bolus size was influenced by food availability, suggesting that there may be some food limitation during the reed warbler breeding season.

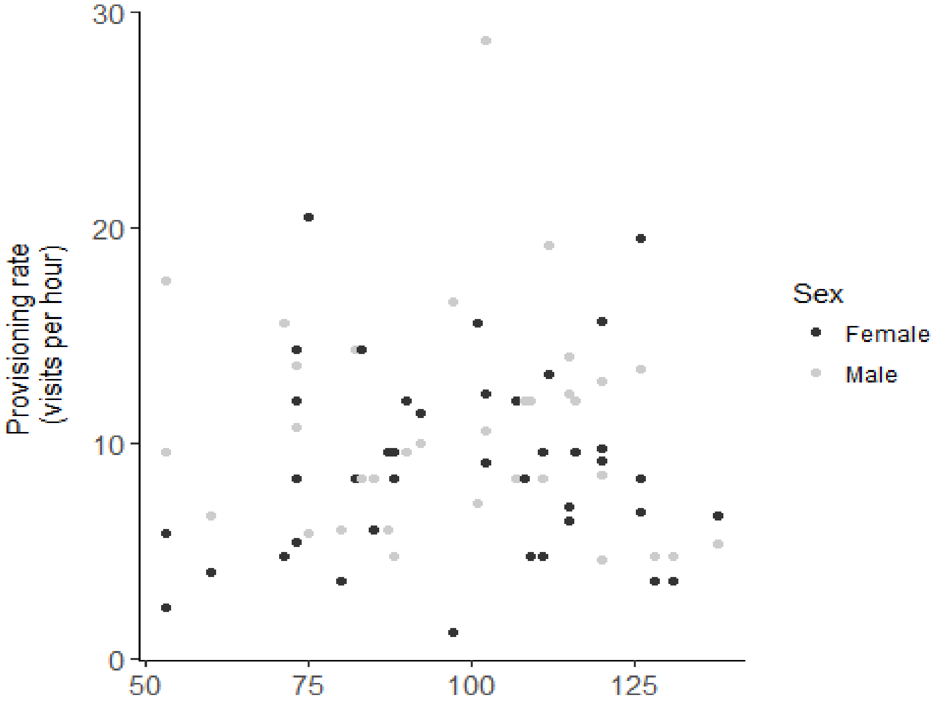

Figure 5.1. Provisioning rates, split by sex, across the breeding season. Sample from nests where both individuals colour-ringed (n = 80).

Table 5.1. Parameter estimates for the best-fitting generalised linear models of the probability of double brooding (n = 37). Null model also included. See Appendix 5 for average model coefficient plot.

Intercept	First egg date	Brood size	Food availability	Proportion male provisioning	Temperature	Rainfall	AICc	Delta AICc	Weight
0.205	-2.598						32.3	0.00	0.172
0.043	-2.819					-1.387	33.2	1.03	0.103
0.313	-2.727			0.494			33.4	1.26	0.092
0.255	-2.879	-0.339					34.1	1.96	0.065
0.163							53.2	21.0	0.000

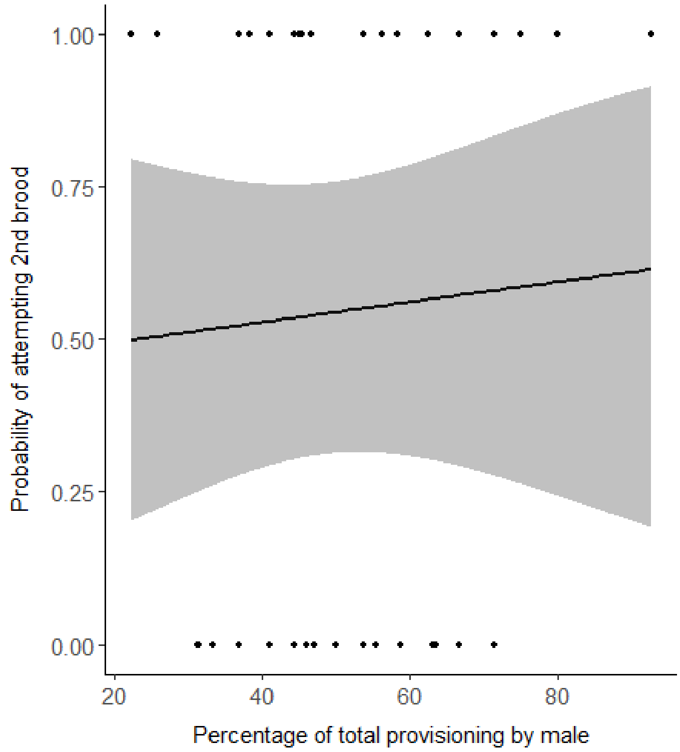

Figure 5.2. Model-averaged, predicted effect of the percentage of provisioning carried out by the male, on the probability of attempting a second brood (n = 37). Line truncated by the minimum and maximum observed male contribution to provisioning. Shaded areas represents 95 % confidence intervals. Raw data indicated by points.

Table 5.2. Parameter estimates for the best-fitting linear mixed effects models (all within 2 AICc of the best-fitting model), ordered by ascending AICc values, of provisioning rate (n = 80). Null model also included. “+” = presence of categorical predictor. See Appendix 5 for average model coefficient plot.

Intercept	Year	Sex	Date	Time	Food availability	Brood size	Sex X Date	AICc	Delta	Weight
9.662	+	+				1.038		470.1	0.00	0.064
9.774	+	+		+		1.106		470.3	0.11	0.061
9.486	+	+	0.979			1.286	+	471.1	0.99	0.039
9.476	+	+	0.549			1.304		471.2	1.07	0.038
9.669	+	+	0.424	+		1.301		471.7	1.56	0.029
9.007		+	1.263			1.426	+	471.7	1.59	0.029
10.540	+			+		1.107		471.8	1.61	0.029
9.677	+	+	0.854	+		1.285	+	471.8	1.64	0.028
10.440	+					1.037		471.8	1.68	0.028
9.002		+	0.836			1.444		471.9	1.71	0.027
9.636	+	+			0.060	1.024		471.9	1.72	0.027
9.736	+	+		+	0.052	1.095		472.0	1.89	0.025
9.336		+		+		1.095		472.1	1.91	0.025
9.767								476.7	6.53	0.001

Table 5.3. Parameter estimates for the best-fitting generalised linear mixed effects models (all within 2 AICc of the best-fitting model), ordered by ascending AICc values, of the proportion of deliveries of large bolus size (n = 80). Null model also included. “+” = presence of categorical predictor. See Appendix 5 for average model coefficient plot.

Intercept	Year	Sex	Date	Time	Food availability	Brood size	Sex X Date	AICc	Delta	Weight
-1.269		+			0.162	0.147		279.3	0.00	0.114
-1.242		+			0.205			279.7	0.39	0.094
-0.975		+		+		0.203		280.6	1.27	0.060
-1.244		+	-0.100		0.190			280.6	1.27	0.060
-1.338	+	+			0.270			280.7	1.36	0.057
-1.344	+	+			0.219	0.134		280.8	1.54	0.053
-1.244		+				0.203		280.9	1.64	0.050
-1.042		+		+	0.121	0.160		281.0	1.69	0.049
-1.266		+	-0.058		0.160	0.124		281.3	1.97	0.042
-0.849								297.0	17.66	0.000

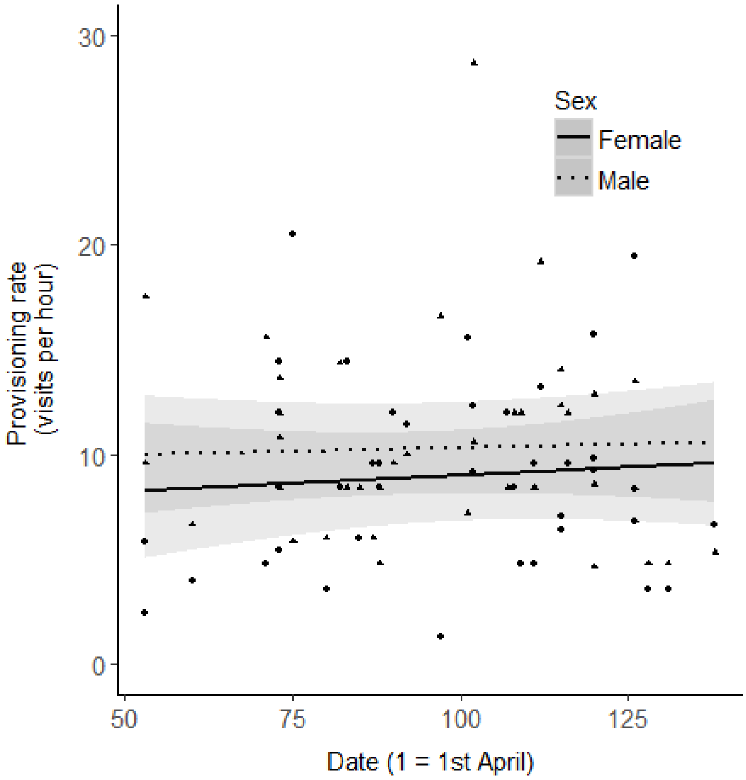

Figure 5.3. Model-averaged, predicted effect of date on provisioning rate, for both sexes (n = 80). Lines have been truncated by minimum and maximum dates of provisioning videos. Shaded areas represent 95 % confidence intervals. Raw data indicated by points (female = circles, male = triangles).

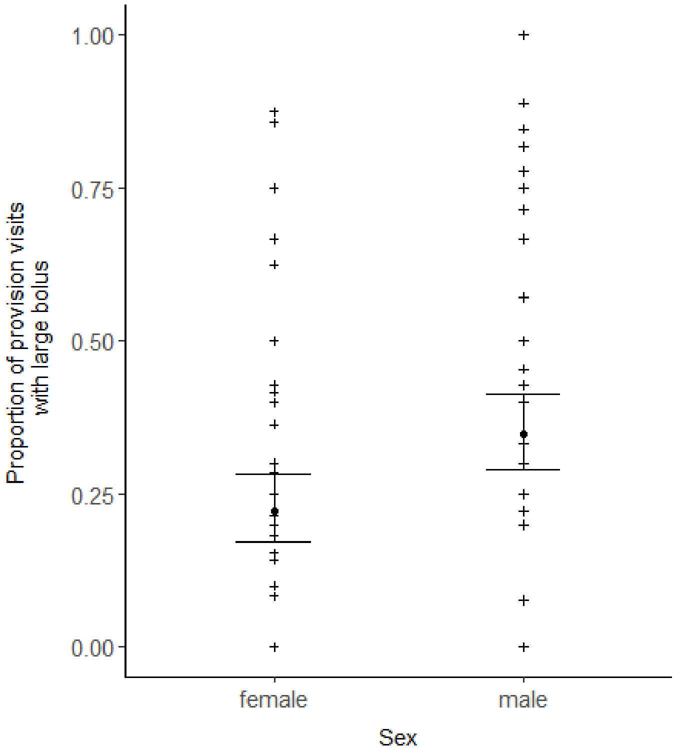

Figure 5.4. Model-averaged, predicted effect of sex on the proportion of deliveries of large bolus size (n = 80). Error bars represent 95 % confidence intervals. Raw data indicated by crosses.

Chapter 6- Counting the number of singing reed warblers *Acrocephalus scirpaceus*: a potential method for monitoring breeding season length

INTRODUCTION

The counting of singing birds (as well as those visually observed) is often used in surveys to monitor long-term abundance changes, such as the Breeding Bird Survey in the UK (Newson *et al.* 2005). In fact, some species are monitored primarily or exclusively on vocalisations, for example censuses of European bitterns, *Botaurus stellaris*, which are rarely seen and nest in dense *Phragmites* reed beds, are carried out via counts of calling males during the breeding season (Gilbert *et al.* 1994). Likewise, the long-term status of an endangered songbird, the Kirtland's warbler, *Dendroica kirtlandii*, has been historically monitored via censuses of singing males (Hayes *et al.* 1986). Indeed, in passerines, singing males are often the principle mode of detection in surveys (Robbins *et al.* 2009). The primary functions of male song are thought to be mate attraction, territory establishment and defence, and paternity/mate guarding (Catchpole 1973, Searcy and Andersson 1986, Kroodsma and Byers 1991, Moller 1991, Sheldon 1994, Catchpole and Slater 2008). As male singing is therefore associated with the nesting season, systematic counting of singing birds may represent an alternative, low intensity method for monitoring breeding phenology and season length, which appears not to have been considered previously.

Breeding season duration is an important parameter in population monitoring because season length may be positively associated with an increased propensity to have multiple nesting attempts (Halupka *et al.* 2008, Husby *et al.* 2009) and/or increased intervals between nesting attempts (Møller 2007) and therefore influences breeding productivity. Furthermore, there is increasing evidence that the breeding season length of many populations is changing in line with climate change (Møller *et al.* 2010, Halupka and Halupka 2017). Unlike other demographic parameters, breeding season length is poorly covered by most monitoring schemes run at national levels. For example, data collected via the UK Nest Record Scheme, which collates records of tens of thousands of nests monitored every year by volunteer birdwatchers, may not adequately cover the end of the breeding season for some multi-brooded species (Crick *et al.* 2003). This may be because fieldworker effort is lower at the end of the summer when fewer species are still nesting and/or because increased vegetation growth makes nests more difficult to find. Intensive nest monitoring is clearly the optimum method of monitoring breeding season length, but while this can be achieved at local scales (e.g. Weggler 2006, Halupka *et al.* 2008, Jankowiak *et al.* 2014), it is unlikely to ever be used at greater spatial scales.

The reliability of using song to measure breeding phenology, and thus breeding season length, will depend upon the strength of the relationship between singing and breeding activity, which in turn depends on song function. For example, in the closely related sedge warbler, *Acrocephalus schoenobaenus*, and reed warbler, *A. scirpaceus*, unpaired males sing to attract a mate in the former, while song is used for both mate attraction (by unpaired males) and for territory defence (unpaired and paired males) in the latter (Catchpole 1973). Song rate is usually greater for unpaired compared to paired males (Hayes *et al.* 1986, Gibbs and Wenny 1993, Hanski and Laurila 1993, Amrhein *et al.* 2007, Robbins *et al.* 2009), however, as long as the extent to which song rate of unpaired birds is greater than paired birds, remains constant through the season, it should not be a limitation of using song as a measure of breeding status at a population level. Song rate also varies between stages of nesting (Best and Petersen 1982, Logan 1983, Wilson and Bart 1985), for example being greatest during nest building (Logan 1983), or during incubation compared to the egg laying and nestling stages (Wilson and Bart 1985, Johnson and Kermott 1991, Hanski and Laurila 1993). Potential functions of song during the incubation period include seeking additional females for polygynous breeding or extra pair copulations (Johnson and Kermott 1991, Hamao 2008) and coordination of female nest attentiveness (Ziolkowski *et al.* 1997). Alternatively, singing rate may be greater during the female fertile period (i.e. the early stages of breeding such as nest building and egg laying), functioning as mate/paternity guarding and a deterrent to territory intruders (Moller 1991). Indeed, peak song rates have been observed during the female fertile period, such as during egg laying, in a number of study species (Greig-Smith 1982, Mace 1987, Møller 1988, Forstmeier and Balsby 2002) but not in others (Hanski and Laurila 1993, Sheldon 1994, Rodrigues 1996, Titus *et al.* 1997, Gil *et al.* 1999).

Beyond the effect of breeding status on singing behaviour, it is also feasible that this relationship may not be constant over time and that singing is also related to environmental factors or pair bond status. For example, singing rate may decline seasonally as a result of lower food resources exerting other demands (i.e. foraging) on time available for singing, or declining opportunities to attract females. In addition, in multi-brooded species, singing rate may be lower later in the season for second broods, as the pair-bond is already established. For example, Amrhein *et al.* (2007) found that the relative detectability of unpaired and paired male nightingales, *Luscinia megarhynchos*, changed to a different extent through the breeding season, with the relative detectability of paired males declining to a greater extent than unpaired males. In comparison, while Wilson and Bart (1985) found singing detectability to vary between stages of nesting, these differences did not vary seasonally in house wrens, *Troglodytes aedon*. Several other studies illustrate that singing later in the season prior to late nesting attempts certainly occurs (Greig-Smith 1982, Møller 1988, Vengerov 2012). Finally, breeding density may be also be lower later in the season, which may reduce the requirement for territorial song.

This study aimed to assess the potential for a census of singing males to track breeding phenology and therefore breeding season length, by matching counts of singing male reed warblers with known nest abundance and phenology determined through intensive nest monitoring. Reed warblers are an ideal study species, as the species is fundamentally difficult to census as a result of breeding in very high densities (e.g. Catchpole 1972) and they are most easily detectable via song, being often out of site in relatively inaccessible, dense *Phragmites* reed beds (Bell *et al.* 1968, Catchpole 1973). The relationship between the number of singing males and the number of nesting attempts at various stages of the nesting cycle: during the pre-incubation (and therefore representative of the female fertile period), incubation and nestling stages were assessed across the whole breeding season. It was expected that the number of singing birds would be most correlated with the female fertile period (the number of pre-incubation nests). Song rate was expected to be lower during incubation as the species is predominantly socially monogamous and males also contribute to incubation (Klimczuk *et al.* 2015) and are therefore not advertising to attract additional females so may spend less time directly defending territories. Moreover, the extent of singing was expected to decline seasonally as pair bonds are already secure, mate-switching is rare and breeding density is low because breeding pairs' seasons end over a protracted period (Chapter 4).

METHODS

Data collection

This study was carried out at a 46 ha site in Norfolk, UK, consisting of lakes and ponds of varying sizes which are fringed with narrow *Phragmites* reed beds. The total area of reed bed on site is 7.6 ha and the site supports a population of approximately 150 pairs of reed warbler per season (Chapter 3). Throughout each of the three breeding seasons 2015-17 (April-August), the number of singing reed warblers was counted weekly and reed warbler nests were found and monitored through to fledging or failure (nest searching was very effective, with very few nests missed; Chapter 2).

Singing reed warblers were counted during singing bird surveys by walking around the land-side edges of the majority of reed bed on site, following the same route on each survey. Singing was infrequently thought to be reactive i.e. in response to the observers' presence, as observation was rarely in close proximity of the nest e.g. within 10 m. Surveys were carried out at least once a week throughout April-August. Effort was similar between seasons with an average of 4.8, 5.1 and 5.8 days between each survey in 2015, 2016 and 2017, respectively. Surveys started between 8.00 and 9.30 am, lasted 1-1.5 hours and were undertaken at a consistent walking pace in fair weather (rain and strong wind were avoided).

Reed warbler nests were found via regular and systematic cold searching of all reed bed on site. First egg dates (FEDs) were calculated on the assumption of one egg being laid per day (Brown and Davies 1949) and where necessary (e.g. when the nest was not visited during hatching), back-calculations on the basis of an incubation length of 11-13 days (from the laying day of the penultimate egg to hatching; Chapter 2). Median, or upper median values were taken from the range of dates which could have represented the FED. End dates of nests were taken as the mid-point (median or upper median) of the latest observation with active contents and the final observation revealing a recently fledged or failed nest (although fledge dates were taken as two days after observation of nestlings big enough to leave the nest in cases where the final check was late; Chapter 2).

Data analysis

Statistical analyses were performed with R version 3.5.0 (R-Core-Team 2018) using ‘dplyr’ for data manipulations (Wickham *et al.* 2018) and both ‘ggplot2’ and ‘gridExtra’ for the production of figures (Wickham 2009, Auguie 2017). Models were run using ‘lme4’ (Bates *et al.* 2018). Generalized Linear Models (GLMs; fitted with Poisson error structures due to the count data response variable) were used to assess the relationship between the number of singing individuals and the number of active nests at three alternative stages; pre-incubation, incubation and nestling stages. Nests at the pre-incubation stage were taken as nesting attempts between five days prior to the FED and three days after the FED, in order to represent the period covering nest construction and egg laying when males may guard their mate. Nests at the incubation stage were taken as the following 10 days (four days after the FED until thirteen days after the FED), as this represented the period after which nestlings would normally hatch, given an incubation duration of 12 days from the penultimate egg to the hatch day (Chapter 2). Nestling stage nests were considered those within the twelve days following the incubation stage period, as nestlings usually fledge at 11 to 13 days old (Robinson 2018; and pers. obs.). Data on the timing of each nest stage for all nests were not known exactly as, for example, clutch size was not always known and hatch dates were not routinely observed. Therefore, in order to include all nests in analyses, the separation of incubation and nestling stages on singer survey dates were calculated by assuming the modal value for clutch size (four) and incubation duration (12 days from the penultimate egg; Chapter 2).

The first GLM was fitted with the number of nests at each of the three stages as fixed effects, as well as the year, date and start time of the survey (minutes after 8 am). Due to the relatively small sample size and to avoid over-parameterisation, no interaction terms were included. Therefore, in order to assess whether the relationship between the number of nests and singing birds interacted with date, a second GLM was fitted with the following fixed effects: number of pre-incubation nests, year, date, time and all two-way interactions between pre-incubation nests, year and date.

In all analyses, numeric variables were centred and scaled. All fixed effects had variance inflation factors < 4 (Zuur *et al.* 2009). Models were validated by plotting an index of residuals versus fitted values and all potential candidate models were subsequently ranked by their AICc value (sample size adjusted Akaike Information Criterion) using 'MuMIn' (Bartoń 2018). Predicted effects in figures represent those from average models, across the best-fitting models, taken as those within 2 AICc of the top model.

RESULTS

The first singing male was observed on the 19th, 17th and 16th of April in 2015, 2016 and 2017, respectively. The number of singing males then increased rapidly (and consistently between seasons), presumably reflecting the cumulative arrival of males through April and early May (Figure 6.1). A considerably lower peak in singer numbers was reached in 2017 compared to the other two seasons, which had an almost identical peak. This is in agreement with nest monitoring data which showed that the population size was lower in 2017 (Chapter 3).

The number of nests at the pre-incubation stage was the best predictor of the number of singing males, displaying a positive relationship (Figure 6.2); it was present in all of the best-fitting models and had the largest effect size (Table 6.1). The number of nests at the incubation and nestling stages were relatively unimportant, as they were absent from the best-fitting models (incubating nests) or had a very small effect size (nestling stage nests; Table 6.1). The best-fitting models included the start time of surveys but the effect sizes were very small (Table 6.1). There was an effect of date; the predicted number of singing birds declined seasonally (Table 6.2, Figure 6.3). The interaction between the number of nests and date was negligible as this parameter had a very small effect size (Table 6.2). The interactions between the number of pre-incubation nests and year as well as between date and year were absent from the best-fitting models.

DISCUSSION

The number of singing male reed warblers was positively related to the number of active pre-incubation nesting attempts. By contrast, the number of nesting attempts at the nestling stage was a poor predictor of the number of singing males and the number at the incubation stage was not correlated with the number of singers at all. This aligns with the expectation that singing would be most correlated with the female fertile period. Male song during this period has been hypothesised to be a form of mate guarding, whereby neighbouring males are deterred from entering territories containing fertile females to a greater extent with increased male singing, which acts as a signal of quality (Moller 1991). This may well be the case for reed warblers as males certainly face a threat from extra pair paternity (Davies *et al.* 2003). The idea that singing during the female fertile period acts as a form of paternity guarding has also been supported by observations of yellowhammers, *Emberiza citronella*, where male intrusions into territories peaked during the female fertile period and in territories where male song was relatively low (Møller 1988). In addition, the author found that song activity and direct mate guarding behaviour were strongly positively associated, further supporting song as a paternity guarding behaviour. High song rates during the female fertile period have also been observed in other species such as the dusky warbler, *Phylloscopus fuscatus* (Forstmeier and Balsby 2002), the great tit, *Parus major* (Mace 1987) and the stonechat, *Saxicola torque* (Greig-Smith 1982). Moreover, in European starlings, *Stumus vulgaris*, not only did male singing peak during the female fertile period, but most males completely ceased singing after this stage (Pinxten and Eens 1998). Interestingly, however, singing does not peak during the female fertile period in all species, for example in those where song is thought to function primarily in mate attraction either by unpaired males or paired males seeking additional mating opportunities (e.g. Catchpole 1973, Johnson and Kermott 1991, Hanski and Laurila 1993, Hamao 2008), but the reasons for these inter-specific differences are not clear (Forstmeier and Balsby 2002).

Mate attraction is one of several functions of song in reed warblers (Catchpole 1973) and studies of other species illustrate that the singing of unpaired males has the potential to influence census results (Amrhein *et al.* 2007). Although the paired versus unpaired status of singing birds was unknown in the current study there is no reason why this should confound the relationship between singing and the number of nesting attempts. It was very rare for a singing male to hold a territory for a long period of time and for a nest never to be found in the territory (pers. obs.). Furthermore, there is no reason why the number of unpaired males should vary in parallel with the number of pre-incubation nests through the entire season.

The relationship between the number of (pre-incubation) nesting attempts and singing males illustrates that there is potential for the use of season-long counting of singing males as a proximate measure of breeding season length. Furthermore, the relationship between the number of (pre-incubation) nests and the number of singers was consistent over time; the relationship did not change with either date or year, which is crucial for a prospective method of monitoring inter-annual variation in breeding phenology. While this study has shown in principle that singer censuses could be useful in estimating breeding season length, a long-term data set of paired systematic counting of singing birds and systematic nest monitoring over a greater number of years is now required to directly compare season length estimates of the two methods. Such a dataset is also required to assess the degree of effort required to estimate season length at a sufficient level of accuracy i.e. the number and distribution of singing censuses across the breeding season, required to estimate season length. This is certainly worthy of future investigation, as currently there is no systematic way of monitoring breeding season length of many multi-brooded species at large spatial scales. National scale nest record schemes, for example, may be biased against late nesting attempts (Crick *et al.* 2003). Moreover, while censuses of singing males have been widely used for the purpose of estimating inter-annual population sizes (Hayes *et al.* 1986, Gilbert *et al.* 1994), their potential use for monitoring breeding season length appears to have been overlooked.

There was evidence that singing declines seasonally; there were fewer singing males as the season progressed, after controlling for the number of active pre-incubation nesting attempts. This is interesting, as a singer census not controlling for the effect of date would therefore underestimate breeding at the end of the season (or overestimate it at the start). There appears to be a lack of other population scale studies documenting singing behaviour throughout the breeding season of multi-brooded species with which to compare these results. Vengerov (2012) reported that multiple singing peaks in a population of song thrush, *Turdus philomelos*, matched similar peaks in egg laying, but did not quantitatively assess whether the relationship between singing and the number of nests was consistent throughout the season or not. The seasonal decline in singing in the current study was not a result of a declining requirement for the defence of paternity, or territory as a result of lowered nesting density, as although nesting density is low at the end of the season, the effect of date on the number of singing males controlled for the number of nests. Other potential explanations for reduced singing relative to the number of nests later in the season could be that individuals sing less during later nesting attempts than earlier nesting attempts and/ or that as the season progresses, the number of unpaired singing birds, inflating the total number of singing males, declines. A possible mechanism for reduced singing by individuals during later nesting attempts, could be that a further song function includes maintenance of the pair-bond, which may strengthen during the first breeding attempt. Mate switching in reed warblers is uncommon (Chapter 2) and, in a study of chaffinch, *Fringilla coelebs*, males that lost their partner later in the season increased their singing (Hanski and Laurila 1993). However, the notion that individuals sing less during later nesting attempts is not supported by studies of yellowhammers and stonechats, in which song rates were either similar or increased during the female fertile period of later nesting attempts respectively (Greig-Smith 1982, Møller 1988). Another explanation for reduced singing later in the season could be reduced energy or time being available for activities such as singing as a result of either reduced body condition due to energy expended on reproduction throughout the breeding season, or lowered availability of food resources later in the season. However, it seems most plausible that the seasonal decline in the ratio of singing males to nests may simply be driven by an increased number of unpaired singing birds at the beginning of the season (Figure 6.1). Seasonal variation in singing behaviour of known individuals during the nesting cycle of multiple nesting attempts within a breeding season could be investigated by future work.

CONCLUSION

The number of singing reed warblers was predicted by the number of pre-incubation nests, representing those during the female fertile period. This relationship between the number of singing birds and nests illustrates that, in principle, the systematic counting of singing birds may be a useful method for monitoring annual variation in breeding season length at large spatial scales. It should be pointed out, that while the counting of singing birds may represent a future method of monitoring breeding season length, this method would obviously not be able to differentiate between extended seasons resulting from increased multi-brooding versus from increased failures. A sufficiently long-term data set of paired systematic counting of singing birds and nest monitoring is required to directly assess the agreement between the two methods in measuring season length. Moreover, as relationships between song rates and reproductive status vary between species, the potential of counting singing birds to infer breeding phenology will also differ considerably between species. Similar studies relating singing behaviour to nesting across a wider range of species are therefore also required. The number of singing reed warblers also declined seasonally, after accounting for the number of nests, which was likely a result of a smaller number of unpaired birds being present later in the season.

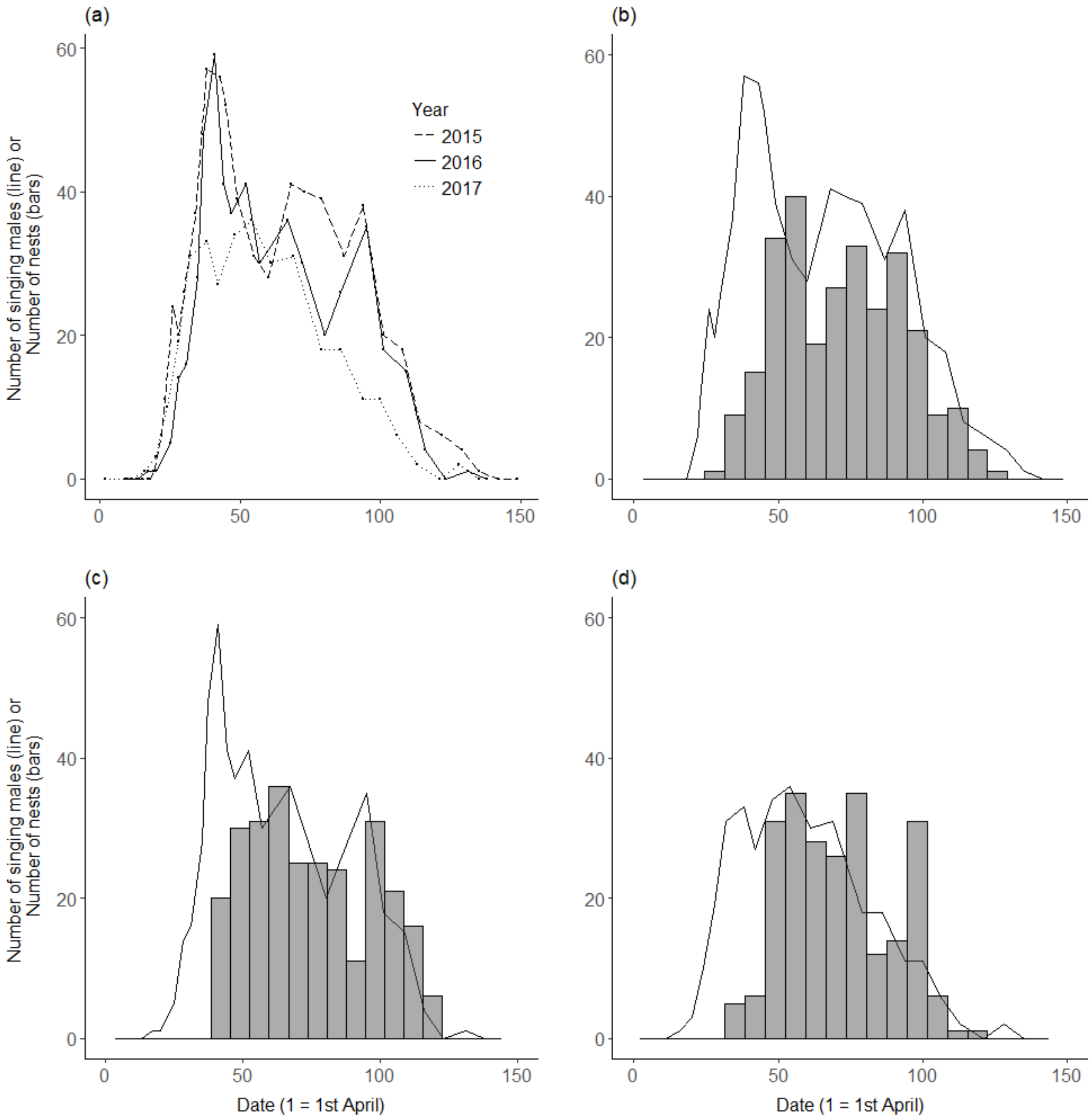

Figure 6.1. Counts of singing males in 2015-17 (a) and split by year with frequency distributions of first egg dates of nests in 2015 (b), 2016 (c) and 2017 (d). Nest sample sizes were 279 in 2015, 276 in 2016 and 231 in 2017.

Table 6.1. Parameter estimates for the best-fitting generalised linear models of the number of singing males without interaction predictors (n = 79). Null model also shown and "+" = presence of categorical variable. See Appendix 6 for average model coefficient plot.

Intercept	Year	Date	Time	Pre-incubation nests	Incubation nests	Nestling nests	AICc	Delta	Weight
2.795	+	-0.298	-0.070	0.629			1059.1	0.00	0.411
2.781	+	-0.327	-0.071	0.627		0.036	1060.4	1.28	0.216
2.919							1741.3	682.16	0.000

Table 6.2. Parameter estimates for the best-fitting generalised linear models of the number of singing males, for the analysis including the interaction terms between the number of pre-incubation nests and both date and year (n = 79). Null model also shown and "+" = presence of categorical variable. See Appendix 6 for average model coefficient plot.

Intercept	Year	Date	Time	Pre-incubation nests	Nests X Year	Nests X Date	Date X Year	AICc	Delta	Weight
2.795	+	-0.298	-0.070	0.629				1059.1	0.00	0.303
2.808	+	-0.314	-0.062	0.618		-0.068		1059.2	0.12	0.286
2.919								1741.3	682.16	0.000

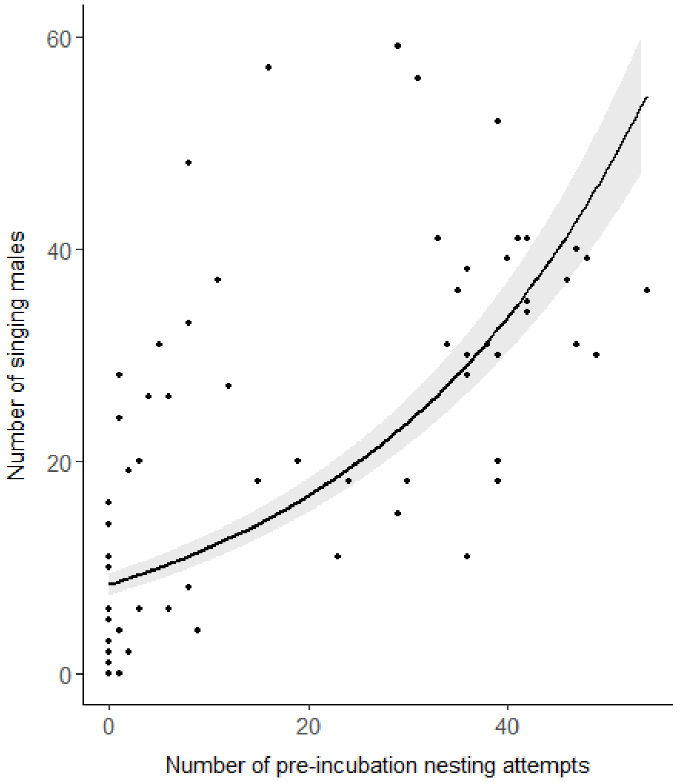

Figure 6.2. Model-averaged, predicted relationship between the number of singing males and the number of active nesting attempts at the pre-incubation stage (n = 79). The shaded area represents the 95 % confidence intervals. Raw data indicated by points.

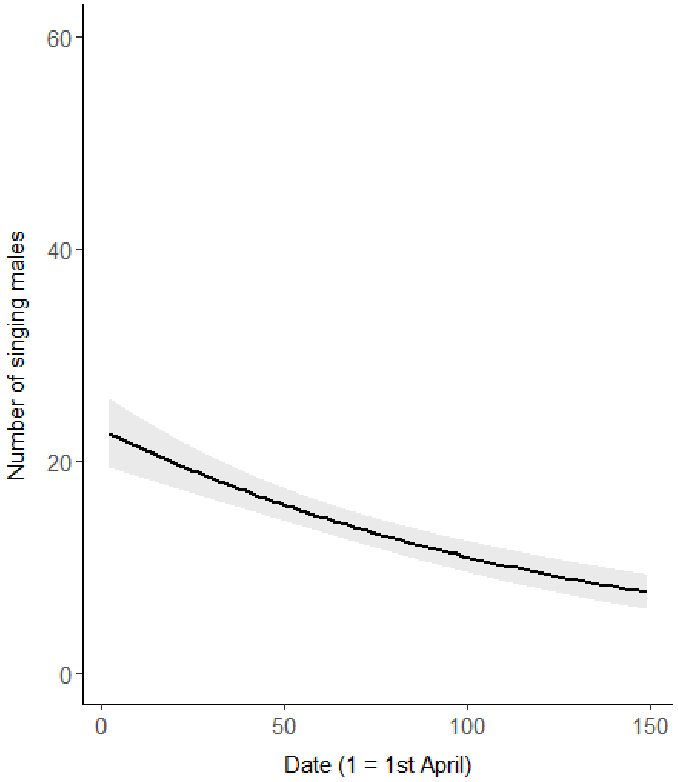

Figure 6.3. Model-averaged, predicted relationship between the number of singing males and date (n = 79). The shaded area represents the 95 % confidence intervals.

Chapter 7- General discussion

INTRODUCTION

Attempting to rear multiple broods within the same season, multi-brooding, is a strategy for maximising reproductive output which is undertaken by many avian species. Despite the fact that multi-brooding can result in increased production of fledglings (e.g. Ogden and Stutchbury 1996, Weggler 2006, Hoffmann *et al.* 2014), it is a strategy that is often employed by some, but not all individuals within multi-brooded populations, within in a given season. The factors influencing the individual-level decisions of making multiple nesting attempts within a season are therefore an interesting element of reproductive ecology. Furthermore, in multi-brooded species, there is evidence that the overall length of the breeding season has extended, for numerous species (Møller *et al.* 2010, Halupka and Halupka 2017). There is currently little understanding of the mechanisms driving breeding season extension. However, possible mechanisms include an increased propensity to multi-brood, increased replacements following failed attempts, or extended interval lengths between nesting attempts (Møller 2007, Halupka *et al.* 2008). There is therefore an even greater need to understand the mechanisms behind aspects of breeding season length such as the incidence of multi-brooding and the lengths of intervals between nesting attempts.

While the production of additional breeding attempts and extending the length of the breeding season may be expected to have a positive impact on individual- and population-level productivity (Halupka *et al.* 2008), previous studies suggest that the relative value of nesting attempts, in terms of the probability of producing recruits, declines throughout the breeding season in many multi-brooded species (Mallord *et al.* 2008, Brown *et al.* 2014, Hoffmann *et al.* 2014). This raises the question of the value of late nesting attempts and multi-brooding, especially in circumstances when an extending season means that the latest attempts are becoming relatively later than the start of the season. Indeed, previous work has illustrated a long term decline in both the profitability and incidence of late nesting attempts in one multi-brooded study system (Husby *et al.* 2009). Furthermore, life history theory predicts that there should be costs to reproduction (Stearns 1976, Reznick 1985) so there may be greater costs to individuals investing more effort into reproduction by extending the breeding season with an additional late nesting attempt. As population level changes in breeding season length are driven by nesting decisions at the individual level, understanding the relative value and costs of late nesting attempts for individuals is fundamental to understanding the end timing of the breeding season of populations.

Using a reed warbler, *Acrocephalus scirpaceus*, study population, this study aimed to investigate the causes and consequences of multi-brooding (Chapters 3, 4 & 5). Whether parental investment, in terms of the provisioning of nestlings, varied seasonally and between each sex was also investigated (Chapter 5). Finally, the potential for censuses of singing birds as a possible future method for monitoring breeding season length was assessed (Chapter 6), as there is currently no reliable method for monitoring season length of multi-brooded species at large spatial scales i.e. outside of intensive nest monitoring projects.

Reed warblers are an ideal study species for the study of multi-brooding and breeding season length for several reasons. There is considerable evidence that, like many species, the timing of the beginning of the breeding season has advanced substantially in recent decades (Crick and Sparks 1999, Calvert 2005, Schaefer *et al.* 2006, Halupka *et al.* 2008, Woodward *et al.* 2018) and evidence from one intensive population study that the length of the breeding season has extended (Halupka *et al.* 2008). Moreover, for an open nesting species, it is relatively easy to find and monitor the nests of reed warblers, making them suitable for intensive studies aiming to find all nesting attempts within a population. Finally, in the UK, increased reproductive output has been hypothesised as a potential mechanism driving population increase and/or a change in distribution range (Woodward *et al.* 2018), which could be due to an increased propensity to multi-brood.

SUMMARY OF RESULTS

Chapter 3 assessed the potential factors influencing the probability of double brooding as well as the length of the interval between first and second broods. The timing (first egg date) of first broods was the most important factor: pairs with earlier first broods were more likely to attempt second broods. Food availability had a positive influence on the likelihood of double brooding. There was also evidence of an interaction between the timing of first broods and the influence of food availability: the likelihood of double brooding, if the first brood was very early, was high regardless of food availability. In addition, rainfall following the fledging of the first brood was found to be negatively associated with the probability of double brooding. The length of the interval between first and second broods was found to be positively influenced by the number of fledglings produced by first broods. Increased food availability was predicted to shorten the interval, while increased rainfall and warmer temperatures following the first brood were both positively associated with interval length. Overall this chapter highlighted the importance of food resources to multi-brooding, with greater food resources increasing the probability of double brooding and reducing the interval between broods.

The potential value of nesting attempts throughout the breeding season and the costs of double brooding and a later end to nesting were assessed in Chapter 4. The probability of nesting attempts being successful (fledging one or more nestlings) was found to increase seasonally, while the number of fledglings produced by successful nests declined seasonally. The likelihood of successful nests producing a recruit (on the study site) declined as the season progressed. Whether nests were first broods, replacement first broods or second broods seemed unimportant to the success, number of fledglings and recruitment probability of nests.

There was no evidence for any costs of double brooding or a later end to nesting for either sex. The number of broods attempted and the end date of the final nesting attempt of individuals appeared to have negligible effects on both survival to the following season and, for individuals that did survive to the next season, breeding timing in this subsequent breeding season. The overall findings were that earlier nests were more valuable in terms of recruitment probability and that despite the apparent low value of late nesting attempts, the costs associated with making late nesting attempts also appeared to be low.

Chapter 5 assessed seasonal variation in parental investment in the provisioning of nestlings and the potential for male contribution to influence the probability of double brooding. Parental investment in provisioning was similar between females and males and did not change seasonally. There was no evidence that male contribution to provisioning had an important influence on the probability of double brooding. Furthermore, there was little seasonal variation in provisioning rates in both females and males.

Chapter 6 evaluated whether censuses of singing reed warblers could be used as a method for the monitoring of breeding season length, by assessing the relationship between the number of active nests and the number of singing males throughout the breeding season. The number of singing birds was positively associated with the number of active nesting attempts at the pre-incubation period, representing those during the female fertile period. The predicted number of singing birds also declined seasonally, when controlling for the number of pre-incubation nests.

CONCLUSIONS

The earliest reed warbler first egg dates were in late April in this study, contrasting with historical reports in the UK stating that reed warbler egg laying begins anywhere from mid-May to early June (Beddall Smith 1919, Brown and Davies 1949, Lack 1963, Bibby 1978, Bibby and Thomas 1985), with mid-May laying being referred to as ‘exceptionally early’ just over one hundred years ago (Hartert 1915, Boyd 1916). Furthermore, the current study found the first evidence of triple brooding ever recorded in the species (Batey and Leech 2018/ Appendix 1). These observations are a useful illustration of the fact that the reed warbler breeding season has extended. However, aside from such novel observations, in the study of breeding season length, it is clearly more important to understand the relative value of nesting attempts throughout the breeding season as well as the determinants of double brooding, which may potentially drive season length extension.

I found evidence for effects of food availability (positively correlated) on the probability of double brooding i.e. controlling for the effect of timing, with greater food availability increasing the likelihood of double brooding (Chapter 3). This result is important in the context of changing environmental conditions during bird breeding seasons resulting from climate change because invertebrate phenology is related to temperature, advancing in warmer temperatures (e.g. Visser *et al.* 2006, Vafidis 2014). Furthermore, in wetland habitats, invertebrate availability may not just advance under warmer conditions but may also remain at higher levels for a long period (Vafidis 2014). Greater invertebrate availability in warmer conditions and the likelihood of double brooding being increased when invertebrate prey is more abundant, gives support to the idea that an increased propensity to double brood (or re-nest after failed attempts) could be the mechanism driving breeding season extension. Moreover, an increasing propensity to double brood could also represent the mechanism driving the observed population increase and range expansion in reed warblers in the UK in the last 25 years (Woodward *et al.* 2018).

While my suggestion that an increased propensity to double brood, as a result of increased food availability, may be the driver of breeding season extension is based on research in wetland habitats, it is unclear to what extent the same relationships may occur in other habitats. Certainly, in woodland habitats, invertebrate availability to insectivorous species shows fundamentally different seasonal patterns, where there is a very distinct peak in herbivorous insect abundance (Both *et al.* 2009). Indeed, in woodland study systems there is some evidence that breeding productivity has declined in some single-brooded study species, as breeding timing has advanced to a lesser extent than invertebrate availability (e.g. Visser *et al.* 1998, Visser *et al.* 2006). The phenology of primary consumers (e.g. herbivorous insects) are significantly more sensitive to climate than secondary consumers (e.g. insectivorous birds; Thackeray *et al.* 2016), which is clearly a greater problem for single-brooded species, which are under selection pressure to time a single reproductive attempt with optimal conditions, than multi-brooded species. However, even a study of a multi-brooded population of great tits, *Parus major* (in a woodland habitat) has reported that as timing of first broods have become out of synchrony with optimal food resources, the incidence of double brooding has declined (Husby *et al.* 2009). It seems that there may be fundamental differences in the links between weather, invertebrate food availability and bird breeding is different habitats and clearly more studies linking these three elements in both wetland and woodland habitats, as well as other habitats would be illuminating.

Finding that the interval length between first and second broods reduced with greater food availability and also that there was not a strong effect of timing of breeding on interval lengths are interesting results. The finding that the timing of first broods did not have a strong effect on interval length, contrasts with a study of barn swallows, *Hirundo rustica*, where later breeding resulted in shorter intervals and consequently reduced adult fitness (Møller 2007) but conforms with a study of great tits (Verboven and Verhulst 1996). A better understanding of the period of post-fledging care in reed warblers is required before conclusions can be drawn about the differences between these studies. Given that greater food availability is expected under a warmer climate (Vafidis 2014), longer intervals between broods as a mechanism for breeding season extension is not supported by the results of this study. Instead, a greater propensity to multi-brood, with shorter intervals between broods is supported as a mechanism of breeding season extension (as discussed above).

Finding evidence for weather effects on both double brooding and interval length between broods in reed warblers are also important findings. Previous studies of multi-brooding have found that spring weather influences the timing of breeding at the start of the season (Pearce-Higgins and Green 2014). It can then be inferred, that as a result of timing of breeding influencing the likelihood of double brooding, that spring weather could ultimately influence the extent of double brooding. However, in this study I have reported direct effects of weather after accounting for the effects of timing of breeding as well as food availability, in both the analyses of double brooding probability and interval length. Interpretation of these weather effects is difficult, but effects could act by influencing either adult condition, fledgling survival or the level of care required by fledglings. For example, the predicted effect of increased rainfall reducing the probability of double brooding, could be driven by: reduced adult condition directly as a result of greater thermoregulatory costs, increased parental care demands for nestlings if food items are harder to catch, or a positive association between rainfall and fledgling survival. The effect of increased rainfall extending interval length could act via any of the same mechanisms, as well as delaying the start of nest building in wet conditions, or making nest building slower. The positive association between temperature and interval length may be a result of temperature positively influencing fledgling survival and therefore parental care requirements. Finally any of the effects of weather reported above could be driven by differential effects on males and females. Hypothetically, if warmer temperatures influenced the time allocated to an activity other than post-fledging care, this could influence the care level required by the other partner and thereby the interval length. Clearly further work is required on the period post fledging first broods, to address some of these speculations. Finally, the apparent weather effects may also be a result of weather variables capturing some further variation in food availability which is not captured by the measures of food availability used in analyses. Further work may seek to understand how weather conditions influence fledgling survival, adult condition and adult time allocation to foraging, the provisioning of fledglings and nest building following the fledging of a first brood. More generally, these results highlight that weather may have effects outside of influencing food availability to insectivorous species.

I found that the most important predictor of double brooding in reed warblers, was the timing of the first brood, which accords with the majority of previous studies of the determinants of double brooding in other multi-brooded species (e.g. Geupel and DeSante 1990, Ogden and Stutchbury 1996, Verboven and Verhulst 1996, Brinkhof *et al.* 2002, Weggler 2006, Bulluck *et al.* 2013, O'Brien and Dawson 2013, Townsend *et al.* 2013, Carro *et al.* 2014, Hoffmann *et al.* 2014, Zając *et al.* 2015, Béziers and Roulin 2016, Jackson and Cresswell 2017). There are very few exceptions, where studies have found timing not to be the most important factor influencing double brooding, but they have been in study populations in which the timing of first breeding attempts are highly synchronous (Cornell and Williams 2016, Nomi *et al.* 2018). This is not the case in reed warblers where there is considerable variance in the timing of successful first broods, not least because there is variation in arrival timing as well as there being high failure rates early in the season (Schulze-Hagen *et al.* 1996). Timing of breeding should still be the most important factor influencing multi-brooding in the vast majority of species, as the examples listed above cover a variety of species with diverse ecological traits; for example the range of study species are not limited to passerines or migratory species.

Despite the fact that reed warbler nests were more likely to produce fledglings as the season progressed, the probability of successful nests producing a recruit declined seasonally (Chapter 4). This is in agreement with many other studies of multi-brooded species, which have also reported a similar seasonal decline in recruitment potential (Hochachka 1990, Barba *et al.* 1995, Smith and Marquiss 1995, Van Noordwijk *et al.* 1995, Verboven and Visser 1998, Cowley 2001, Mallord *et al.* 2008, Brown *et al.* 2014, Hoffmann *et al.* 2014) and suggests that individuals are under selection pressure to nest early. This raises the question of why individuals make late nesting attempts at all.

The fact that there was no evidence of any costs of double brooding or having late nesting attempts, suggests that despite the apparently low value of late nests, they are also equivalently low cost. Therefore late nesting attempts may not be selected against despite the fact that they appear to contribute little to recruitment. Despite the fact that costs of reproduction are predicted by life history theory (Stearns 1976, Reznick 1985) numerous other studies have also found no evidence for any costs of individuals extending the breeding season by making late nesting attempts (Geupel and DeSante

1990, Smith and Marquiss 1995, Weggler 2006, Carro *et al.* 2014, Cornell and Williams 2016). The lack of observed costs of late nesting is likely due to the fact that only individuals that can 'afford' the costs of making an additional attempt, do so. Therefore the costs of late nesting may be masked either by territory or individual quality, that is to say that only high quality individuals and/or territories may produce late nesting attempts. Given the lack of costs of late nesting in this study and, therefore, no suggestion of selection pressure against late nesting, these circumstances may explain why the end timing of the season appears to have remained relatively less variable than the start in some multi-brooded species including reed warblers (Halupka *et al.* 2008). The start of the season is certainly getting earlier not only in reed warblers (Crick and Sparks 1999, Schaefer *et al.* 2006, Halupka *et al.* 2008, Woodward *et al.* 2018) but many species (Pearce-Higgins and Green 2014) and the way in which breeding season length has extended in multi-brooded species has generally been reported to be the result of an advance in the start of the season while the end timing of the season remains relatively unchanged (Halupka *et al.* 2008, Møller *et al.* 2010). A relative lack of costs of nesting attempts at the end of the season may go some way to explaining why the end timing of the season has not more often been reported to advance in line with the start of the breeding season in other studies. However, costs of late nesting attempts have been found in other studies (e.g. Nilsson and Svensson 1996, Brown *et al.* 2014) and such costs may be mediated by quicker and poorer quality post breeding moult (Nilsson and Svensson 1996). Future studies could pay particular attention to the potential for a cost in feather quality and/or speed of moult, especially in long-distance migrants (including investigating this further in reed warblers) where poorer quality feathers could be a severe handicap.

I failed to find evidence of relative paternal investment influencing the probability of double brooding in reed warblers (Chapter 5). This analysis followed a recent study of Japanese tits, *Parus minor*, which reported that the proportion of first brood nestling provisioning undertaken by the male was a strong predictor of double brooding (Nomi *et al.* 2018). Only one other study, to my knowledge, has reported on whether male contribution to provisioning influenced the probability of double brooding. This was a male removal experiment on dark eyed juncos, *Junco hyemalis*, where single females suffered no reduction in the likelihood of attempting a second brood, although they did suffer reduced body condition as a result of raising first broods alone, illustrating another cost of reduced male care (Wolf *et al.* 1991). Given the scarcity of studies, conclusions about differences in results are probably best avoided. But both previous studies highlight the potential for costs of reduced paternal investment. There have been many other studies of double brooding which have not considered variance in male investment as a potential predictor, but this should be borne in mind for future projects.

The finding that the number of singing reed warblers was a good predictor of the number of nesting attempts (Chapter 6), is an encouraging indication that systematic censuses of singing birds could be used as a future method of estimating breeding season length. Currently, breeding season length has been mainly studied by intensive nest monitoring projects, with the aim of finding every nesting attempt of a single study site/population (e.g. Halupka *et al.* 2008, Jankowiak *et al.* 2014). While this is clearly the optimum method of monitoring season length and is essential for incorporating the detailed study of individuals season long breeding attempts (such as in the present study), it could never be applied to large spatial scales. Moreover, national schemes which collate data from nesting attempts observed by volunteers over large spatial scales, although having been used for analyses of season length (Møller *et al.* 2010) may be biased against the end of the breeding season for some multi-brooded species (Crick *et al.* 2003). The fundamental benefit of a singer census approach to monitoring season length is that it would be considerably less effort than intensive nest monitoring. On the other hand, the disadvantage of such a method would be that it could not distinguish between season extension as a result of the replacement of increased failed nesting attempts (a neutral or negative impact on population productivity) versus an increased extent of multi-brooding (a positive impact on population productivity). Nevertheless, it would certainly be desirable to monitor breeding season length of a wide range of species at large spatial scales, given that there is increasing evidence that season duration has changed and there is the potential for this to influence population productivity. The results of this study suggest that the potential for singing bird censuses to be applied in this way deserves further attention and the next step should be for the relationship between singing and breeding status to be investigated across a large number of sites and species, possibly where intensive nest monitoring already takes place.

FUTURE RESEARCH PRIORITIES

Following this project, several gaps in knowledge have emerged as being most in need of further research. These priorities are suggestions for research relevant to understanding breeding season length and multi-brooding generally and are not necessarily specific to the study system used in this project.

As a priority, food supplementation experiments should be undertaken in order to confirm whether greater food availability increases the probability of double brooding, in reed warblers as well as other multi-brooded species. There is evidence from two previous experimental studies that greater food resources can improve the likelihood of double brooding (Nagy and Holmes 2005a, O'Brien and Dawson 2013). These studies were on species in woodland and grassland habitats respectively, so similar experiments in wetland study systems such as the subject of this project would be valuable.

The period of post-fledging offspring dependence is to some extent a neglected aspect of breeding ecology, in terms of research effort, as a result of being difficult to quantify. It has been stressed by some authors that nest success and whole season breeding success, defined as the number of offspring raised to independence across the breeding season are not necessarily related in any particular way, which highlights the importance of the period of post-fledging dependence for measuring breeding success (Streby and Andersen 2011, Streby *et al.* 2014). Several results in the current study have also highlighted the need to understand this part of the breeding cycle. For example, variance in interval lengths between broods is likely to be influenced by post-fledging mortality, while the division of labour between males and females during this period could also influence both the probability of making an additional nesting attempt and the interval between attempts. Even more fundamentally, it would be interesting to quantify the extent to which complete post-fledging mortality contributes to double brooding. Some nests defined as second broods could actually be better considered replacement nesting attempts; a first brood failing a day before fledging versus a day after are the same in terms of their contribution to parents' fitness. It is clearly far more difficult to follow the fate of offspring and to monitor parents' behaviour post fledging than it is to monitor both while offspring are still in the nest. Efforts to study this period of the breeding cycle may require either intensive daily observation of colour-ringed family groups or radio tracking of either the adults or fledglings.

Another priority for research should be to investigate the potential mechanisms driving the seasonal decline in recruitment probability of successful nesting attempts. This ties in to the study of the post-fledging period, as seasonally declining recruitment potential could be driven by a seasonal decline in survival during the period of post-fledging dependence. Alternatively, there may be a seasonal decline in survival of juveniles after becoming independent, during migration or over-winter. Assessing differences in survival and behaviour post-fledging and while remaining on the study site could at least assess several of these possibilities. Furthermore, the difficulty of non-local recruitment still requires greater attention. Currently, most studies which find recruitment potential to decline as the season progressed, including this study, are reliant on the measure of local (within the study site) recruitment. A study of great tit recruitment reported that there was evidence of non-local recruitment being biased towards later fledglings (in males only), but even after accounting for this, overall recruitment potential of fledglings still declined seasonally (Verboven and Visser 1998). This illustrates that non-local recruitment may not confound the seasonal effect on recruitment probability, but it is still worthy of attention in other study systems. The problem with addressing non-local recruitment is the intensity of fieldwork required and the difficulty in deciding how far from the natal site to search. Perhaps non-local recruitment should be studied via the colour-ringing of conspicuous species which are likely to elicit re-sighting reports to national ringing schemes.

My final suggestion for future research is the study of post-breeding adult moult. The duration of moult has been identified as a mechanism by which the potential costs of extending the breeding season can operate (Nilsson and Svensson 1996). These authors found, in blue tits, *Cyanistes caeruleus*, that when post-breeding moult was delayed by a later end to breeding, it was completed faster which resulted in poorer quality feathers, which in turn resulted in both reduced thermoregulatory and flight performance. More studies should consider the timing and duration of post-breeding moult and it would be especially interesting to study this in study species in which studies have failed to find evidence for costs of extending the breeding season such as the reed warbler population studied here. Moreover, in long-distance migrants, the timing or duration of autumn migration may represent another potential mechanism by which costs of a later end to breeding may operate. The timing of migration in relation to the end of breeding would therefore be an interesting area to investigate and would likely require geolocation tracking.

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Appendix 1- Evidence of triple brooding in the Reed Warbler

(Citation: Batey, C., and D. I. Leech. 2018. Evidence of triple brooding in the reed warbler. British Birds 111:481-482.)

Reed Warblers, *Acrocephalus scirpaceus*, are known to be double brooded throughout Europe. In fact, the first reports of the species being double brooded were published in British Birds almost 100 years ago (Beddall Smith 1919; Pettitt 1919). Here we document the first evidence of triple brooding, in the Reed Warbler, a behaviour previously unrecorded in the species.

At a 46-ha wetland site consisting of scrub- and reed-fringed lakes and pools near Cranwich, Norfolk, Reed Warbler nests are found throughout the season via an intensive programme of nest finding and monitored to their conclusion. Adults are colour-ringed and, since 2014, all nests have been videoed to determine the identity of colour-ringed parents (during incubation or provisioning). During the four breeding seasons 2014–17, more than 100 pairs nested at the site and the proportion of pairs that were double brooded in this population ranged between 12% and 48% (Batey *et al.* in prep). In this time, one definite case of triple brooding, involving colour ringed parents being identified in all nesting attempts was recorded in 2015. Additionally, in the same year, another almost certain case of triple brooding was recorded, involving a left leg metal ringed female (one of only two such birds seen in the season and therefore almost a unique identity), which was observed at all three broods within a territory and paired with a colour ringed male. The triple-brooded pair in which both parents were colour-ringed was observed to have four nesting attempts (i.e. the female laid four clutches) during the 2015 season. These attempts spanned a 110-day period, from the first egg date (FED) of the first attempt on 4th May to the eventual failure of the final attempt at the nestling stage on 22nd August.

The first attempt was the third-earliest FED at the site in 2015 and this nest was predated between 18th and 22nd May, during which time it was due to hatch; consequently, this nest could have been predated at the egg or nestling stage. The replacement nest had a FED of 27th May and fledged a single chick between 18th and 21st June. The second brood (third attempt) had a FED of 27th June and three chicks fledged between 21st and 23rd July. The third brood (fourth attempt) was the latest at the site in 2015 and was the only nest initiated in August that season (estimated FED of 4th August). This nest failed within two days of hatching, due to apparent chick starvation.

Both parents were identified via video recordings on attempts 1–3 while only one parent, the female, was identified on the fourth attempt, which failed before any further footage could confirm the male's identity. Although post-fledging mortality is not generally considered when defining multi-brooding, the short brood interval of 6–9 days between the first brood fledging and the second brood (third attempt) FED may have cast doubt over whether this first brood had truly been successful. However, the single chick that fledged from the first brood was recaptured a month later during a CES ringing session, therefore proving this was indeed true triple brooding. The other triple brooding pair had three nesting attempts, were successful in fledging all three broods (FEDs of 11th May, 17th June and 31st July respectively) and produced eleven fledglings in total, productivity which would not be possible with only two broods. As with the first case, the male was not confirmed via video recordings on the third brood.

These observations show that the length of the breeding season in the Reed Warbler is now long enough for triple brooding to occur, and it is especially notable that the first case described here also included an earlier first attempt that failed around the time of hatching. Furthermore, the final attempts in both cases, with FEDs at the end of July and early August, are not unusually late for the study site; during four of the seven breeding seasons in 2011–17, FEDs were recorded in early August. Although triple brooding is not expected to occur in more than a tiny proportion of pairs, it could well be a recent phenomenon in this species as the timing of the breeding season has advanced across Europe with climate warming (Schaefer *et al.* 2006; Halupka *et al.* 2008; Baillie *et al.* 2014), and there is some evidence that length of the breeding season has increased as a consequence (Halupka *et al.* 2008).

Appendix 2- Coefficient plots for models fitted in Chapter 3

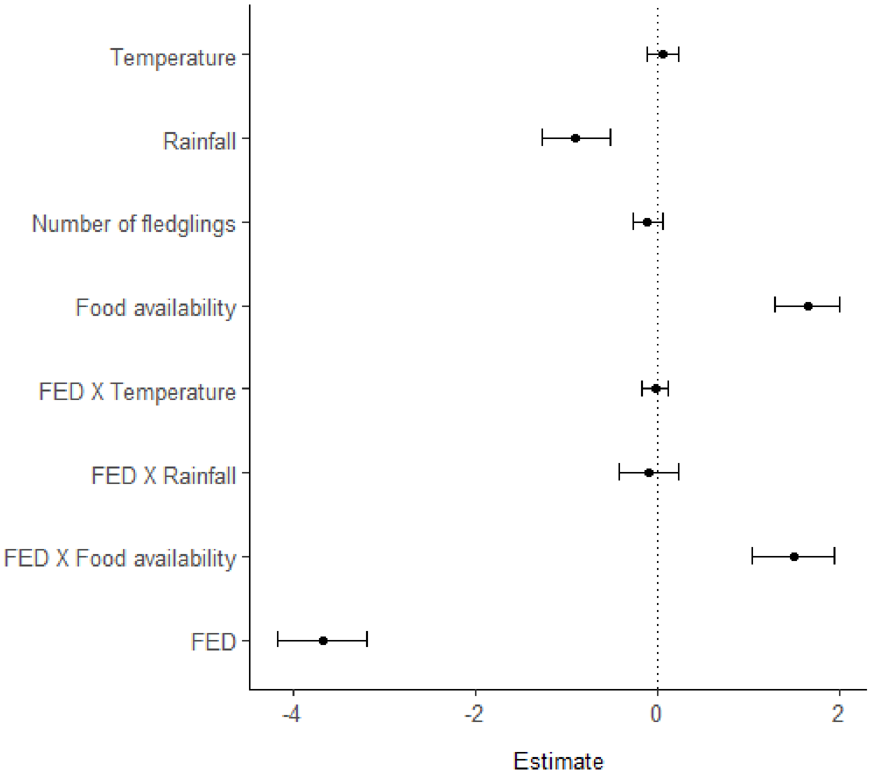

Figure A2.1. Parameter estimates and standard error from the average model of the probability of double brooding: Table 3.4 (average of models within 2 AIC units of the best-fitting).

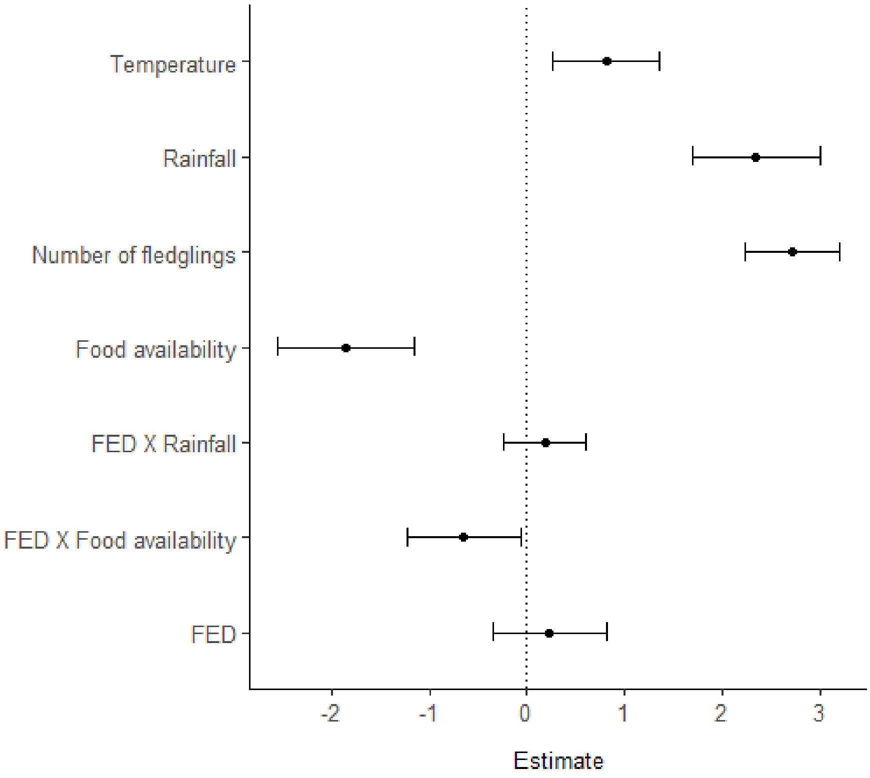

Figure A2.2. Parameter estimates and standard error from the average model of the interval length between 1st and 2nd broods: Table 3.5 (average of models within 2 AIC units of the best-fitting).

Appendix 3- Coefficient plots for models fitted in Chapter 4

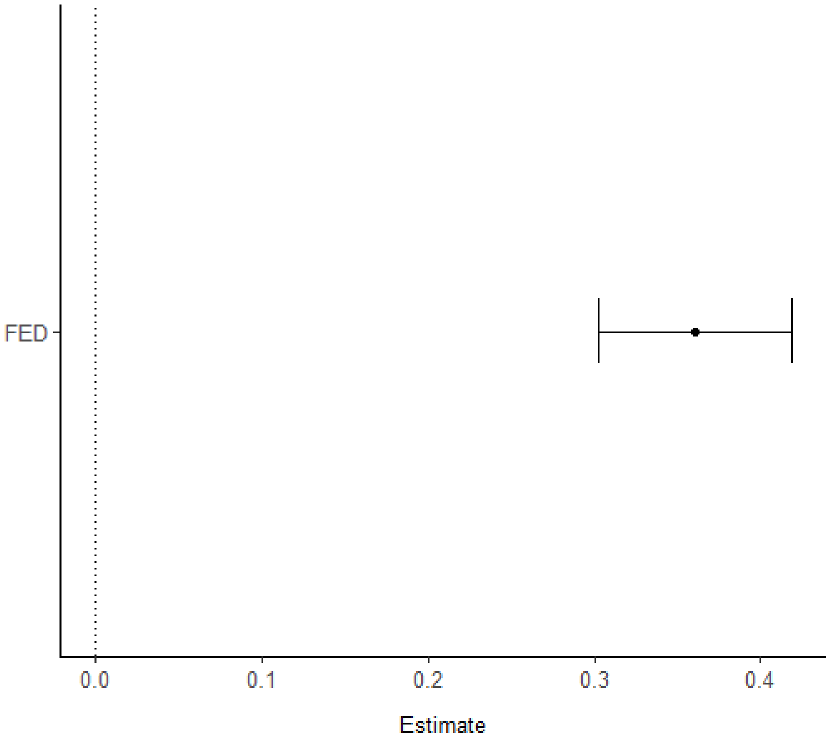

Figure A3.1. Parameter estimate and standard error from the model of the probability of nest success, 2012-17: Table 4.1.

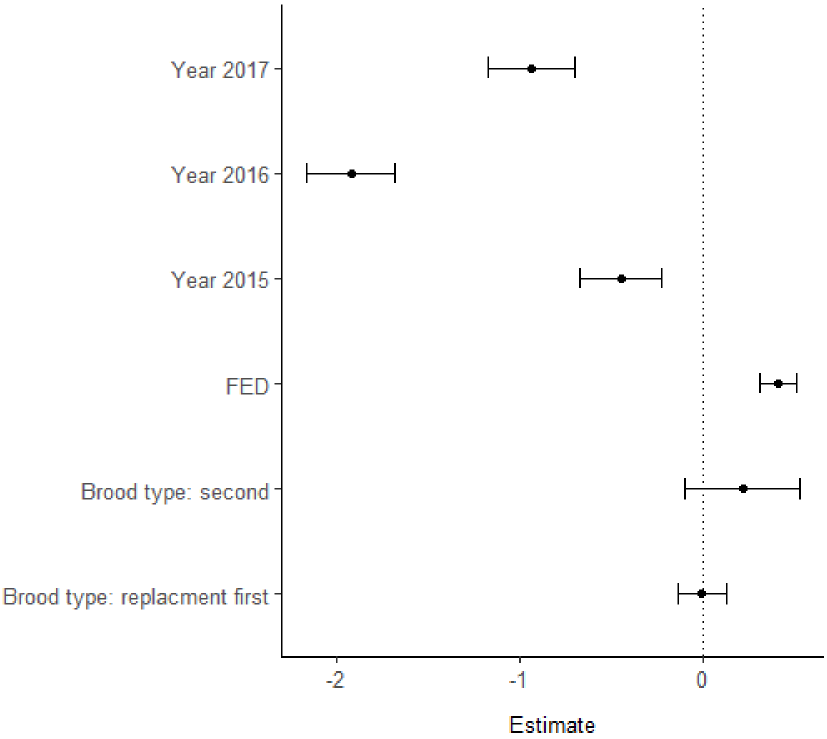

Figure A3.2. Parameter estimates and standard error from the average model of the probability of nest success, 2014-17: Table 4.2 *Brood Type* analysis (average of models within 2 AIC units of the best-fitting).

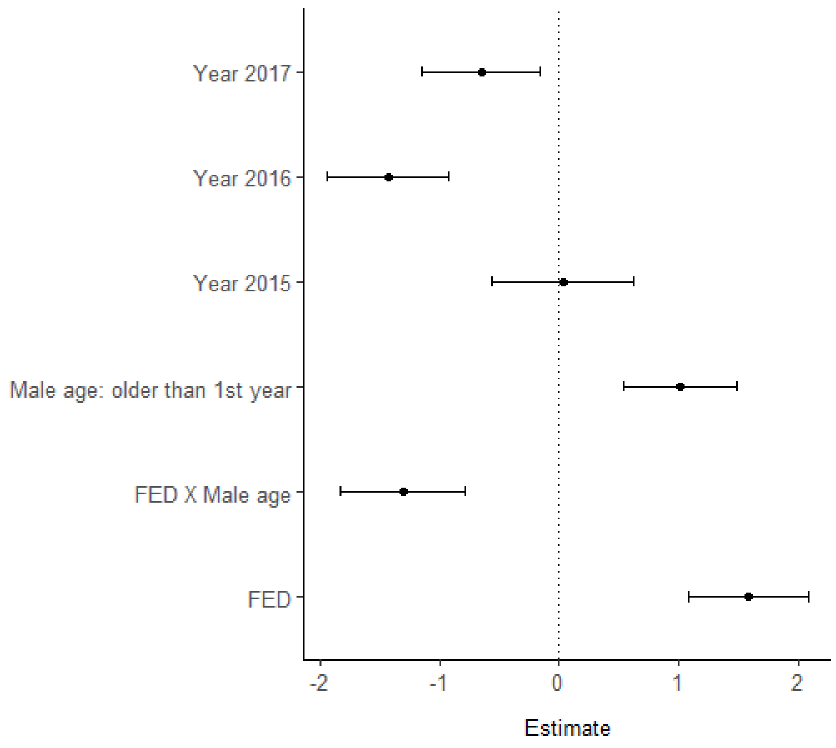

Figure A3.3. Parameter estimates and standard error from the average model of the probability of nest success, 2014-17: Table 4.2 *Male age* analysis (average of models within 2 AIC units of the best-fitting).

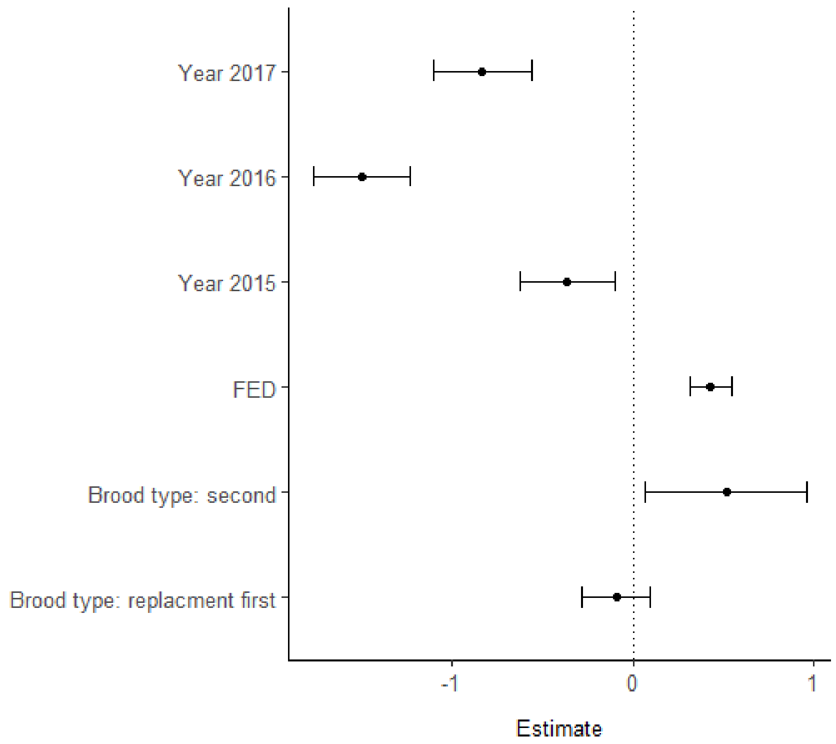

Figure A3.4. Parameter estimates and standard error from the average model of the probability of egg stage success (hatching): Table 4.3 *Brood type* analysis (average of models within 2 AIC units of the best-fitting).

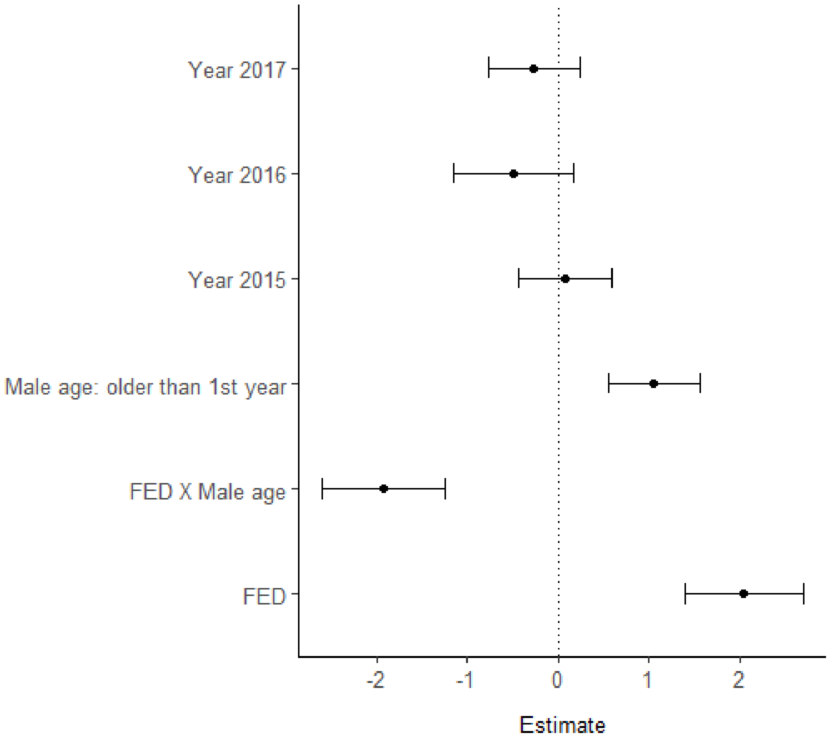

Figure A3.5. Parameter estimates and standard error from the average model of the probability of egg stage success (hatching): Table 4.3 *Male age* analysis (average of models within 2 AIC units of the best-fitting).

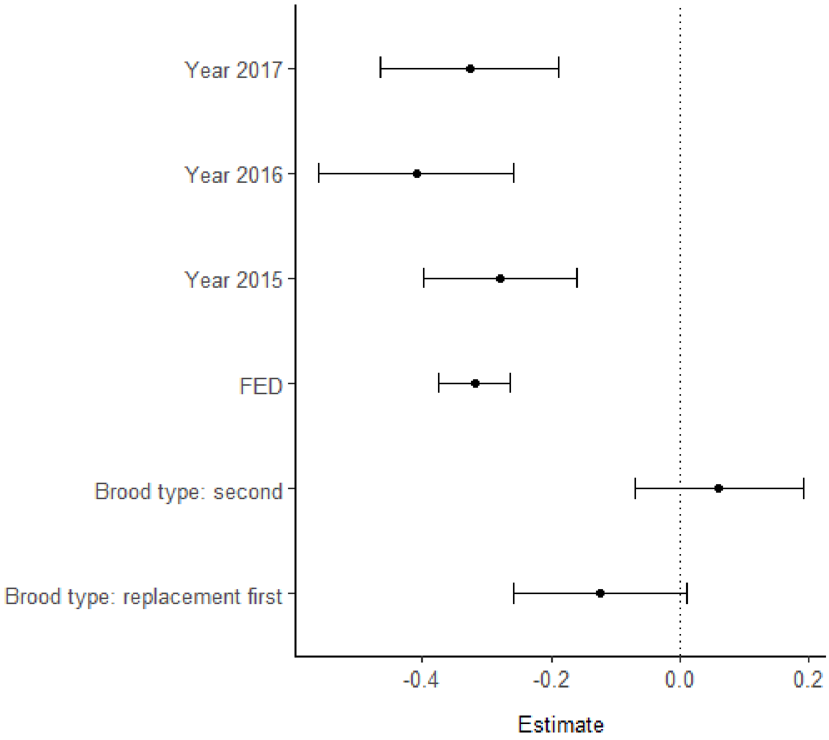

Figure A3.6. Parameter estimates and standard error from the average model of the number of fledglings produced by successful nests: Table 4.4 *Brood type* analysis (average of models within 2 AIC units of the best-fitting).

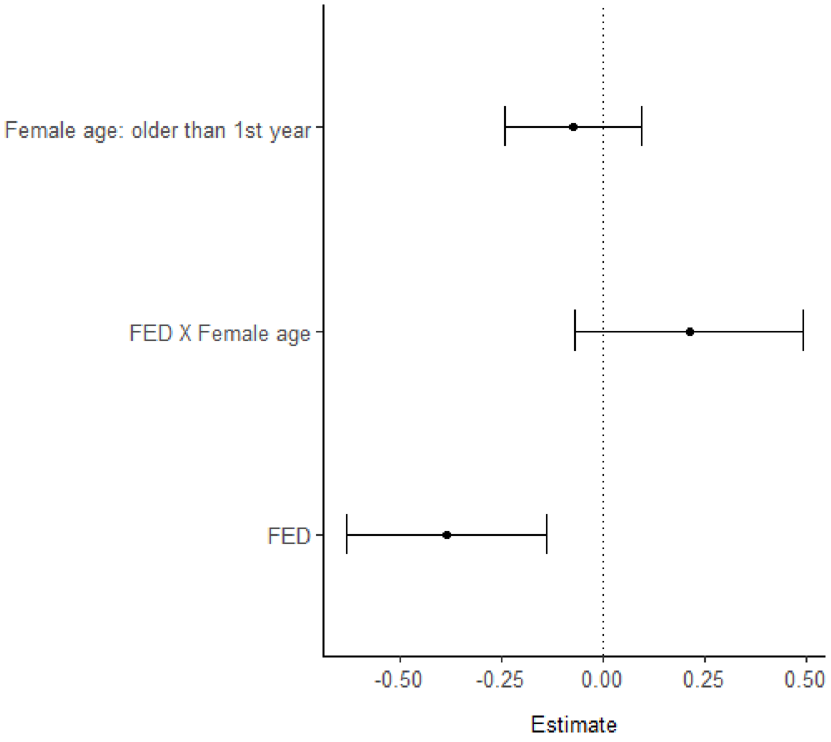

Figure A3.7. Parameter estimates and standard error from the average model of the number of fledglings produced by successful nests: Table 4.4 *Female age* analysis (average of models within 2 AIC units of the best-fitting).

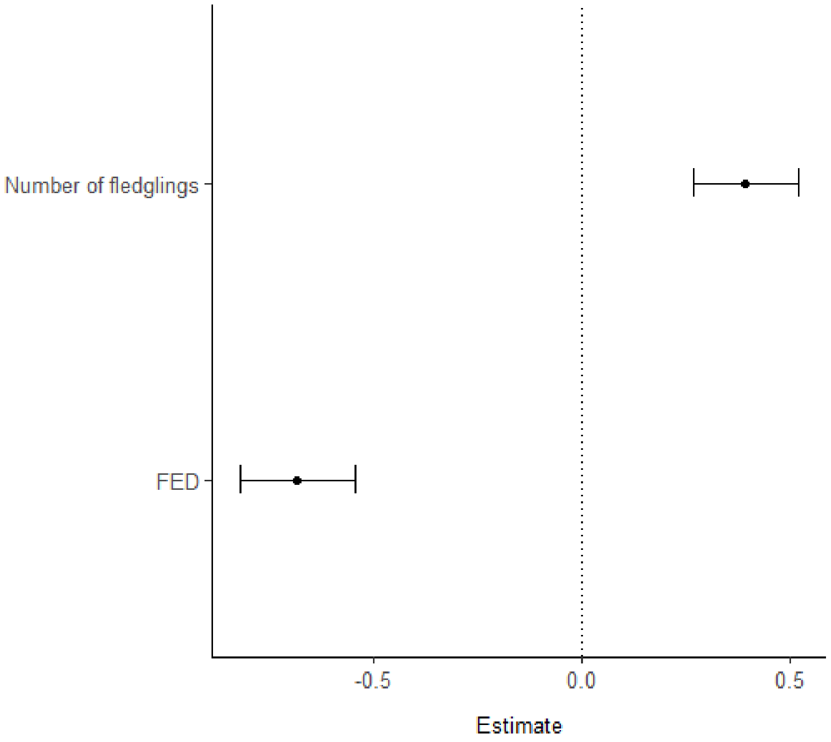

Figure A3.8. Parameter estimates and standard error from the best-fitting model of the probability of successful nests producing a recruit, 2012-16: Table 4.5.

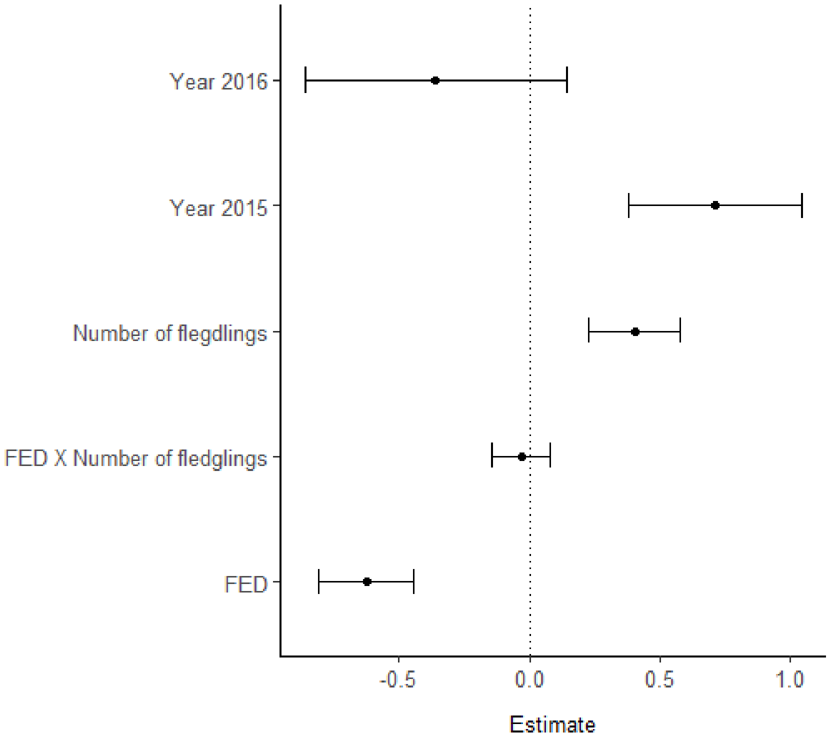

Figure A3.9. Parameter estimates and standard error from the average model of the probability of successful nests producing a recruit, 2014-16: Table 4.6 (average of models within 2 AIC units of the best-fitting).

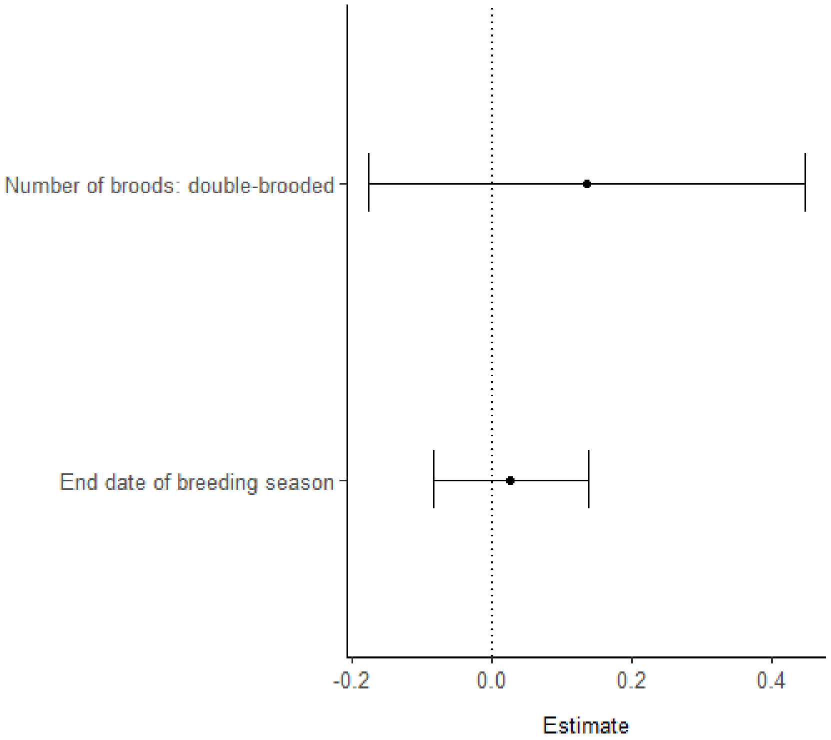

Figure A3.10. Parameter estimates and standard error from the average model of the probability of breeding adults being re-sighted in the following season: Table 4.7 *Females analysis* (average of models within 2 AIC units of the best-fitting).

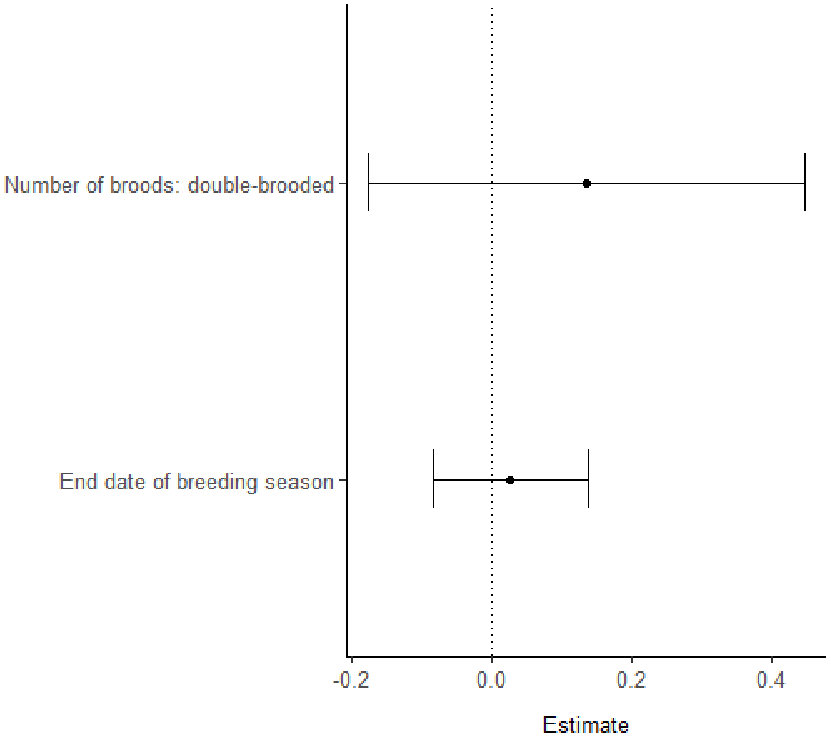

Figure A3.11. Parameter estimates and standard error from the average model of the probability of breeding adults being re-sighted in the following season: Table 4.7 *Males analysis* (average of models within 2 AIC units of the best-fitting).

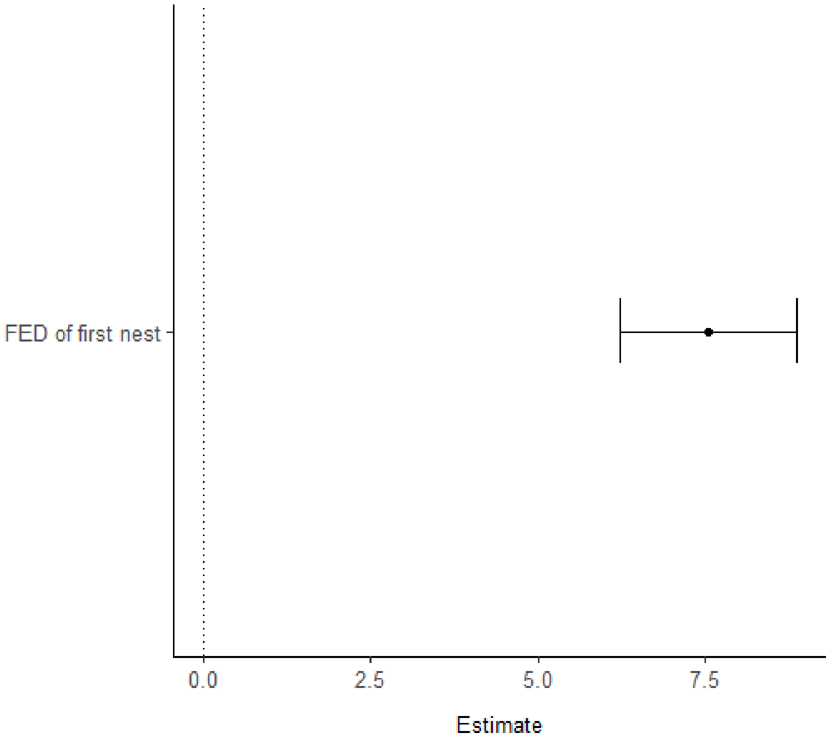

Figure A3.12. Parameter estimate and standard error from the best-fitting model of the timing of breeding in the following season: Table 4.9 *Females analysis*.

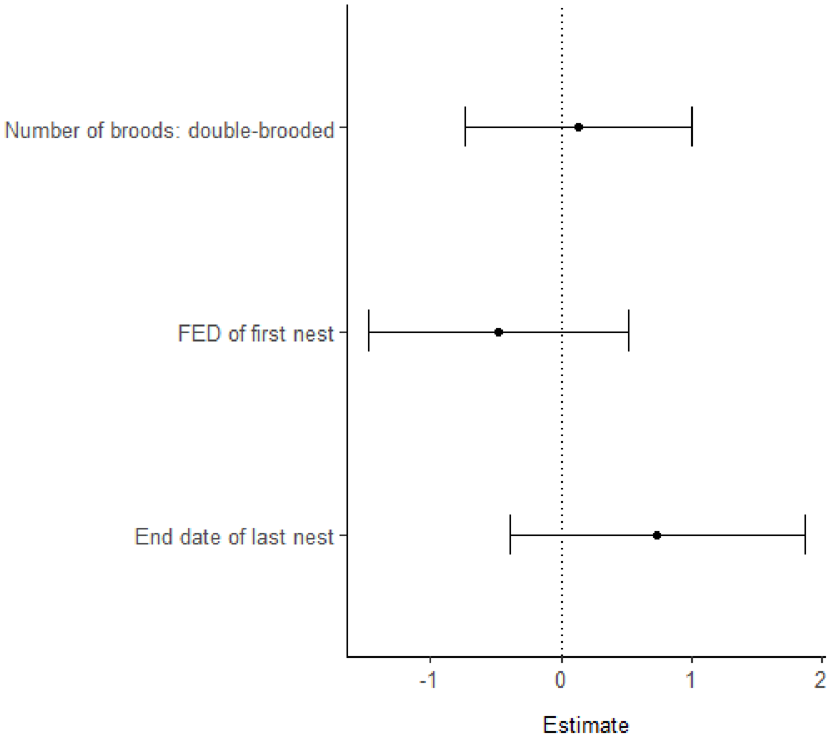

Figure A3.13. Parameter estimates and standard error from the average model of the timing of breeding in the following season: Table 4.9 *Males analysis* (average of models within 2 AIC units of the best-fitting).

Appendix 4- Prey items identified being provisioned to reed warbler nestlings

There were a total of 932 provisioning deliveries observed during provisioning videos (data and methods described in Chapter 5), and it was possible to identify the taxonomic group of prey, either for part or all of the delivery, in 198 cases. The identification of prey was clearly biased towards large prey items: 76.3 % of deliveries which were partly or entirely identified, were classified as 'large' (n = 198), compared to only 38.1 % (n = 734) of unidentified deliveries being classified as such. The proportion of identified prey items belonging to various taxonomic groups may not be an accurate reflection of their true contributions to nestling diet, but the data do indicate some frequent prey groups in the population e.g. damselflies, hoverflies and other Bracycera flies (Table A4.1).

Table A4.1. Prey items identified being provisioned to reed warbler nestlings during provisioning videos (data collected as part of Chapter 5 analyses; n = 198 deliveries with part or all of delivery identified). Observations of identified prey in deliveries represent presence in the delivery; individual deliveries often included multiple taxonomic groups and/or multiple individuals of the same prey type.

Prey taxonomic group	Number of individual deliveries identified within
Odonata:	
-Damselfly (adult)	58
-Damselfly (larvae)	32
Diptera:	
-Syrphidae	43
-Other Bracycera	28
-Tipulidae	1
Lepidoptera:	
-Larvae	13
-Moth (adult)	7
-Butterfly (adult):	
Pieris sp.	1
Maniola jurtina	1
Araneae	11
Hymenoptera	1
Trichoptera	4
Coleoptera:	
Donacia sp.	1
-Other beetle	2
Ephemeroptera	2
Neuroptera	1

Appendix 5- Coefficient plots for models fitted in Chapter 5

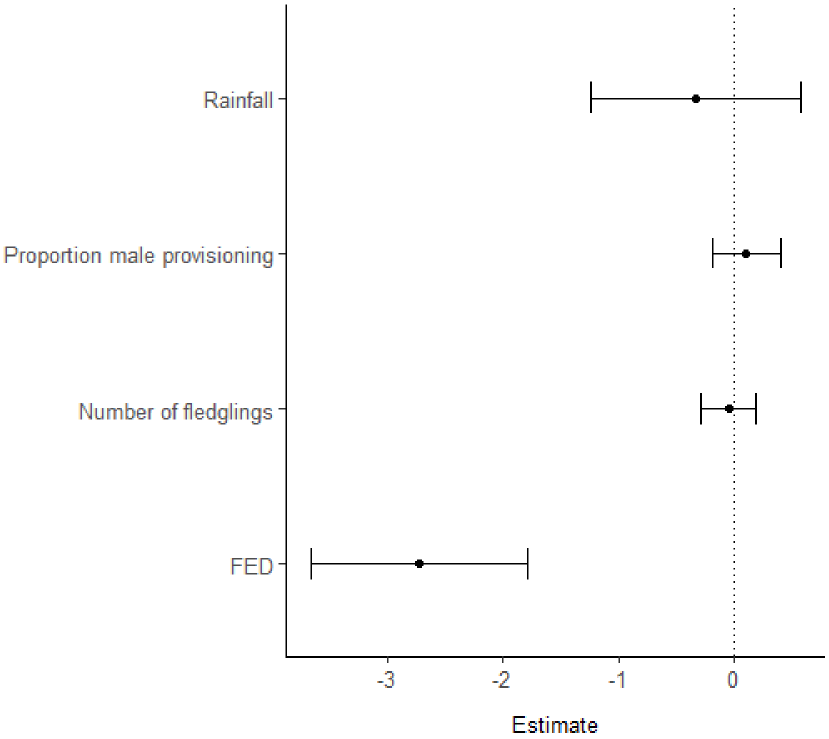

Figure A5.1. Parameter estimates and standard error from the average model of the probability of double brooding: Table 5.1 (average of models within 2 AIC units of the best-fitting).

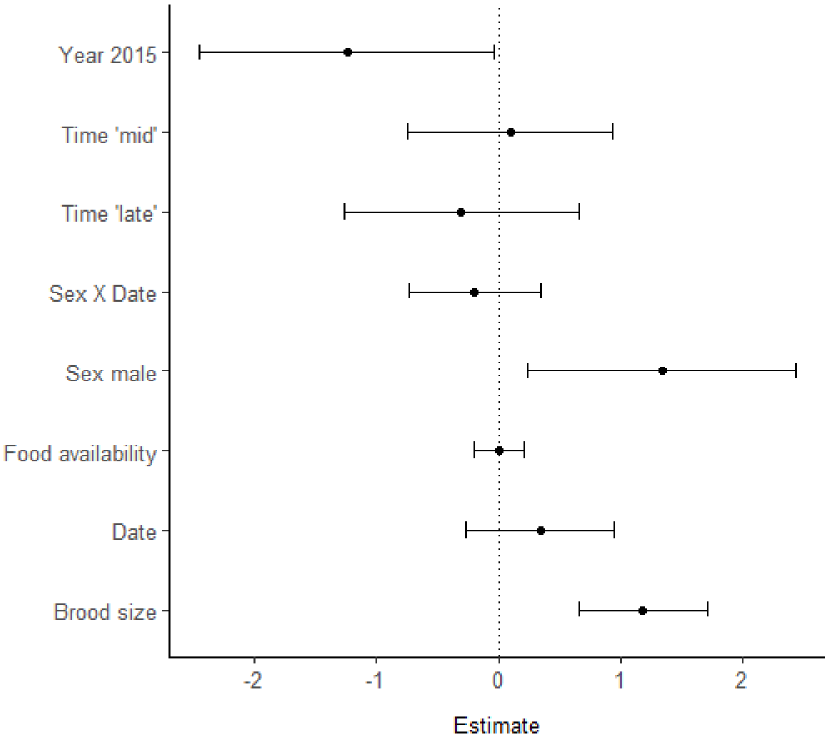

Figure A5.2. Parameter estimates and standard error from the average model of provisioning rates: Table 5.2 (average of models within 2 AIC units of the best-fitting).

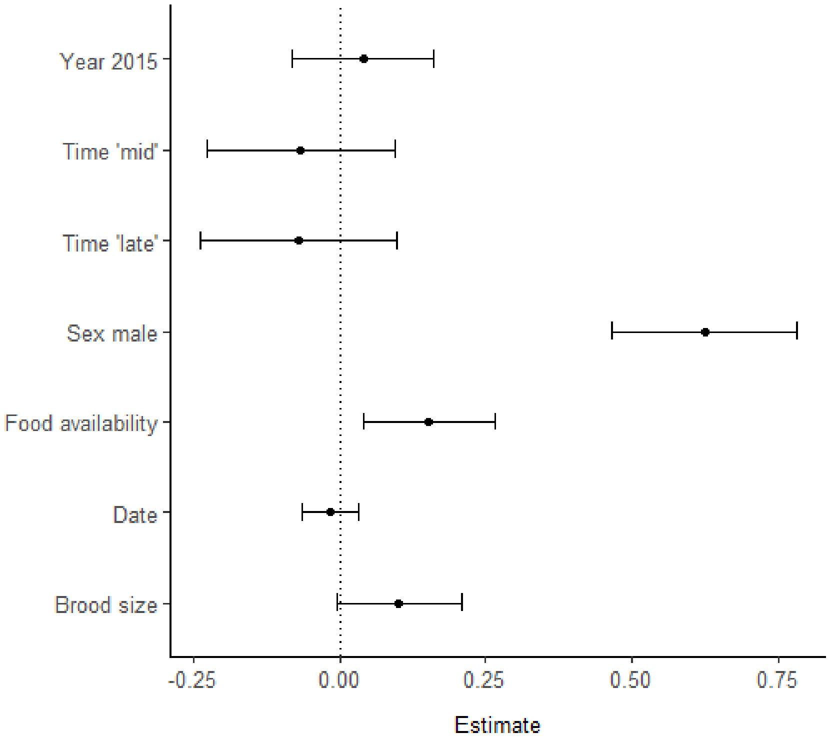

Figure A5.3. Parameter estimates and standard error from the average model of the proportion of deliveries of large bolus size: Table 5.3 (average of models within 2 AIC units of the best-fitting).

Appendix 6- Coefficient plots for models fitted in Chapter 6

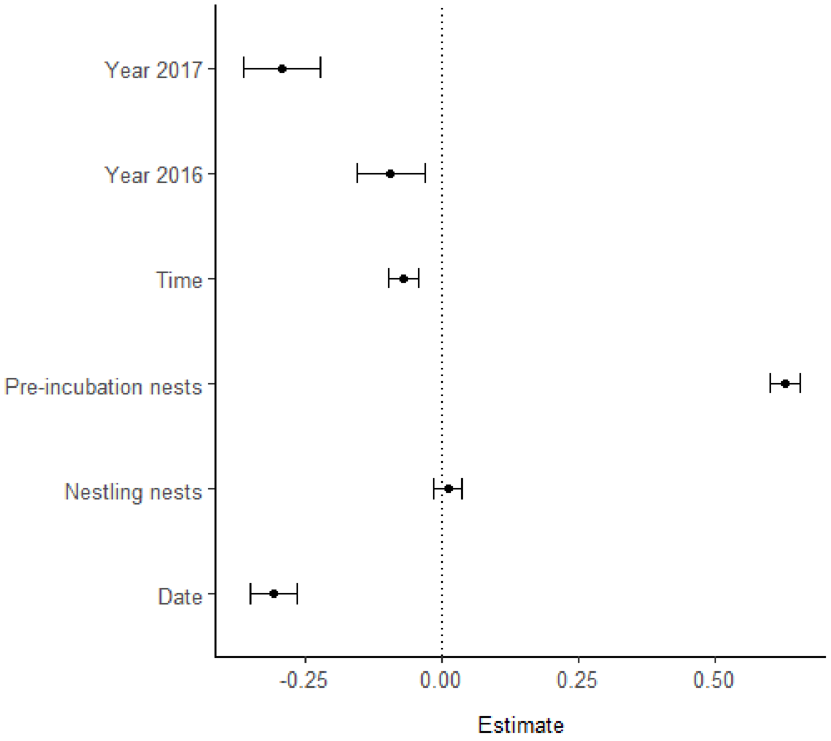

Figure A6.1. Parameter estimates and standard error from the average model of the number of singing males without interaction predictors: Table 6.1 (average of models within 2 AIC units of the best-fitting).

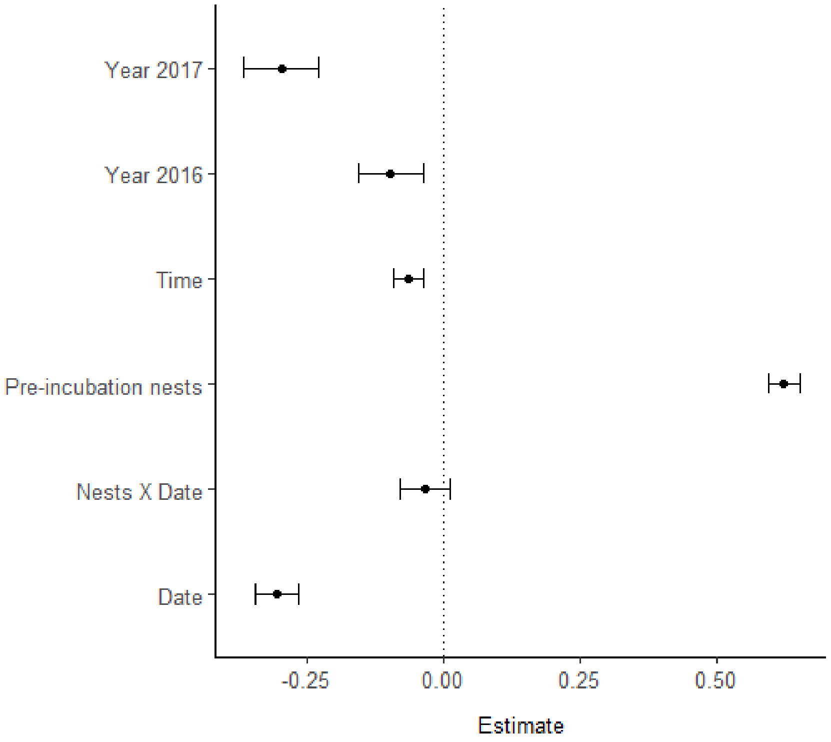

Figure A6.2. Parameter estimates and standard error from the average model of the number of singing males for analysis including interaction terms: Table 6.2 (average of models within 2 AIC units of the best-fitting).