FROM NEST BUILDING TO LIFE-HISTORY PATTERNS:

DOES FOOD SUPPLEMENTATION INFLUENCE

REPRODUCTIVE BEHAVIOUR OF BIRDS?

by

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ABSTRACT

Supplementary feeding wild birds is a widespread phenomenon. Recently, non-governmental organisations have recommended that the bird-feeding public should feed wild birds throughout the breeding season. Currently, such recommendations are not supported by a large body of research findings to suggest that food supplementation has benefits for breeding birds. To investigate this further I provided two commercially available wild bird foods (peanut cake and mealworms [*Tenebrio molitor*]) to Blue Tits (*Cyanistes caeruleus*) and Great Tits (*Parus major*) breeding in a woodland in Central England from 2007 to 2009. Supplementary feeding significantly advanced nest construction and decreased brood provisioning rates for both species. Supplemented Blue Tits significantly decreased daily incubation activity and increased both the proportion of extra-pair young and proportion of males per brood while supplemented Great Tits decreased incubation recess lengths. Analyses of data from the British Trust for Ornithology's Nest Record Scheme suggested that probable widespread supplementary feeding of both Blue and Great Tits in urban habitats from 1962 to 2008 influenced breeding parameters across the study period but measuring food availability across wide spatial scales remains problematic. I discuss the implications of my results within an urban garden bird feeding context and provide suggestions for future research.

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LIST OF ABBREVIATIONS

BTO: British Trust for Ornithology

C: Control dietary treatment

CHD: Chromo-helicase-DNA-binding

DIA: Daily incubation activity

DNA: Deoxyribonucleic Acid

EPC: Extra-pair copulation

EPP: Extra-pair paternity

EPY: Extra-pair young

NGO: Non-Governmental Organisations

NRS: Nest Record Scheme

PC: Peanut cake dietary treatment

PC+MW: Peanut cake + mealworms dietary treatment

PCR: Polymerase Chain Reaction

RSPB: Royal Society for the Protection of Birds

WPY: Within-pair young

Chapter One

GENERAL INTRODUCTION

1.1. WILD BIRD FEEDING

The anthropogenic provisioning of food to wild birds, often referred to as supplementary feeding, is a globally widespread phenomenon (reviewed in Tasker et al. 2000, Jones and Reynolds 2008). Supplementary feeding can be considered as the provision of food in addition to that naturally available and can occur either intentionally, where humans provide bird food in garden bird feeders (e.g. Jones and Reynolds 2008, Toms and Sterry 2008), or unintentionally through commercial activities such as discards from the fisheries industry (e.g. Tasker et al. 2000, Votier et al. 2004) and disposal of food waste at landfill sites (Greig et al. 1986, Belant et al. 1993). For much of this thesis which addresses the impacts of food supplementation the focus will be on the intentional feeding of wild birds.

1.1.1 History of wild bird feeding

Providing food to wild birds dates back to the 7th century AD when St Cuthbert established the first protected area for birds in the Farne Islands, Northumbria, where he inevitably provided supplementary food, particularly to Common Eiders (*Somateria mollissima*). Although there are other accounts of wild bird feeding, it was not until the 19th century when feeding garden birds emerged as a more widespread activity. Two pioneers of wild bird feeding at the time were Baron von Berlepsch who attracted birds to his German estate by covering twigs with fat, and the British ornithologist John Freeman Dovaston who created the first 'ornithotrophe' which was a bird feeder attracting 23 bird species during the winter of 1825. By the 20th century

bird feeding was becoming increasingly popular aided by the publications of 'How to Attract and Protect Wild Birds' by Hiesemann (1908) and 'The Bird Table Book' by Soper (1965) compelling the American company Droll Yankees Inc. to manufacture the first modern tubular garden bird feeder in 1969. Ever since, the popularity of bird feeding has escalated and is now a common phenomenon in the Western World (Jones and Reynolds 2008): the density of bird feeders in urban areas of the UK is 925 feeders km^{-2} (Fuller et al. 2008) with approx. half of UK households feeding garden birds at some point during the year (Davies et al. 2009, Royal Society for the Protection of Birds 2010) and spending approx. £200-220 million annually (CJ Wildlife Ltd. pers. comm). In the US 53.4 million citizens spend approx. US$3.3 billion annually on feeding garden birds (United States Department of the Interior, Fish and Wildlife Service 2008) while results from a survey in Australia highlighted that 37% of participants fed wild birds (Rollinson et al. 2003). There are many reasons why wild bird feeding has become so prevalent but it is the pleasure of watching wild birds and their aesthetic value which is the driving force for the majority of the feeding public (Horvath and Roelans 1991).

1.1.2. Seasonal patterns and types of foods provided to wild birds

Traditionally, the provision of food to birds by humans occurred during the winter (Cowie and Hinsley 1988a, Jones and Reynolds 2008). However, organisations such as the Royal Society for the Protection of Birds (RSPB) and the British Trust for Ornithology (BTO) now advocate year-round feeding (Toms and Sterry 2008), suggesting that feeding throughout the year may be beneficial (BTO 2010, RSPB 2010), especially during the breeding season when birds can experience nutrient and energy limitation (Martin 1987).

Types of food being provided have also varied over the last few decades. For example, results from a survey of householders in suburban Cardiff in the 1980s indicated that bread was the most common food provided to birds (by approx. 90% of households), followed by kitchen scraps (approx. 55% of households) but only approx. 40% of households provided peanuts specifically purchased for wild bird feeding (Cowie and Hinsley 1988a). More recently, as a result of the assimilation of knowledge regarding the suitability of different food types and the supply of more detailed advice on what to feed birds from organisations such as the RSPB and BTO (BTO 2010, RSPB 2010), bird food companies such as CJ Wildlife Ltd. have developed and introduced a wide range of bird foods, including live foods as well as a plethora of seed and fat types (CJ Wildlife Ltd. 2010). While these food types may reflect those purchased across the UK for wild birds (CJ Wildlife Ltd. pers. comm.), by definition they only partially reflect the global provision of food types to birds. For example, a study of householders in Brisbane, Australia, highlighted that 58% of participants provided bread and 22% provided bird seed, but 32% provided mince meat, specifically to attract species including Australian Magpies (*Gymnorhina tibicen*) and 4% provided sugar water to attract lorikeets (*Trichoglossus* spp.) (Rollinson et al. 2003). Sugar water is also provided in the Americas where it is intended mainly for species of hummingbirds (*Trochilidae* spp.) and other nectar-feeding birds (Fig. 1.1).

1.1.3. Bird assemblages at wild bird feeders

Garden bird feeders may provide an important source of food to many garden bird species (Chamberlain et al. 2005, Fuller et al. 2008, Robb et al. 2008a) and there is increasing evidence that the density of garden bird feeders strongly and positively

Figure 1.1. Saw-billed Hermit (*Ramphodon naevius*) at a hummingbird feeder containing sugar water in Brazil 2010 (Photo: J.A. Smith).

influences avian abundance at a landscape scale (Chamberlain et al. 2005, Fuller et al. 2008, Fuller et al. In Press). For example, garden bird feeders predict 57% and 26% of the variation in the abundance of House Sparrows (*Passer domesticus*) and Common Starlings (*Sturnus vulgaris*), respectively (Fuller et al. In Press). In the UK approx. 80 common garden bird species are known to utilise bird feeders (Glue 2006, CJ Wildlife Ltd. 2010) with the most common including European Robins (*Erithacus rubecula*), Common Blackbirds (*Turdus merula*) and Blue Tits (*Cyanistes caeruleus*) (Glue 2006, Chamberlain et al. 2005) (Fig. 1.2). Other species including granivorous finches such as European Greenfinches (*Carduelis chloris*) and Common Chaffinches (*Fringilla coelebs*) are also common and have shown increased tendencies to visit feeders over the past 30 years, possibly as a result of the increase in the provision of

Figure 1.2. Blue Tits feeding on a garden bird feeder in a garden in the UK (Photo: CJ Wildlife Ltd.).

specialised bird food targeted at them (Chamberlain et al. 2005, Glue 2006). In contrast to the small passerines regularly observed at bird feeders in the UK, in Australia bird feeders are exploited by large, socially dominant species such as Australian Magpies and butcherbirds (*Cracticus* spp.) (Rollinson et al. 2003). This may represent a problem to smaller garden bird species due to an increase in competition for food together with an increased risk of nest predation from larger, more aggressive carnivorous species (Rollinson et al. 2003, D. L. Jones pers. comm.).

1.2. EFFECTS OF SUPPLEMENTARY FEEDING DURING REPRODUCTION

During reproduction a breeding bird must divide time and energy between investment in the current breeding attempt and self-maintenance in order to maximise fitness benefits while not compromising its own chances of survival (reviewed in Stearns 1989). For capital breeding species such as Common Eiders (Meijer and Drent 1999) the energy and nutrients required for both self-maintenance and reproduction are

drawn from endogenous reserves which are accumulated before breeding (Jönsson 1997). However, income breeders, such as small passerines, must assimilate most of their required energy and nutrients from exogenous resources (i.e. food) (Jönsson 1997). Blue Tits are good examples of income breeders since they rely on direct food intake for 90% of their total energy and protein during egg formation (Meijer and Drent 1999). Therefore, when food is limited during reproduction species that are income breeders should allocate more time and energy towards self-maintenance and less towards reproductive investment (reviewed in Martin 1987). In this respect, the availability of food during reproduction is likely to limit reproductive success. However, food may not always be limiting during reproduction and, indeed, previous studies have shown that when food is superabundant its availability does not limit reproduction (e.g. Bollmann et al. 1997, Rauter et al. 2000).

1.2.1. The effects of food limitation on reproduction: insights from supplementary feeding studies

Supplementary feeding provide a potent way to examine whether food limits reproduction and numerous studies have reported that food availability can have dramatic consequences on reproductive success (reviewed in Martin 1987, Meijer and Drent 1999, Christians 2002, Robb et al. 2008b). Perhaps the most common documented effect of supplementary feeding is the advancement of clutch initiation date (e.g. Arcese and Smith 1988, Källander and Karlsson 1993, Nilsson 1994, Harrison et al. 2010). Two main, but not mutually exclusive, hypotheses have been proposed to explain why food availability is likely to be a proximate cue in the reproductive timing of birds: (i). birds may use food availability to time breeding so that they will be feeding nestlings in periods of peak food abundance (Lack 1954);

and (ii). females can accumulate enough resources to form and lay eggs earlier (Perrins 1970). Birds that are able to advance breeding are likely to accrue substantial fitness benefits since nestlings that hatch early in the breeding season have a higher likelihood of survival than those hatching later (Perrins 1965 – but see Nilsson 1994).

Another determinant of breeding phenology is the length of the incubation period. In intermittent incubators incubation consists of periods on the eggs, in which the incubator warms the eggs, interspersed with periods off the nest (and eggs), in which the incubating bird feeds (Deeming 2002a). When food is limited an incubating bird may have to allocate more towards feeding, spending more time away from the nest (e.g. Eikenaar et al. 2003, Londoño et al. 2008). Extended periods away from the nest may result in the cooling of eggs and the subsequent decrease in the rate of embryonic development (Deeming 2002a). Therefore, when food is limited the incubation period may be prolonged (Moreno 1989, Zimmerling and Ankney 2005, Harrison et al. 2010). Protracted incubation periods may severely reduce fitness of incubating birds through an increase in the costs associated with incubation, such as increased predation risk (reviewed in Reid et al. 2002), high energetic expenditure (reviewed in Tinbergen and Williams 2002) and reduced further mating opportunities (reviewed in Reid et al. 2002).

Supplementary feeding studies have also reported effects of food availability on numerous determinants of breeding performance. For example, clutch size may be constrained by food availability since egg formation requires energy and nutrient input (reviewed in Meijer and Drent 1999, Williams 2005). Indeed, previous studies have provided evidence that clutch size is positively related to food availability (e.g. Dijkstra et al. 1982, Arcese and Smith 1988). In contrast, previous work on my study population suggested that supplementary feeding may decrease clutch size of both

Blue and Great (*Parus major*) Tits (Harrison et al. 2010). However, the mechanism underlying this result is currently unclear. Food availability may also influence breeding performance during the nestling period when altricial young rely on food from their parents to satisfy the energetic and nutritional demands of growth and development (O'Connor 1984). Brood provisioning by adults is energetically demanding (reviewed in Bryant and Tatner 1991) and supplementary feeding during the nestling period has been shown to enhance nestling growth (Arcese and Smith 1988), reduce partial brood mortality, increase fledging success (e.g. Arcese and Smith 1988, González et al. 2006 – but see Harrison 2010), and increase post-fledging survival (e.g. Dewey and Kennedy 2001, Harrison 2010).

1.3. EFFECTS OF SUPPLEMENTARY FEEDING ON REPRODUCTIVE BEHAVIOUR

Food availability during reproduction is likely to affect the trade-off between time spent on self-maintenance (e.g. preening, feeding) and that spent on reproductive behaviours (e.g. incubation, brood provisioning) (Nur 1984, Kacelnik and Cuthill 1990, Ydenberg et al. 1994). Numerous supplementary feeding studies have provided empirical evidence that supplemented birds allocate more time to reproductive behaviours than nonsupplemented controls (e.g. Markman et al. 2002, Eikenaar et al. 2003, Londoño et al. 2008). Below, I introduce the effects of supplementary feeding on reproductive behaviours and show that, compared with other aspects of avian breeding biology, our knowledge of the effects of food supplementation on the underlying reproductive behaviours is limited.

1.3.1. Nest construction behaviour

The nest construction period represents one of the earliest reproductive phases in a breeding attempt constituting a significant proportion of the breeding cycle both temporally and energetically (e.g. Skutch 1949, Withers 1977, Collias and Collias 1984). However, despite this, to my knowledge few studies have investigated the effects of supplementary feeding on this reproductive stage. However, supplementary feeding has been shown to advance the timing of nest construction (Ewald and Rohwer 1982, Kelly and van Horne 1997). Mainwaring and Hartley (2009) also demonstrated that supplementary feeding from the time of nest initiation to nest completion resulted in supplemented Blue Tits constructing heavier nests with greater amounts of moss base compared with controls. Since nest building is temporally and energetically costly, their results indicate that nest building is condition-dependent in this species.

1.3.2. Incubation behaviour

Incubation is the process by which eggs are maintained in optimal conditions of temperature and humidity for embryonic development via the exchange of heat between the incubating bird and the clutch of eggs during what is termed 'contact incubation' (Deeming 2002a). Incubation is energetically expensive (Williams 1996, Tinbergen and Williams 2002) and, therefore, in species that are income breeders that rely upon food for the majority of their energy (Jönsson 1997) time must be spent off the nest to forage (Deeming 2002a). This incubation strategy is referred to as 'intermittent incubation' and consists of periods on the nest (so-called incubation bouts) interspersed with periods off the nest (so-called incubation recesses) (Deeming 2002a). Since food availability partly controls the energy available to the incubator, it

is not surprising that numerous supplementary feeding studies have shown that supplemented birds have shorter incubation recess lengths (e.g. Chalfoun and Martin 2007, Londoño et al. 2008) and/or longer incubation bout lengths (e.g. Eikennar et al. 2003, Rastogi et al. 2006). Such effects are likely to be important since such changes in incubation behaviour can result in the truncation of incubation periods (Moreno 1989, Zimmerling and Ankney 2005), reducing the likelihood of time-dependent predation (reviewed in Reid et al. 2002) and resulting in earlier fledging which can have important fitness benefits (Verboven and Visser 1998).

1.3.3. Brood provisioning behaviour

During the nestling period altricial nestlings are dependent on their parents to provide food to satisfy the energetic and nutritional demands of growth and development (e.g. O'Connor 1984, Starck and Ricklefs 1998). However, such brood provisioning is energetically demanding (reviewed in Bryant and Tatner 1991) and, therefore, the adult must provide food for not only its nestlings but also for self-maintenance to fuel its metabolism to sustain provisioning efforts (Ydenberg et al. 1994, Ydenberg 1998). When food is limited brood provisioning rates may decrease as a consequence of a reduction in the amount of energy available to the provisioning adult (Markman et al. 2002). Supplementary feeding may also affect brood provisioning by directly increasing the amount of food available to the nestlings when the food supplement(s) is suitable for nestling consumption (Cucco and Malacarne 1997). This response is likely to be mediated through a reduction in begging intensity as a result of additional food (e.g. Quillfeldt and Masello 2004, Hamer et al. 2006) and may have ultimate fitness benefits.

1.3.4. Mating behaviour and extra-pair paternity (EPP)

EPP is likely to be affected by mate-guarding behaviour and the frequency of
copulations between a social female and an extra-pair male (e.g. Møller 1987,
Westneat 1994, Petrie and Kempenaers 1998) and since these behaviours are likely to
be energetically and temporally costly (reviewed in Birkhead and Møller 1992, Petrie
and Kempenaers 1998), also by food availability. However, there have been few
studies that have investigated the effects of supplementary feeding on mating
behaviour and EPP and, moreover, they have reported conflicting findings. For
example, Westneat (1994) found that supplementary feeding resulted in a reduction of
EPP on territories of male Red-winged Blackbirds (*Agelaius phoeniceus*) that
presumably invested more in mate guarding compared with unfed (control) males. In
contrast, Hoi-Leitner et al. (1999) demonstrated that supplementary feeding during the
fertile period of female European Serins (*Serinus serinus*) increased the number of
broods containing extra-pair young (EPY). While the effects of supplementary
feeding on mating behaviour and EPP are currently unclear, it may be important to
consider food availability during reproduction with respect to its potential influence
on the genetic structure of avian populations (e.g. Petrie et al. 1998).

1.3.5. Secondary sex ratio

In addition to EPP, brood sex ratio may influence the genetic structure of avian
populations (reviewed in Donald 2007). Although there is controversy over the
mechanism (reviewed in Krakow 1995, Komdeur and Pen 2002, Alonso-Alvarez
2006), adult females may be able to manipulate brood sex ratio in response to food
availability when the relative reproductive value of producing males and females
differs in relation to food resources (e.g. Clout et al. 2002, Rutstein et al. 2004).

Brood sex ratio manipulated at laying is referred to as primary sex ratio (Burley 1986). Food availability may further adjust sex ratio during the nestling period resulting in biased secondary sex ratio (i.e. sex ratio at fledging – Burley 1986). For example, in sexually dimorphic species where the larger sex is more susceptible to starvation, food shortages during the nestling period may lead to biased mortality of the larger sex (e.g. Teather and Weatherhead 1989, Torres and Drummond 1997). Alternatively, when food is limited, mortality may be biased towards the smaller sex as a result of the larger having a competitive advantage and being able to monopolise food (Hipkiss et al. 2002).

1.4. AIMS OF THE THESIS

The main aim of this thesis is to investigate the effects of supplementary feeding on the reproductive behaviours of small passerines. I fed birds during the spring and summer to mimic the recommendations of the non-governmental organisations (NGOs) (e.g. BTO, RSPB) to the bird-feeding public about when they should feed garden birds. Currently, such recommendations are not supported by a large body of scientific empirical evidence that suggests food supplementation has benefits for breeding birds. It is important to establish the effects of supplementary feeding on reproductive behaviours since it is through such changes in reproductive behaviour that important downstream effects on breeding performance, survival and, ultimately, fitness may take effect. In so doing, supplementary feeding could ultimately have indirect, but important, effects at the population and community levels.

I conducted a field study in central England from 2007 to 2009 inclusive. I used two commercially available garden bird foods: (i). peanut cake (an energy-rich food source consisting of 50% peanut cake flour and 50% beef tallow); and (ii).

mealworms (a protein-rich food source known to be fed to nestlings by adults; CJ Wildlife Ltd. pers. comm.). Peanut cake was provided *ad libitum* from approx. 3 weeks prior to egg laying until post-fledging while mealworms were provided during the nestling period as recommended by the garden bird food supplier (CJ Wildlife Ltd. 2010). Ideally, my study would have been conducted in suburban gardens but the logistical problems (e.g. gaining access to gardens, controlling the types and amounts of bird food provided by the public) were insurmountable and, instead, the study was conducted in a broadleaved woodland consisting predominantly of oaks (*Quercus* spp.)

A secondary aim of this thesis was to investigate the potential effects of supplementary feeding on avian reproduction at a national scale and to investigate how effects of supplementary feeding may change over time. This was achieved by using data from the BTO's Nest Record Scheme (NRS) (Crick et al. 2003) collected from both urban and deciduous woodland habitats between 1962 and 2008 inclusive. Using the NRS data, I examined breeding phenology (through clutch initiation date) and performance (through clutch size, brood size and failure rates [brood size as a proportion of clutch size]). Although the extent of supplementary feeding cannot be quantified using data from the NRS, birds breeding in urban habitats are more likely to have access to supplementary food than birds breeding in deciduous woodland habitats since wild bird feeding is a widespread activity in UK gardens in towns and cities (e.g. Fuller et al. 2008, Jones and Reynolds 2008, Davies et al. 2009, Fuller et al. In press). While investigating the effects of supplementary feeding at a local scale (i.e. using a supplementary feeding field study) allows the direct manipulation of food availability, considering such effects at a national scale is crucial since reproductive performance and, therefore, potentially the effects of supplementary feeding, may

vary spatially (e.g. Young 1994, Fargallo 2004). Furthermore, considering how supplementary feeding affects reproduction over a longer timeframe than a self-contained 3-year study is also important since it may provide valuable additional insights into how supplementary feeding may affect reproduction in the future in relation to, for example, climate change (e.g. IPCC 2001, King 2005).

In order to address the aims of this thesis, I chose to study Blue and Great Tits as my focal species. They are both common species that breed in UK gardens (Bland et al. 2004) and deciduous woodlands (Perrins 1979) and readily feed on garden bird feeders consuming both peanut cake and mealworms (CJ Wildlife Ltd. 2010). Furthermore, they nest in nestboxes (Perrins 1979) making them easily trappable and relatively easy to observe, two key attributes of species that I looked to target in this behavioural investigation. In addition, both Blue and Great Tits are socially monogamous (Cramp and Perrins 1993), but will partake in extra-pair copulations (EPCs) resulting in EPP (e.g. Kempenaers et al. 1992, Krokene et al. 1998). They lay large clutches (Great Tit: 5-12 eggs; Blue Tits: 7-13 eggs – Gosler and Clement 2007) making them good model species to investigate the effects of supplementary feeding on both brood sex ratio and mating behaviour. I adopted such a two-species approach since during reproduction Blue and Great Tits occupy different niches (Minot 1981) with the dietary breadth of Great Tits exceeding that of Blue Tits (Gosler and Clement 2007). Therefore, Blue Tits are often considered to be specialist feeders compared with more generalist Great Tits (Török 1986). Thus, I aimed to provide insights into how supplementary feeding might affect the reproductive behaviours of species with different feeding ecologies. This is important to consider since a wide range of species with different feeding ecologies feed on garden bird feeders in the UK (e.g. Chamberlain et al. 2005, Glue 2006).

1.5. STRUCTURE OF THE THESIS

My thesis has been prepared in 'paper' format and each data chapter is intended for publication. In **Chapter Two**, I investigate the effects of supplementary feeding on the nest construction behaviour of both Blue and Great Tits over a one-year period in a deciduous woodland. I hypothesised that the reduction of the energetic and temporal costs of foraging by the provision of *ad libitum* supplementary food before and during nest building would enable food-supplemented birds to spend more time finding material for, and engaged in, nest construction. Thus, in comparison with nonsupplemented (control) birds, I predicted that food supplementation would result in: (i). earlier nest construction; (ii). more rapid nest construction; (iii). construction of deeper nests; and (iv). a longer time engaged in nest lining.

In **Chapter Three**, I examine the effects of supplementary feeding on the incubation behaviour of both Blue and Great Tits during 2008 and 2009 in a deciduous woodland using two methods. I use focal behavioural observations to determine incubation bout and recess lengths of Great Tits and small temperature loggers to investigate daily incubation activity (DIA) of Blue Tits. I hypothesised that supplementary feeding would partly alleviate the energetic and temporal constraints on foraging, allowing incubating females to invest more heavily in incubation and less time in self-maintenance. I predicted that supplementary feeding would: (i). increase DIA; (ii). decrease incubation recess lengths because energy demands can be satisfied more rapidly; and (iii). increase incubation bout lengths.

In **Chapter Four**, I assess the effects of supplementary feeding on the brood provisioning behaviour of both Blue and Great Tits from 2007 to 2009 in a deciduous woodland by using focal behavioural observations. I also explore the effect of supplementary feeding on nestling condition and post-fledging survival. I

hypothesised that the provision of supplementary food for adults (peanut cake) would reduce the energetic and temporal costs of foraging and would enable supplemented adults to invest more time and energy in provisioning nestlings. Therefore, I predicted that supplemented adult birds would have higher brood provisioning rates than nonsupplemented (control) birds. I also hypothesised that providing additional supplementary food intended for nestling consumption (mealworms) would further enhance the investment of adults in their provisioning effort of nestlings compared with birds simply fed with peanut cake. I also predicted that nestlings in supplementary fed blocks would be in better body condition than those in the control block, and that survival of the fledglings from the supplementary fed blocks would be higher.

In **Chapter Five**, I investigate the effects of supplementary feeding on the incidence of EPP in Blue Tits breeding in 2008 and 2009 in a deciduous woodland by using molecular genetic techniques. I hypothesised that supplementary food (peanut cake) provided prior to, and throughout, the egg laying period would partly alleviate the energetic and temporal constraints of foraging allowing male Blue Tits to increase time spent mate-guarding during the fertile period of female Blue Tits. I also hypothesised that feeders containing the supplementary food would act as 'food hotspots' resulting in a reduction in the roaming behaviour of foraging female Blue Tits allowing them to be closely mate-guarded by male Blue Tits. Therefore, I predicted that the supplementation of peanut cake would result in fewer nests containing EPY and that broods of supplemented birds would have a lower proportion of EPY than broods of control (nonsupplemented) birds. In addition, I hypothesised that an additional food supplement (mealworms) intended for nestlings, and provided to birds already receiving peanut cake, would result in increased nestling survival and

a lessening of differential mortality between WPY and EPY compared with only

peanut cake-supplemented birds. Therefore, I predicted that the additional

supplementation of food intended for nestlings would result in a lower proportion of

EPY within nests compared to that in nests of birds only supplemented with peanut

cake.

In **Chapter Six**, I assess the effects of supplementary feeding on secondary

brood sex ratio of Blue Tits breeding in 2008 and 2009 breeding in a deciduous

woodland by using molecular genetic techniques. I hypothesised that the

supplementation of peanut cake intended for adult consumption during the fertile

period would increase female condition enabling supplemented females to produce

higher quality nestlings. Since higher quality male Blue Tits have higher reproductive

success than high quality females (Dreiss et al. 2006), I predicted that broods in

blocks supplemented with peanut cake would be more male-biased than in the control

(nonsupplemented) block. In addition, I hypothesised that the additional

supplementation of mealworms intended for nestling consumption would reduce

male-biased mortality. Therefore, I predicted that broods in the treatment block

supplemented with both peanut cake and mealworms would be more male-biased than

broods in both the peanut cake-supplemented and control blocks.

In **Chapter Seven**, I use NRS data to compare breeding performance (i.e.

clutch size, brood size and failure rates [brood size as a proportion of clutch size]) and

phenology of both Blue and Great Tits breeding in urban and deciduous habitats

across the UK from 1962 to 2008.

Finally, in **Chapter Eight**, I summarise my results and discuss them within an

urban garden bird feeding context. I also discuss the mechanisms underlying the

effects of supplementary feeding on avian reproductive behaviour and provide

suggestions for future research.

Chapter Two

FEATHERING THE NEST: FOOD SUPPLEMENTATION INFLUENCES NEST

CONSTRUCTION BY BLUE AND GREAT TITS

2.1. ABSTRACT

Food supplementation during reproduction can potentially provide additional energy and/or liberate time usually devoted to foraging, enabling birds to invest more in activities such as nest construction that provide longer term fitness benefits. Although nest construction can represent large temporal and energetic investments in a breeding attempt, few studies have investigated how it is influenced by food supplementation. In 2008 I studied the effects of food supplementation on nest construction by Blue and Great Tits. I found that food supplementation advanced nest construction and also advanced laying in both species. Food supplementation also resulted in truncation of the nest construction period of Blue, but not Great, Tits. The duration of the nest-lining period was not influenced by food supplementation in either species. Unexpectedly, food supplementation resulted in Blue Tits constructing significantly shallower nests than control conspecifics whereas the depth of Great Tit nests remained unaffected. This study provides empirical evidence that food availability prior to and throughout nest construction constrains nest construction behaviour of Blue and Great Tits breeding in a deciduous woodland in Central England suggesting that nest construction behaviour is both energetically and temporally costly.

2.2. INTRODUCTION

The avian breeding cycle has been well characterised in many species (Murton and

Westwood 1977). Although the constituent phases of egg laying, incubation and brood

rearing are important to consider, one of the earliest phases of the breeding attempt,

namely nest construction, remains relatively under-studied despite the fact that it may

constitute a significant proportion of the breeding cycle both temporally and

energetically (e.g. Skutch 1949, Withers 1977, Collias and Collias 1984). Thus, it is

fundamental to consider this phase, together with others, when quantifying investments

in breeding attempts and when considering the breeding biology of birds within a life-

history context (Martin and Li 1992, Stearns 1992).

Nests can play a key role in reproduction by contributing to parental care efforts

in the maintenance of thermal conditions during incubation and brooding, and in

providing protection of both eggs and young against predators and inclement weather

(e.g. Collias and Collias 1984, Kern et al. 1993, Lima 2009). During incubation nest

architecture and integrity are fundamental for the conservation of heat within the nest

(Deeming 2002a). In addition, nest architecture can help regulate nest humidity, a vital

component of the nest microclimate, since nest humidity partially determines water loss

from eggs with excessive or insufficient water loss resulting in abnormal embryonic

development (Ar and Sidis 2002). Therefore, investment in nest building may reflect the

phenotypic quality (e.g. Lens et al. 1994 – but see Álvarez and Barba 2008) or health

status (e.g. Tomás et al. 2006, Moreno et al. 2008) of the nest builder and, in accordance

with the 'sexual display hypothesis' (Soler et al. 1998), act as a sexually selected trait

(e.g. Mainwaring et al. 2008). Alternatively, investment in nest building may reflect the

condition of the nest builder's mate (e.g. Szentirmai et al. 2005, Broggi and Senar 2009)

providing support for the 'differential allocation hypothesis' which suggests that

reproductive investment is influenced by mate attractiveness (Burley 1986).

Reproductive success of birds can be limited by several factors such as adult

condition (Doutrelant et al. 2008), predation (Zanette et al. 2003) and inclement

weather (Morrison and Bolger 2002), but perhaps the most important factor is food

availability (reviewed in Martin 1987, Robb et al. 2008a). The effects of food

availability have been revealed most directly in supplementary feeding studies in which

birds were provided with additional supplies of food beyond those available in the

natural environment (see Section 1.3). Many such studies have highlighted the effects of

increased food availability on reproductive parameters such as clutch initiation date

(e.g. Ramsay and Houston 1997), clutch size (e.g. Ewald and Rohwer 1982), egg size

(e.g. Reynolds et al. 2003a) and brood size (e.g. Harrison et al. 2010). The influence of

supplementary feeding on some reproductive behaviours such as incubation (e.g.

Eikenaar et al. 2003) and brood provisioning (Cucco and Malacarne 1997) is well

known, but relatively little is known about its influence on nest construction behaviour.

However, Mainwaring and Hartley (2009) have recently shown that the provision of

mealworms as a supplementary food source from the time of nest initiation to nest

completion resulted in supplemented female Blue Tits constructing heavier nests which

contained more moss than nonsupplemented females. This indicated that nest building

was condition-dependent in this species.

I investigated whether the timing and duration of nest construction of Blue and

Great Tits are affected by supplementary feeding. Nest construction in Blue and Great

Tits can be subdivided into two distinct phases: (i). the structural layer period (the

period in which the structural component of the nest is built) and; (ii). the nest-lining

period (when the nest-lining component is built). I hypothesised that the reduction of the

energetic and temporal costs of foraging by the provision of *ad libitum* supplementary food before and during nest building would enable food-supplemented birds to spend more time finding material for, and engaged in, nest construction. Thus, in comparison with nonsupplemented (control) birds, I predicted that food supplementation would result in: (i). earlier nest construction; (ii). more rapid nest construction; (iii). construction of deeper nests; and (iv). a longer time engaged in nest-lining.

Prior to and during nest construction Blue and Great Tits occupy different niches, the former feeding mainly in the canopy and the latter mainly on the ground (e.g. Minot 1981). At this time the dietary breadth of Great Tits exceeds that of Blue Tits (Gosler and Clement 2007) and, therefore, Blue Tits are often considered to be specialist feeders compared with Great Tits that are considered to be generalist feeders (e.g. Török 1986). However, where the dietary preferences of these species overlap, and there is inter-specific competition for food, Great Tits are dominant (Wilson 1992). Since I was studying Blue and Great Tits simultaneously at the same site, I hypothesised that Great Tits would exploit artificial feeders to a greater extent than Blue Tits both through having a more generalist diet and through out-competing Blue Tits at the feeding stations. I know that compared with Blue Tits food supplementation of Great Tits in my study population has more significant effects on some breeding parameters (e.g. clutch initiation date, incubation period length – Harrison et al. 2010). Thus, in comparison with Blue Tits, I predicted that any observed effects of supplementary feeding would be more pronounced in Great compared with Blue Tits.

2.3. METHODS

2.3.1. Focal species

Blue and Great Tits are cavity nesters that readily breed in nestboxes (Perrins 1979).

Females dominate males at the nest in both species and construct the nest alone,

predominantly out of moss (Gosler and Clement 2007). Blue Tits line the nest with

feathers and Great Tits with hair or fur (Perrins 1979). Typically, in the UK, only one

nest is constructed in a breeding season although replacement nests are made in the

event of nest disturbance (e.g. predation of eggs or young). However, although Great

Tits double brood more often than Blue Tits, it occurs in <5% of Great Tits in deciduous

broadleaved woodlands (Perrins 1979).

2.3.2. Study site and supplementation

The study was conducted in 2008 in Chaddesley Woods National Nature Reserve,

Worcestershire, UK (52°36'N, 2°14'W), a broadleaved woodland consisting

predominantly of oaks (*Quercus* spp.). Two hundred and eighty-eight identical plywood

nestboxes (height 29.5 cm, width 12.7 cm and depth 14.6 cm) were established on the

site in 2005. Nestboxes are approx. 2 m above the ground and have 32 mm entrance

holes facing NE away from the prevailing SW wind. Prior to the onset of the breeding

season, between October and December, old nesting material from previous breeding

attempts was removed from nestboxes to ensure that they were clean prior to new

breeding attempts. The woodland was divided into two blocks containing 192 and 96

nestboxes which were arranged throughout on a 40 m-grid system resulting in a nestbox

density of 6.25 ha^{-1} (Fig. 2.1). (Note the inequality in numbers of nestboxes between

treatment blocks was the result of a broader, ongoing food supplementation study). In

the larger nestbox block a food supplement of peanut cake (an energy-rich food source

consisting of 50% peanut cake flour and 50% beef tallow, and known to be consumed

extensively by both focal species – CJ Wildlife pers. comm.), was provided while in the
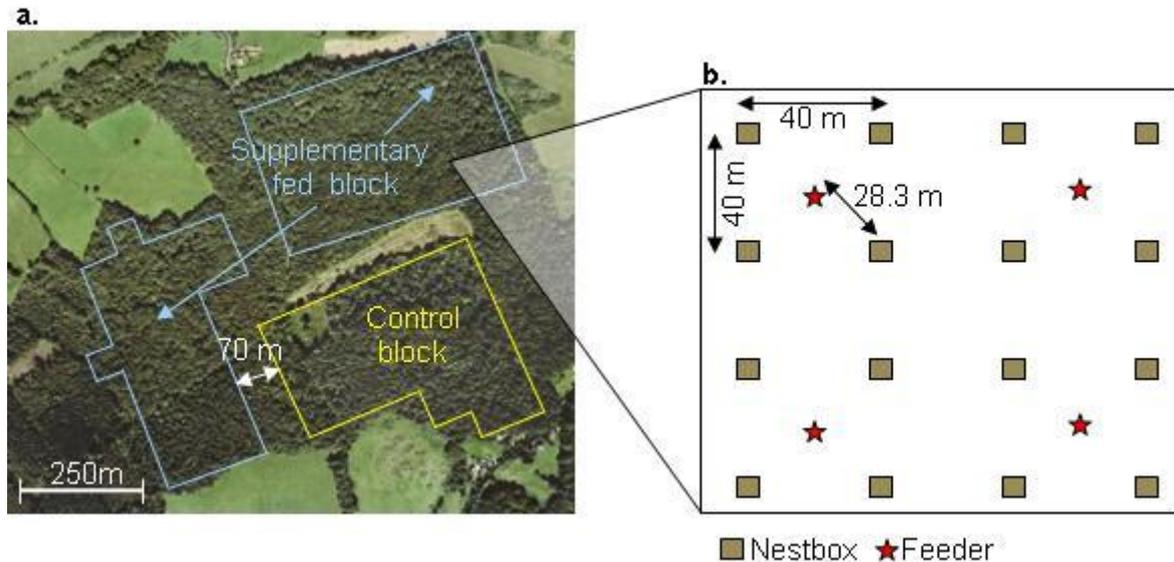
Figure 2.1. (**a**) Chaddesley Woods National Nature Reserve was split into three blocks where Great and Blue Tits either received peanut cake (supplementary fed block) or no supplement (control block) before and during nest building in 2008 (see text for details). (**b**) Nestboxes were separated on a 40 m-grid system with feeders placed in the centre of a block of four nestboxes.

smaller control block there was no food supplementation. Blocks were separated by a buffer strip of approx. 70 m (Fig 2.1) to reduce the probability that territorial breeding birds would travel far beyond their own territories and, thereby, cross between treatment blocks (Naef-Daenzer 2000 – but see Wilkin et al. 2009a). In the food-supplemented block there were 48 peanut cake feeders that were evenly spaced with one for every four nestboxes. Each feeder was placed at the centre of a square of four nestboxes so that it was approx. 28 m away from each nestbox (Fig. 2.1)

In 2007 nest construction at this study site started in the third week of March (pers. obs.) so in 2008 when this study took place supplementary feeding was started in the first week of March ensuring that *ad libitum* supplementary food was available for approx. 2 weeks prior to the predicted onset of nest construction and throughout nest construction.

2.3.3. Experimental procedure

During the winter prior to the study all nest material from the previous breeding attempts was removed from the nestboxes. Approx. 2 weeks after the onset of food supplementation nestboxes were checked for the appearance of nest material. Nest initiation date was defined as the date on which the first nest material was seen. Since both Blue and Great Tits are known to continue adding material to the nest after clutch initiation (Perrins 1979), determination of nest completion date is problematic and, thus, I defined nest completion date as when clutch initiation occurred (i.e. when I assumed that the nest was functionally complete – Fig. 2.2). A photograph was taken on each visit to a nestbox with a digital camera (Nikon Coolpix®) looking vertically down into the opened nestbox from a distance of approx. 30 cm (Fig. 2.2). Nestboxes were checked and photographed every 2-3 days between nest initiation and clutch initiation. The period between nest and clutch initiation was defined as the nest construction period. At clutch initiation, the depth of nest material was measured to the nearest mm at the mid-points of the back and lateral walls of the nestbox using a plastic ruler inserted between the nesting material and nestbox wall. The average nest depth of each functionally complete nest was determined from these three measurements. Thereafter, nestboxes were periodically visited to continue the monitoring of breeding attempts. Nests that appeared to be taken over by another female (e.g. where feathers from a Blue Tit nest were removed and replaced with fur by a Great Tit) were excluded from the study. The complete dataset contained 111 Blue Tit (66 food-supplemented and 45 control) and 115 Great Tit nests (79 food-supplemented and 36 control).

Photographs of nest building sequences (an example is shown in Fig. 2.2) were examined to determine the onset and duration of the nest construction period which was

Figure 2.2. A complete photographic time series of the nest construction period of a Great Tit breeding at Chaddesley Woods National Nature Reserve in 2008: (**a**) first appearance of nest material indicates nest initiation; (**b**) – (**c**) nest building ongoing; (**d**) – (**e**) appearance of fur indicates nest-lining; and (**f**) clutch initiation at the end of the nest-lining and nest construction period. The date on which each photograph was taken appears above.

subdivided into the structural layer period (the period in which the structural component of the nest is built) and the nest-lining period (when the nest-lining component is built). The onset of nest-lining was determined from the appearance of the first piece of lining material (a feather or piece of fur). The lining was defined as completed when clutch initiation occurred (e.g. period between Fig. 2.2d and Fig. 2.2f, respectively). The difference between the two dates defined the nest-lining period.

2.3.4. Statistical analysis

Mixed models were constructed using PROC GLIMMIX in SAS Version 9.2 (SAS Institute 2008) to test for the effects of supplementary feeding on the individual response variables. Nest initiation date, nest construction period, average final nest depth and clutch initiation date were tested for normality using the Anderson-Darling Test and for homogeneity of variance using the F-test. All were square-root transformed to normalise their distributions and then fitted with normal error distributions after examination of the model fit (Generalised Chi-Square/DF = approx. 1). Nest-lining and nest structural layer periods were fitted with Poisson error distributions.

Differences in degrees of freedom between models can be attributed to the inclusion/exclusion of covariates and covariate interactions (see below). Since nest construction period may decrease seasonally (Mainwaring and Hartley 2008), I included nest initiation date as a covariate in the nest construction period analysis. Since mass of accumulated nest-lining material may decline seasonally (McGowan et al. 2004, Mainwaring and Hartley 2008), I used both nest initiation date and nest construction period as covariates in the nest-lining period analysis. Both of these covariates may influence final nest size (e.g. Møller 1982, Powell and Rangen 2000) and, thus, I used them as covariates in the nest depth analysis. In addition, clutch size may influence nest

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construction behaviour with larger nests being constructed to contain larger clutches
(see Møller 1982). Therefore, clutch size was added as a covariate in all models. To
determine if the effects of covariates were consistent between treatment blocks,
covariate × dietary treatment interactions were tested independently of one another
within each model. Where multiple covariate × dietary treatment interactions were
significant they were both included in the model as long as each remained significant
(Grafen and Hails 2002). I performed backward model selections removing the least
non-significant covariate interactions ($P > 0.05$) one-by-one from models ensuring that
there was no significant change in deviance between models where applicable (Grafen
and Hails 2002).

2.4. Results

2.4.1. Nesting phenology

Food-supplemented Blue and Great Tits initiated nests significantly earlier than control
birds (Blue: $F_{1,75} = 7.52$, $P = 0.01$; Great: $F_{1,97} = 8.17$, $P < 0.01$; Table 2.1 and Fig.
2.3a). Birds of both species in both treatments also initiated clutches significantly earlier
than control birds (Blue: $F_{1,76} = 4.09$, $P = 0.05$; Great: $F_{1,98} = 6.87$, $P = 0.01$; Table 2.1
and Fig. 2.3b).

2.4.2. Nest construction period

Food supplementation did not have a significant effect on the nest construction period
of Great Tits ($F_{1,100} = 3.41$, $P = 0.07$; Table 2.1 and Fig. 2.4a), on the nest structural
layer period of Great Tits ($F_{1,97} = 0.70$, $P = 0.40$; Table 2.1 and Fig. 2.4b) or on the nest-
lining period for either species (Blue: $F_{1,82} = 0.09$, $P = 0.76$; Great: $F_{1,100} = 0.31$, $P =$

Table 2.1. A comparison of nest construction traits in terms of output from final mixed models for control (nonsupplemented/N) and supplemented (S) Blue Tits (BT) and Great Tits (GT) breeding in Chaddesley Woods National Nature Reserve in 2008. Significant main effects are given in bold text. Directions of significant effects are given: '+' and '-' denote significant positive and negative relationships, respectively, and 'NS' denotes nonsignificance ($P > 0.05$).

Response variable	Species	Factor	F	df	P	Least square means (SE)		Direction
						Nonsupplemented	Supplemented	
Nest initiation date (1 = 1st March)	BT	Dietary treatment	**7.52**	**1,75**	**0.01**	36.09 (0.72)	33.41 (0.58)	N = latest; S = earliest
		Clutch size	**4.88**	**1,75**	**0.03**			-
		Nest construction period	**140.54**	**1,75**	**<0.0001**			-
	GT	Dietary treatment	**8.17**	**1,97**	**<0.01**	38.32 (1.00)	35.16 (0.60)	N = latest; S = earliest
		Clutch size	**3.82**	**1,97**	**0.05**			-
		Nest construction period	**97.12**	**1,97**	**<0.0001**			-
Nest construction period (days)	BT	Dietary treatment	**4.96**	**1,75**	**0.03**	16.00 (0.81)	13.76 (0.6)	N = longest; S = shortest
		Clutch size	**4.53**	**1,75**	**0.04**			+
		Nest initiation date	**164.23**	**1,75**	**<0.0001**			Negative relationship
	GT	Dietary treatment	3.41	1,100	0.07	12.96 (0.80)	11.29 (0.48)	NS
		Nest initiation date	**106.38**	**1,100**	**<0.0001**			-
Nest structural layer period (days)	BT	Dietary treatment	**9.14**	**1,75**	**<0.01**	9.95 (0.57)	7.90 (0.42)	N = longest; S = shortest
		Clutch size	3.23	1,75	0.08			NS
		Nest construction period	**298.48**	**1,75**	**<0.0001**			+
	GT	Dietary treatment	0.70	1,97	0.40	7.23 (0.52)	6.74 (0.31)	NS
		Clutch size	1.97	1,97	0.16			NS
		Nest construction period	**187.54**	**1,97**	**<0.0001**			+
Nest-lining period (days)	BT	Dietary treatment	0.09	1,82	0.76	5.10 (0.41)	5.26 (0.33)	NS

Table 2.1. continued

Response variable	Species	Factor	*F*	df	*P*	Least square means (SE)		Direction
						Nonsupplemented	Supplemented	
		Nest initiation date	**21.00**	**1,82**	**<0.0001**			-
	GT	Dietary treatment	0.31	1,100	0.58	4.18 (0.37)	4.42 (0.25)	NS
		Nest construction period	**82.79**	**1,100**	**<0.0001**			+
Nest depth (mm)	BT	Dietary treatment	**4.93**	**1,85**	**0.03**	56.55 (1.82)	51.41 (1.44)	N = deeper; S = shallower
	GT	Dietary treatment	1.92	1,100	0.17	50.27 (2.00)	47.06 (1.24)	NS
		Nest construction period	**4.87**	**1,100**	**0.03**			+
Clutch initiation date (1 = 1st March)	BT	Dietary treatment	**4.09**	**1,76**	**0.05**	51.41 (0.86)	49.28 (0.70)	N = latest; S = earliest
		Clutch size	3.73	1,76	0.06			NS
	GT	Dietary treatment	**6.87**	**1,98**	**0.01**	50.55(1.00)	47.75(0.55)	N = latest; S = earliest
		Clutch size	**4.49**	**1,98**	**0.04**			-
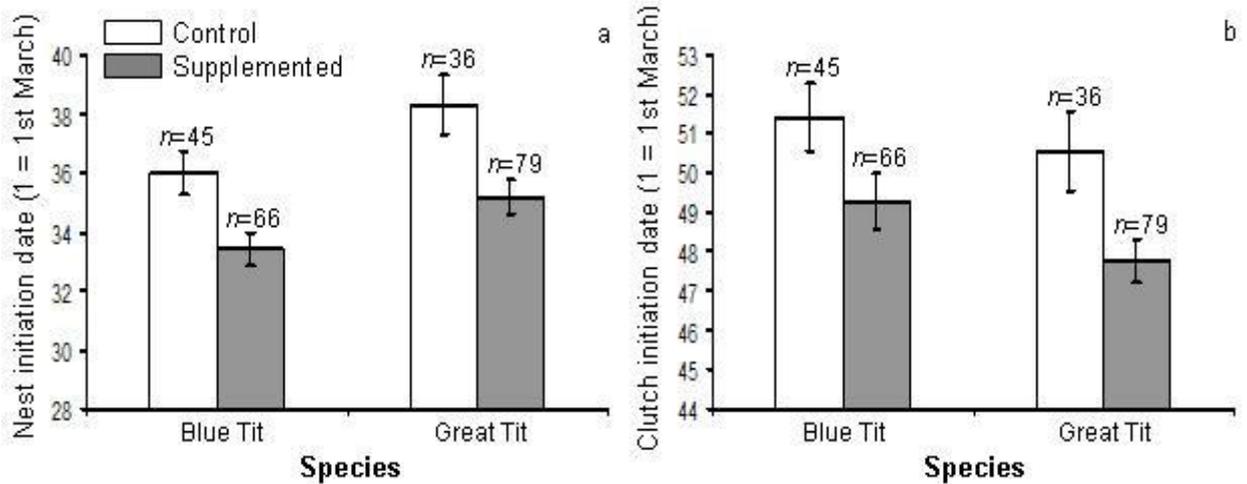
Figure 2.3. Nest building parameters (mean ± 1 SE from statistical estimates) of control (nonsupplemented) and food-supplemented Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve in 2008: (**a**) nest initiation date; and (**b**) clutch initiation date. The number of nests (*n*) is given about each bar.
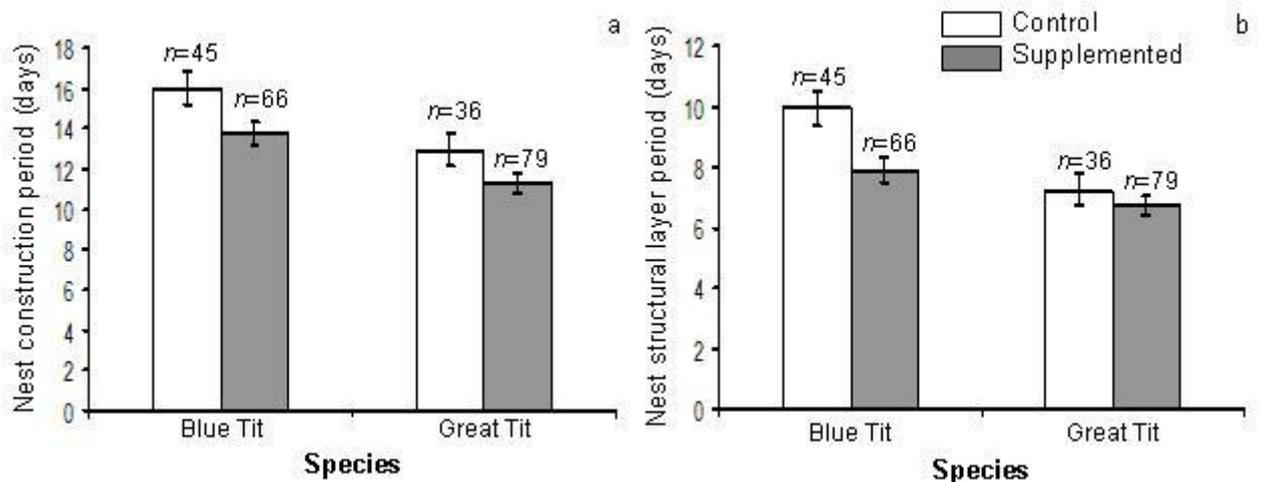
Figure 2.4. Nest building parameters (mean ± 1 SE from statistical estimates) of control (nonsupplemented) and food-supplemented Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve in 2008: (**a**) nest construction period; and (**b**) nest structural layer period. The number of nests (*n*) is given about each bar.

0.58; Table 2.1 and Fig. 2.5a). However, food-supplemented Blue Tits built the nest structural layer faster than control birds ($F_{1,75} = 9.14$, $P < 0.01$; Table 2.1 and Fig. 2.4b) resulting in a truncation of the total time taken to construct the nest ($F_{1,75} = 4.96$, $P = 0.03$; Table 2.1 and Fig. 2.4a).
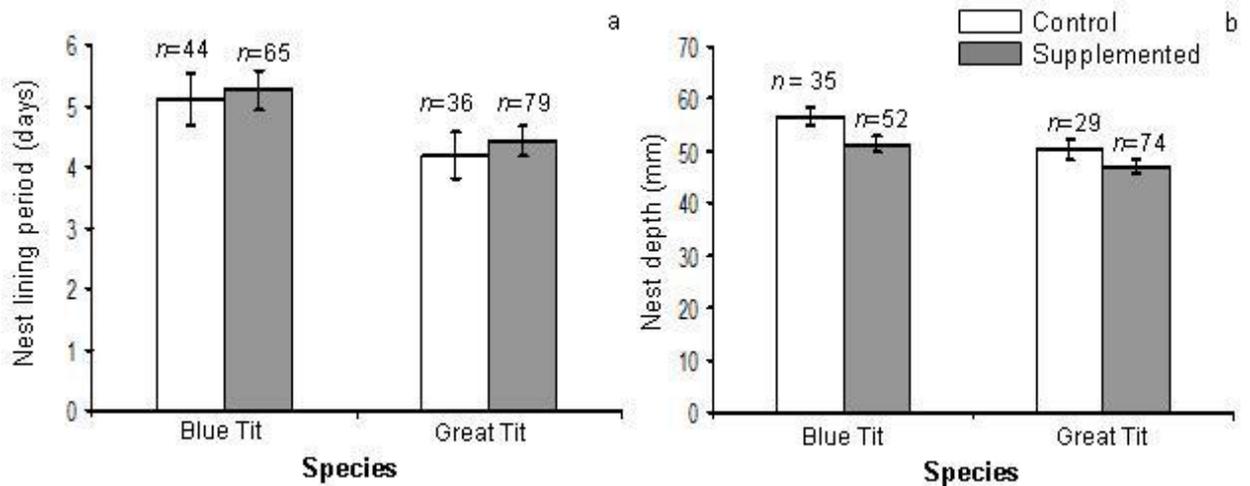
Figure 2.5. Nest building parameters (mean ± 1 SE from statistical estimates) of control (nonsupplemented) and food-supplemented Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve in 2008: (**a**) nest-lining period; and (**b**) nest depth. The number of nests (*n*) is given about each bar.

Furthermore, control Blue Tits built nests that were significantly deeper on completion than those breeding in the food-supplemented block ($F_{1,85} = 4.93$, $P = 0.03$; Table 2.1 and Fig. 2.5b). However, Great Tits built nests of equivalent depths irrespective of dietary treatment ($F_{1,100} = 1.92$, $P = 0.17$; Table 2.1 and Fig. 2.5b).

2.4.3. Seasonal trends in nest construction

Birds of both species in both the food-supplemented and the control blocks showed seasonal trends in nest building behaviour with nests that were initiated later in the season being constructed over a shorter period (Blue: $F_{1,75} = 164.23$, $P < 0.0001$; Great: $F_{1,100} = 106.38$, $P < 0.0001$; Table 2.1 and Fig. 2.6). Nests of Blue Tits that were initiated later in the season also took less time to line ($F_{1,82} = 21.00$, $P < 0.0001$; Table 2.1). In addition, there was a tendency for both Blue and Great Tits that started breeding earlier to lay larger clutches (Blue: $F_{1,76} = 3.73$, $P = 0.06$; Great: $F_{1,98} = 4.49$, $P = 0.04$).
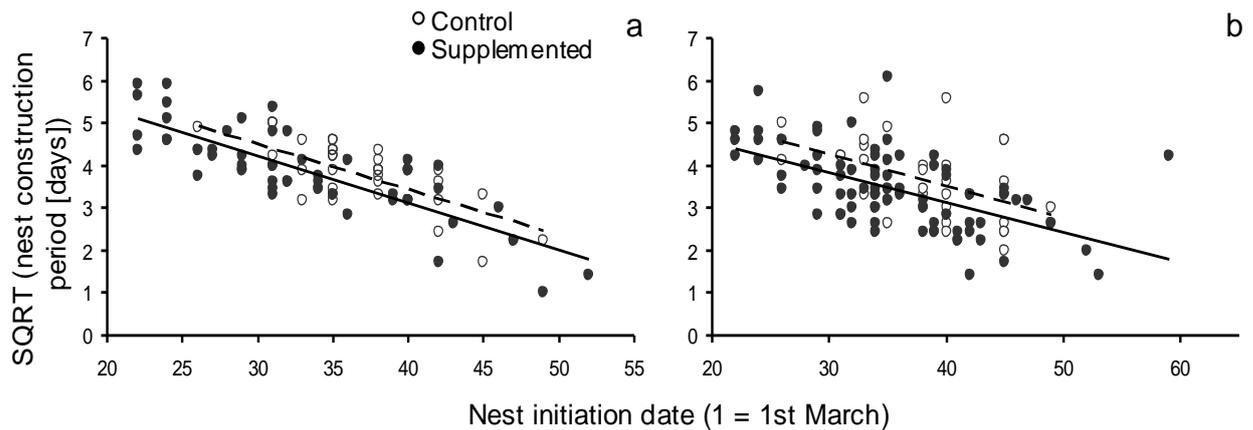
Figure 2.6. Nest construction period in relation to nest initiation date for food-supplemented (filled circles and solid regression line) and control (nonsupplemented) (open circles and dashed regression line) birds breeding in Chaddesley Woods National Nature Reserve in 2008: (**a**) Great Tits (Pearson's rs = 0.57, $P < 0.001$ and 0.52, $P < 0.001$, respectively). The slopes of the regression lines do not significantly differ (ANCOVA: $F_{1,114} = 3.31$, $P = 0.76$); and (**b**) Blue Tits (Pearson's rs = 0.82, $P < 0.001$ and 0.72, $P < 0.001$, respectively). The slopes of the regression lines do not significantly differ (ANCOVA: $F_{1,86} = 0.04$, $P = 0.84$).

2.5. DISCUSSION

The 'bird-nest unit' (Deeming 2002a) plays an integral role in avian development by contributing towards parental care efforts (e.g. Collias and Collias 1984, Kern et al. 1993) and, therefore, a bird should invest considerably in nest construction. Supplementary feeding should reduce a bird's time and effort spent foraging and, consequently, it might allow earlier, greater or more concentrated investment of time and effort in nest construction. I predicted that food supplementation would result in: (i). earlier nest construction; (ii). more rapid nest construction; (iii). construction of deeper nests; and (iv). a longer time engaged in nest-lining. I also predicted that any observed effects of supplementary feeding would be more pronounced in Great than in Blue Tits. I have shown only partial support for these predictions. Supplementary feeding resulted in: (i). advanced nest initiation in both Blue and Great Tits; (ii).

advanced egg laying in both Blue and Great Tits, and this was associated with (iii).

truncation of the nest construction period by Blue but not Great Tits; (iv). shallower,

rather than deeper, nests in Blue Tits but no effect in Great Tits; and (v). no effect on the

duration of nest-lining by either species.

2.5.1. Nesting phenology

The timing of reproduction can have a significant impact on the fitness of birds since

birds that fledge earlier have a higher probability of being recruited into the breeding

population (e.g. Verboven and Visser 1998). My results indicate that food availability at

least partly determines the timing of reproduction by significantly advancing nest

initiation date and egg laying date in both species. These results are consistent with

previous studies where supplementary feeding resulted in earlier initiation of nest

construction (e.g. Belted Kingfishers (*Megaceryle alcyon*) – Kelly and van Horne 1997;

Red-winged Blackbirds – Ewald and Rohwer 1982) and earlier egg laying (e.g.

Common Starlings – Källander and Karlsson 1993; Blue Tit – Svensson and Nilsson

1995; Blue and Great Tits – Harrison et al. 2010).

Two main, but not mutually exclusive, hypotheses have been proposed to

explain why food availability is likely to be a proximate cue in the reproductive timing

of birds: (i). Lack (1954) proposed that birds may use food availability to time breeding

so that they will be feeding nestlings in periods of peak food abundance; and (ii).

Perrins (1970) suggested that it is determined by the time at which a female can

accumulate enough resources to form and lay eggs. Birds that are supplemented with

food prior to egg laying experience an increase in the immediate availability of energy

enabling them to invest more time in activities such as territory defence, preening and

vigilance (e.g. Cucco and Malacarne 1997, Fleischer et al. 2003) resulting in the

advancement of onset of breeding attempts. However, food availability is unlikely to be exclusively responsible for the timing of breeding in birds and numerous other determinants have been identified, including ambient temperature (e.g. Dhondt and Eyckerman 1979), parental condition (e.g. Murphy 1986), reproductive experience (e.g. Perrins and McCleery 1985) and parental genotype (e.g. Lambrechts et al. 1999). In the present study food availability is likely to be the main (direct or indirect) driver of the effects found because environmental determinants were likely to have been constant across the different dietary treatment blocks and parental genotype, parental condition and reproductive experience were likely to have been randomly allocated between the experimental blocks (Harrison 2010). However, in my study, the provisioning of food is expected to increase both the relative quality of territories and the physiological condition of breeding adults, as well as increasing the immediate availability of energy and nutrients. Therefore, the particular mechanisms by which food-supplements advanced breeding (nest and clutch initiation) cannot be elucidated.

2.5.2. Nest construction period

The construction of a nest can be both energetically and temporally costly (e.g. Withers 1977) with the collection of nest material, rather than its transportation, being the principal cost. Some species are known to allocate a substantial amount of time to nest construction undertaking hundreds of trips to gather nest material (e.g. 873 trips per pair of Village Weavers [*Ploceus cucullatus*] – Collias and Collias 1984; 2,564 trips per pair of Black-billed Magpies [*Pica hudsonia*] – Stanley 2002). In accordance with my predictions, my results suggest that food-supplemented Blue Tits were able to invest more time and energy in nest building through a reduction in the energetic and temporal costs of foraging and this resulted in a shortening of the nest construction period. In

contrast, Mainwaring and Hartley (2009) found that Blue Tits supplemented with mealworms from the time of nest initiation through to nest completion did not alter their nest construction period. Similarly, Enoksson (1990) demonstrated that Eurasian Nuthatches (*Sitta europaea*) supplemented with Sunflower (*Helianthus annuus*) seeds during the winter spent an equivalent period of time constructing nests as the nonsupplemented conspecifics. However, my findings for Great Tits agreed with both Enoksson (1990) and Mainwaring and Hartley (2009) in finding no influence of food supplementation on their nest construction period.

2.5.3. Nest-lining period

The lining of a nest functions primarily to retain heat (e.g. Winkler 1993) and potentially improves reproductive performance (e.g. Winkler 1993, Lombardo et al. 1995). To maximise reproductive performance and, ultimately, fitness, birds that are able to reduce foraging time should invest more time collecting nest-lining materials to construct a well-insulated nest. I examined nest construction by examining periods to construct the structural layer and the nest-lining. The former was achieved faster in food-supplemented Blue Tits than in control birds suggesting that food supplementation reduced costs of collection and/or integration of materials into the nest. However, the duration of the nest-lining period was equivalent between the dietary treatments for both species. This suggests that it may be more fixed and less sensitive to food availability than other nesting behaviours. Food-supplemented birds might allocate the time saved as a result of reduced foraging effort in activities that have greater potential to increase fitness (e.g. seeking out EPCs, increasing vigilance). Alternatively, food-supplemented birds may invest more time in locating and collecting nest materials of higher insulatory quality instead of lining the nest more quickly compared with control birds.

2.5.4. Average nest depth

Average nest depth of food-supplemented Great Tits was equivalent to that of control birds but there was considerable variability within and between treatments. Similarly, Alabrudzińska et al. (2003) found much variability in nest depth (45-150 mm) within a population of Great Tits in Poland. In Blue Tits I found that food-supplemented birds made shallower nests than control birds. Building a shallow nest may be adaptive since constructing such a nest increases the distance between the nest entrance and the eggs/nestlings, reducing predation risk (Mazgajski and Rykowska 2008). Assuming that nest weight correlates positively with nest size, my results conflict with those of Mainwaring and Hartley (2009) who found that Blue Tits supplemented with mealworms built heavier nests and of de Neve et al. (2004) who found that food supplementation of Eurasian Magpies (*Pica pica*) resulted in larger nests. In accordance with the 'sexual-display' hypothesis (Soler et al. 1998), Eurasian Magpies employ nest size as a post-mating sexually-selected signal indicating the male builder's likelihood to invest in reproduction (de Neve et al. 2004). Nest size may act as a sexually-selected signal in both Blue and Great Tits. Nest size was positively correlated with female quality in the former (Mainwaring et al. 2008) and to female carotenoid-based plumage characteristics in the latter (Broggi and Senar 2009). Female investment in nest building in these species may relate to male condition (as measured by plumage coloration) through the 'differential allocation hypothesis' (Burley 1988).

2.5.5. Seasonal trends in nest construction

Similar to Mainwaring and Hartley (2008), I found that food-supplemented and nonsupplemented birds of both species showed a seasonal decline in nest construction period. Both food-supplemented and nonsupplemented birds synchronise their breeding

so that the greatest nutritional demands of the brood coincide with the peak abundance

of caterpillars, the main food of the nestlings (Perrins 1979). As hypothesised by

Mainwaring and Hartley (2008), it is likely that the seasonal decline in nest construction

period can be explained by this need to optimise the timing of breeding. An alternative

hypothesis could be that there is an increase in the availability of nest material as the

breeding season progresses. However, this is not likely to have applied since natural

feather and fur availability throughout the breeding season, often from dead animals, is

consistently high (Hansell and Ruxton 2002).

2.5.6. Future considerations and directions

There are a number of lines of further research that could be valuable in explaining

further the mechanism and functions of the effects of supplementary feeding that we

have described here. Ectoparasites, such as Hen Fleas (*Ceratophyllus gallinae*),

commonly inhabit nests of parids (Perrins 1979) with infestations having detrimental

effects on nestling growth (e.g. Richner et al. 1993). Parasite load can be reduced

through parental sanitation activities, with smaller nests being cleaned more easily

(O'Connor 1984) perhaps partially as a result of them having fewer parasites (Heeb et

al. 1996). It is possible that, instead of building deeper nests as I predicted, food-

supplemented Blue Tits allocated more resources to finding materials with high

insulatory properties (e.g. down feathers rather than moss – Hilton et al. 2004), allowing

them to reduce nest volume and parasite load, and to sanitise them more easily. To

further explain the mechanism and function of supplementary feeding in reducing nest

depth, further research is needed to determine the relationship between nest depth, nest

parasite load and nest sanitation behaviour. Furthermore, more work is needed to

determine why shallower nests were only found in supplemented Blue, but not Great, Tits.

Ideally, I would have made daily nestbox visits but this would have increased the disturbance of birds and the likelihood of nest abandonment. Instead I visited nests every 2-3 day which is unlikely to have biased my data significantly because any errors in determining duration of breeding phases would have been systematic across both dietary treatments. However, future studies could employ nestbox cameras to monitor nest construction daily to provide further insight into how supplementary feeding affects nest construction. Finally, I assumed that the availability of nest material was constant throughout the study site but further study of the distribution and availability of nest material throughout the study site and over the nesting period might explain some of the inconsistencies in parts of this study.

I have provided empirical evidence that food supplementation prior to and throughout nest construction has significant effects on nest building by both Blue and Great Tits in an English deciduous woodland. Determining the fitness benefits of food supplementation on reproductive performance mediated through changes in nest construction of birds remains problematic in my study because food supplementation was extended beyond nest completion and is the subject of the rest of this thesis. Therefore, it is likely that subsequent reproductive behaviours associated with downstream reproductive phases (i.e. incubation and brood provisioning) could have been influenced by food supplementation. Termination of food supplementation upon nest completion would allow more direct investigation of such effects. Further comparative studies of the effects of food availability on nest construction may provide considerable, and important, insights within a life-history context across different avian taxa.

2.5.7. The next chapter

During incubation nest architecture and integrity are fundamental to heat conservation within the nest (Deeming 2002a). Therefore, nests play a key role in reproduction by contributing to parental care efforts in the maintenance of thermal conditions during incubation (e.g. Collias and Collias 1984, Kern et al. 1993). With my findings showing a significant effect of supplementary feeding on nest construction, I will now investigate the effect of supplementary feeding on incubation behaviour in Chapter Three.

Chapter Three

THE EFFECTS OF SUPPLEMENTARY FEEDING ON THE INCUBATION

BEHAVIOUR OF BLUE AND GREAT TITS

3.1. ABSTRACT

Incubation is energetically demanding and, as an incubating bird partly relies on exogenous food sources for energy, food availability is likely to influence time spent in incubation. Here, I test the effects of food supplementation on the incubation behaviour of Blue and Great Tits during 2007-2009 with 2007 as a pilot year. Incubation in both Blue and Great Tits consists of periods off the nest (recesses) and periods on the nest (bouts) in which they perform a multitude of other more subtle behaviours such as bill pressing, tremble-thrusting and egg turning. By providing food *ad libitum* from approx. 4 weeks before egg laying to post-fledging, I investigated the effects of supplementary feeding on bout and recess lengths in Great Tits and daily incubation activity in Blue Tits. In both 2008 and 2009 supplementary feeding had a significant effect on recess lengths although the direction of effect was inconsistent between years; in 2008 supplemented female Great Tits had longer recess lengths, and in 2009 shorter recess lengths, compared with control (nonsupplemented) conspecifics. However, incubation bout lengths were equivalent across dietary treatments in both years. In Blue Tits supplementary feeding resulted in a decrease in daily incubation activity compared with control birds. My results provide evidence that food is a proximate factor that influences incubation behaviour. However, my results suggest that the effects of food supplementation on incubation behaviour are not always consistent between years highlighting that the exact effects of supplementary feeding may depend on other factors.

3.2. INTRODUCTION

In Chapter Two I provided empirical evidence that the supplementation of food prior to and throughout nest construction has marked effects on the nest building of both Blue and Great Tits. Nests can play a key role in reproduction by contributing to parental care efforts in the maintenance of thermal conditions during incubation (e.g. Collias and Collias 1984, Kern et al. 1993) as part of the 'bird-nest unit' (Deeming 2002a). In this chapter, I investigate the effects of supplementary feeding on incubation behaviour in both Blue and Great Tits.

3.2.1. Incubation behaviour and food availability

Incubation is the process by which eggs are maintained under optimal conditions of temperature and humidity for embryonic development (Deeming 2002a). In the majority of avian species stable egg temperature is achieved through the transfer of heat from the incubator's brood patch. This is a ventral body area that undergoes dramatic morphological changes prior to the onset of incubation that allow for efficient heat exchange between the incubator's body and the eggs (Lea and Klandorf 2002). Such heat exchange is termed 'contact incubation' (Deeming 2002a). Transfer of heat during contact incubation is regulated through the adjustment of blood flow through the brood patch (Lea and Klandorf 2002) so that embryo temperature is maintained at 37-38°C, the optimal temperature for embryonic development (Deeming 2002a). During incubation nest humidity partially determines water loss from eggs and, as excessive or insufficient water loss may result in abnormal embryonic development (Ar and Sidis 2002), nest humidity must also be regulated. In contrast to the maintenance of egg temperature, nest humidity is not actively regulated through egg contact with the incubating bird during incubation. Instead, regulation of

nest humidity is achieved through a combination of nest characteristics (e.g. selection of nesting material, construction of the nest, nest location) and physiology (e.g. water vapour loss by evaporation from skin, respiratory tract evaporation) of the incubator (Ar and Sidis 2002).

The incubator relies upon energy to fuel incubation. While some endogenous reserves may be mobilised to achieve this (e.g. Lesser Snow Goose [*Chen caerulescens*] – Ankney and MacInnes 1978, Brant Goose [*Branta bernicla*] – Ankney 1984) in small passerines the majority of energy is supplied from food which is either provided by the non-incubating partner visiting the nest or by foraging of the incubator in recess periods away from the nest (Deeming 2002a). The latter case is referred to as intermittent incubation when the incubator alternates time on the nest when eggs are warmed (the so-called incubation bout) with time off the nest when eggs cool (the so-called incubation recess). During this time the incubator restores depleted body reserves, conducts self-maintenance behaviours (e.g. preening) and may defend the territory (Skutch 1962, White and Kinney 1974, Deeming 2002a). Intermittent incubation strategies can be broadly classified into either biparental or single-sex incubation with the latter split further into male- or female-only incubation (Deeming 2002a). Female-only (gyneparental – Williams 1996, Deeming 2002a) intermittent incubation is common in many passerine species including the focal species of this study.

Incubation behaviour of intermittent incubators is not defined simply by measurement of bout and recess lengths, but it may also involve a multitude of other more subtle behaviours. For example, incubated eggs are continuously turned and moved around changing both their orientation and location within the clutch (Deeming 2002b). 'Egg turning' is a common behaviour elicited by almost all species

of bird and functions to prevent the embryo from adhering to the inner shell membrane (e.g. New 1957, Drent 1975) and to promote the formation of sub-embryonic fluid (e.g. Deeming et al. 1987, Babiker and Baggott 1995). Egg turning is vital during incubation since failure to turn eggs ultimately reduces the rate of embryonic development and hatchability (e.g. New 1957, Tullett and Deeming 1987, Deeming 1989). An incubating bird may also exhibit 'tremble-thrusting', a behaviour first described by Hartshorne (1962) while studying behaviour of Eastern Bluebirds (*Sialia sialis*) at the nest. Tremble-thrusting consists of the incubator adopting a head-down position, forcefully poking its bill deep within the nest material while vigorously twisting its head and trembling (Hartshorne 1962, Haftorn 1994). This behaviour is thought to assist egg turning (Hartshorne 1962) and to maintain the insulatory properties of the nest by creating small air pockets (Haftorn 1994). Tremble-thrusting often accompanies 'bill pressing' (Haftorn 1994) where the incubator presses its bill against the material of the nest cup lining, tightening it, reducing heat loss and improving the insulation of the eggs (Haftorn 1994, Haftorn and Slagsvold 1995).

Incubation is energetically expensive because there is a cost to re-warming and sustaining egg temperature as the incubator has to raise metabolic rates to increase heat production (Williams 1996, Tinbergen and Williams 2002). For example, numerous studies have demonstrated that the metabolic rate of an incubator exceeds that of a non-incubating conspecific under such conditions by 6-50% depending on the prevailing conditions and species (e.g. Vleck 1981, Haftorn and Reinertsen 1985, Weathers 1985). Energetic costs of incubation are further increased during periods when birds have to re-warm eggs (Vleck 1981) following a recess. Therefore, incubation is constrained by the availability of energy and, when energy is

limiting, the incubator may have to increase time spent foraging and in self-maintenance (e.g. Skutch 1962, Nilsson and Smith 1988, Moreno 1989).

Since food availability directly controls energy flow within the incubator, it is predicted to directly influence the time spent by the bird in self-maintenance and incubation (e.g. Martin 1987, Nilsson and Smith 1988, Moreno 1989). Numerous studies provide empirical evidence that food availability can influence incubation behaviour in this way (Table 3.1). For example, Rastogi et al. (2006) showed that Song Sparrows (*Melospiza melodia*) whose food availability was supplemented with a mixture of white proso millet, high fat/high protein pellets and oystershell during incubation had shorter recesses, longer bouts and, therefore, higher incubation attentiveness (total time spent on nest in a given period) than nonsupplemented conspecifics. As a result of increased incubation attentiveness, the risk of predation was reduced as the incubator had more time for nest guarding (Rastogi et al. 2006). In contrast, Karoo Prinia (*Prinia maculosa*) supplemented with mealworms had shorter recesses than nonsupplemented females whereas bouts did not differ in length (Chalfoun and Martin 2007).

While previous studies have investigated the effects of food availability on incubation behaviour, the majority have failed to consider the effects on the duration of incubation. Only a few studies have considered them, but they have provided inconsistent findings. For example, while some studies have demonstrated that supplementary feeding shortens incubation periods (e.g. Moreno 1989, Zimmerling and Ankney 2005), others

Table 3.1. Studies of the incubation biology of species in response to food availability including information about species, food type (S – supplemented; N – natural), incubation strategy (G – gyneparental [female-only with mate feeding]); U – uniparental [female-only without mate feeding]), and effects on various incubation parameters (↓ : decreased; = no change in response to food availability; ↑ : increased; – : data not available). Note: Common and scientific names of species follow Gill and Wright (2006).

Species common name	Species scientific name	Food type	Incubation parameter					Reference
			strategy	bout length	recess length	attentiveness	period	
New Zealand Robin	*Petroica australis*	N	G	↓	↓	=	n/a	Boulton et al. (2010)
Karoo Prinia	*Prinia maculosa*	S	U	=	↓	↑	n/a	Chalfoun and Martin (2007)
Australian Reed Warbler	*Acrocephalus australis*	S	U	↑	↓	↑	n/a	Eikenaar et al. (2003)
Bewick's Wren	*Thryomanes bewickii*	S	G	↑	=	↑	=	Pearse et al. (2004)
House Wren	*Troglodytes aedon*	S	U	↑	=	↑	=	Pearse et al. (2004)
Northern Mockingbird	*Mimus polyglottos*	S	U	n/a	↓	↑	n/a	Londoño et al. (2008)
Northern Wheatear	*Oenanthe oenanthe*	S	U	=	↓	↑	↓	Moreno (1989)
European Pied Flycatcher	*Ficedula hypoleuca*	S	G	n/a	↓	↑	n/a	Smith et al. (1989)
Water Pipit	*Anthus spinoletta*	N	G	↑	n/a	↑	n/a	Rauter and Reyer (1997)
Red-winged Blackbird	*Agelaius phoeniceus*	N	G	↑	↓	↑	↓	Zimmerling and Ankney (2005)
Song Sparrow	*Melospiza melodia*	S	U	↑	↓	n/a	n/a	Rastogi et al. (2006)

have demonstrated a significant effect on various aspects of incubation behaviour but no overall effect on incubation period (e.g. Pearse et al. 2004).

Recent findings from my study population in Chaddesley Woods National Nature Reserve have provided evidence that supplementary feeding from approx. 4 weeks before egg laying and through incubation resulted in a truncation of incubation period in both Blue and Great Tits (Harrison et al. 2010). However, little is known beyond this. Both of these parid species exhibit assisted gyneparental (i.e. female-only) incubation which lasts approx. 12-13 days (Perrins 1979, Williams 1996). While bouts and recesses occur throughout daylight hours (Hinde 1952, Perrins 1979), more discrete incubation behaviours, such as tremble-thrusting (Hartshorne 1962), are commonly elicited both throughout daylight hours and at night (e.g. Haftorn 1994, Deeming 2002b).

Here, I investigate the effects of supplementary feeding on incubation behaviour of Great Tits, by quantifying incubation bout and recess lengths, and Blue Tits by quantifying overall time spent in 'Daily Incubation Activity' (hereafter referred to as 'DIA'). In this study DIA describes the activity which occurs on the nest over a 24-hr period during incubation and reflects arrival and departures from the nest by the incubator, and also more subtle discrete behaviours such as tremble-thrusting, bill pressing and egg turning. I hypothesised that supplementary feeding would partly alleviate the energetic and temporal constraints on foraging, allowing incubating females to invest more heavily in incubation and less time in self-maintenance. I predicted that supplementary feeding would: (i). increase DIA of Blue Tits; (ii). decrease incubation recess lengths of Great Tits because energy demands can be satisfied more rapidly; and (iii). increase incubation bout lengths of Great Tits.

3.3. METHODS

3.3.1. Study site and supplementation

The study area, food supplements and food supplementation procedures were the same as described in Section 2.2.1. However, since this is a multi-year study I attempted to control for potential habitat effects by rotating dietary treatments between years (see Fig. 3.1 for details).
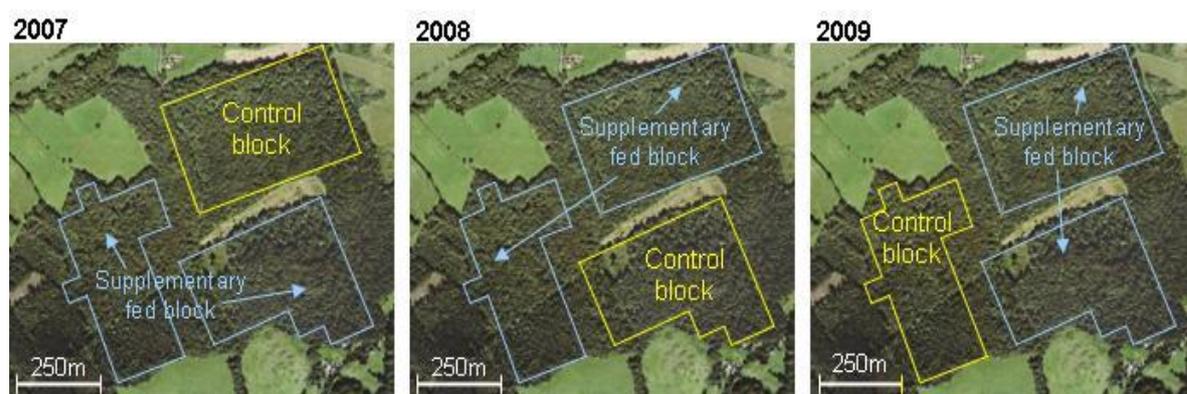
Figure 3.1. Allocation of supplementary and control (nonsupplemented) dietary treatments (see text for details) to areas of Chaddesley Woods National Nature Reserve, Worcestershire, UK where Great and Blue Tits were breeding between 2007 and 2009.

3.3.2. Incubation behaviour

Nestboxes were checked every 3-4 days to determine clutch initiation date from either direct observation of the first egg appearing or by back counting when more than one egg was present as Blue and Great Tits typically lay one egg every day around sunrise (Perrins 1979) until clutch completion. Thereafter, nestboxes were checked every 1-2 days to determine clutch completion date, clutch size and the onset of incubation, characterised by either the presence of an incubating female sitting tightly on the clutch or uncovered, but warm, eggs.

3.3.3. Temperature loggers – Blue and Great Tits (2007 – pilot year)

To investigate DIA Thermochron® iButton® loggers (Maxim Integrated Products.,

Sunnyvale, CA, USA; Fig. 3.2) (hereafter referred to as the 'iButton® logger') were

Figure 3.2. A Thermochron iButton® logger used to study the incubation behaviour
of Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve in
2007-2009. It is placed next to a 10 mm scale (Photo: N. Day).

used. They are programmable, small (16 mm in diameter and 6 mm in thickness),

durable temperature loggers that contain an in-built power supply and provide a

means of recording thermal data (Maxim Integrated Products 2010). I employed

iButton® logger model DS1922L (hereafter referred to as 'nest logger') to investigate

DIA. These are thermally sensitive between -40°C and +85°C allowing the collection

of thermal data that reflect both the temperature of the incubating female's brood

patch, which is maintained near to the optimal temperature for incubation (approx.

38°C – Deeming 2002a), and ambient temperature throughout the breeding season.

The use of such a device allowed the collection of data which reflected the arrival and

departure from the nest of an incubating bird. In addition, because egg temperature

changes vertically across an egg, being warmest at the surface which is in contact with

the brood patch and coolest at the surface which is in contact with the nest substrate

on the lower side (see Ar and Sidis 2002), thermal data obtained from a nest logger was likely to reflect egg turning rates and other subtle incubation behaviours that constituted DIA. The large memory capacity (8192 bytes which allows the collection of 8192 data points) of the DS1922L device allowed data collection over the majority of the incubation period for both Blue and Great Tits (approx. 12-13 days for both species – Perrins 1979, Harrison et al. 2010).

To determine DIA of Blue and Great Tits in 2007, nest loggers were programmed using OneWireViewer® software (Maxim Integrated Products., Sunnyvale, CA, USA) to record temperatures every 2 mins over an 11-day period. At the onset of incubation, upon departure of an incubator from a focal nestbox, a programmed nest logger was inserted at the top of the nestcup in close proximity to the top of the clutch, taking care not to disturb the eggs. Nest-lining material (fur or feathers) was used to cover the nest logger to avoid abrasion against the eggs. As a mean incubation period of 12-13 days was expected for both species (Perrins 1979), nestboxes were checked daily from day 10 of incubation to determine hatch date. Nest loggers were then carefully removed from nestboxes when brooding parent(s) were absent from the nestbox, and data were downloaded using the OneWireViewer® software.

During hatch checks, however, it was noted that some nest loggers had been either buried deep within the nesting material or removed from the nest by incubating birds. These behaviours were more common in Great Tits and, as a result, their use to record the DIA of this species was discontinued early in the 2007 breeding season.

3.3.4. Initial focal watches – Great Tits (2007 – pilot year)

As a result of problems with nest logger retention in Great Tit nestboxes (see above), focal nestboxes were, thereafter, directly observed or filmed using a Sony Handycam DCR-SR90E mounted on a tripod to investigate incubation behaviours of Great Tits from 2007 to 2009.

To minimise disturbance during filming, video cameras were set up away from the focal nestbox and then carefully positioned as far away as possible whilst still being able to obtain a clear view of the nestbox entrance hole. After their establishment, I quietly moved away ensuring I was not in close proximity to the nestbox so as not to disturb the incubating female. To minimise disturbance during focal watches, I made myself inconspicuous by positioning myself as far away as possible from nestboxes using trees and shrubs as cover whilst still being able to observe the nestbox entrance hole. Focal watches were initiated when the bird was observed arriving or departing from the nestbox. Arrival and departure times of birds at nestboxes were noted using a watch during direct observation in the field or from the time trace upon playback of video footage after the breeding season. Only the female incubates the eggs (see above) and any visits made by the male to the nestbox are usually to provide food to the female and are short in duration (e.g. Hinde 1952, pers. obs.). Therefore, female and male visits to the nestbox could be distinguished according to the observed behaviour. Incubation bout and recess lengths (of females) were calculated from her arrival and departure times at the nestbox. The expected bout and recess lengths of Great Tits are approx. 30 and 10 mins, respectively (e.g. Hinde 1952, Perrins 1979, Gosler 1993). Therefore, to obtain successive measures of both bout and recess lengths, focal watches were conducted over a 3-hr period starting 8-9 hrs after sunrise to control for diurnal variation in incubation behaviour (Hinde 1952). At the point that I abandoned the methodology using nest loggers (see above)

in favour of focal watches in 2007 many of the breeding Great Tits were in the later stages of incubation (i.e. at day 8 or beyond). To standardise the day on which focal watches were conducted, in the event that incubation behaviour changed with day of the incubation period, all focal watches during 2007 were conducted on day 10 of incubation.

3.3.5. Use of temperature loggers in 2008 and 2009 – Blue Tits

Although the majority of nest loggers placed in Blue Tit nests during 2007 had not been removed by the incubating bird, many had been partially buried in the nesting material. Initial inspection of temperature traces from buried nest loggers revealed that data resolution was poor (compare Fig. 3.3a and Fig. 3.3b), presumably as a result of the insulating properties of the nest material surrounding the device. Therefore, during 2008 and 2009, the initial approach was modified to overcome the burial of nest loggers by creating a mechanism to enable nest loggers to be physically attached to the nest. Prior to their deployment, nest loggers were glued using small pieces of double-sided adhesive tape to shirt buttons of similar diameter (approx. 15 mm) through which a length (approx. 20 cm) of garden wire was threaded to secure the nest logger to the nestbox (Fig. 3.4a). The thermal sensors within all models of iButton® logger are positioned closer to the bottom surface, which is more thermally sensitive than the top surface (C. Cooper pers. comm.), and is distinguished from the top surface by the absence of inscribed product information (Fig. 3.2). Therefore, all nest loggers were glued to shirt buttons using the top surface before being programmed as in 2007.

As in 2007, nestboxes were visited every 1-2 days to determine the onset of incubation. At this time a nest logger was secured to the nest by threading the attached
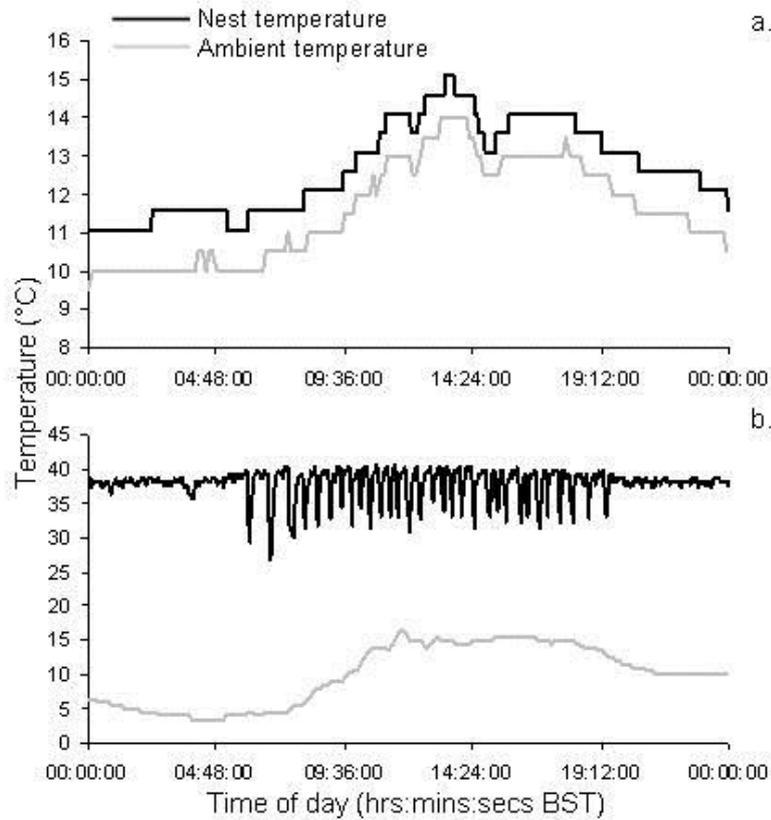
Figure 3.3. Recorded 24-hr ambient temperature traces and those from nest loggers: (**a**) buried beneath nest material showing low resolution of nest temperature; and (**b**) in close proximity to an incubating Blue Tit showing high resolution of nest temperature. Traces are from nest loggers placed in separate Blue Tit nests in Chaddesley Woods National Nature Reserve in 2007.
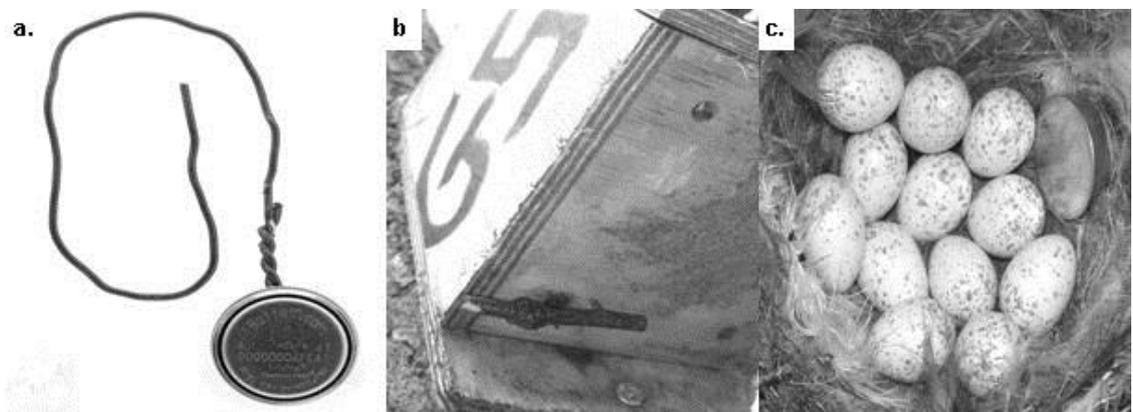
Figure 3.4. Attachment of nest loggers at the nest with wire: (**a**) fed through a shirt button that has been glued to the device; (**b**) secured on the outside of the nestbox after passing out of a nestbox drainage hole; and (**c**) directly tied into the nest substrate (Photos: N. Day and J. A. Smith).

garden wire though the nesting material and out of the nestbox drainage hole, securing it externally with a short twig (Fig. 3.4b). The nest logger was then positioned in the nest as in 2007 (Fig 3.4c). To minimise damage to nests through the repeated probing of garden wire, a large darning needle was used to allow the wire to be threaded through the nest with ease.

In order to control for the ambient temperature in the nestbox during subsequent data analysis (see Fig. 3.3), it was monitored using another iButton® logger type (model DS1921G, hereafter referred to as 'ambient logger') that was thermally sensitive between -40°C and +85°C. This has a smaller memory capacity than the DS1922L used in nests (2048 compared to 8192 bytes) and was, therefore, programmed to record temperatures at intervals of 8 mins. Each ambient logger was positioned at the onset of incubation at the centre of the back wall of the nestbox approx. 2 cm below the nestbox lid. As in 2007, egg hatch checks were conducted and ambient loggers were removed once hatching had occurred and data were downloaded.

3.3.6. Focal watches in 2008 and 2009 – Great Tits

To maximise data collection in 2008 and 2009 focal watches were conducted on a randomly chosen day between days 3 and 9 of incubation, but in all other respects focal watches were as conducted in 2007. This approach was justified since full daytime incubation does not develop until 2-3 days after the onset of incubation (Haftorn 1981). Once a regular incubation pattern is established, incubation constancy (i.e. the total time that an incubating bird is on the nest during incubation – Skutch 1962), is consistent throughout the rest of the incubation period (Zerba and Morton 1983).

3.3.7. Data analysis from temperature loggers

Data from all nest loggers were visualised using the chart function in Excel to determine data quality. Data were considered to be of low quality when the average temperature detected was <25°C and the low resolution of temperature fluctuations made it problematic to determine if, and when, incubation behaviours occurred (Fig. 3.3a). Such data were omitted from statistical analyses.

Initial observations of nest logger data revealed that temperature fluctuations occurred continuously over a 24-hr period and at regular intervals (5-10 mins), thereby providing information about incubation activities of Blue Tits (Fig. 3.5).
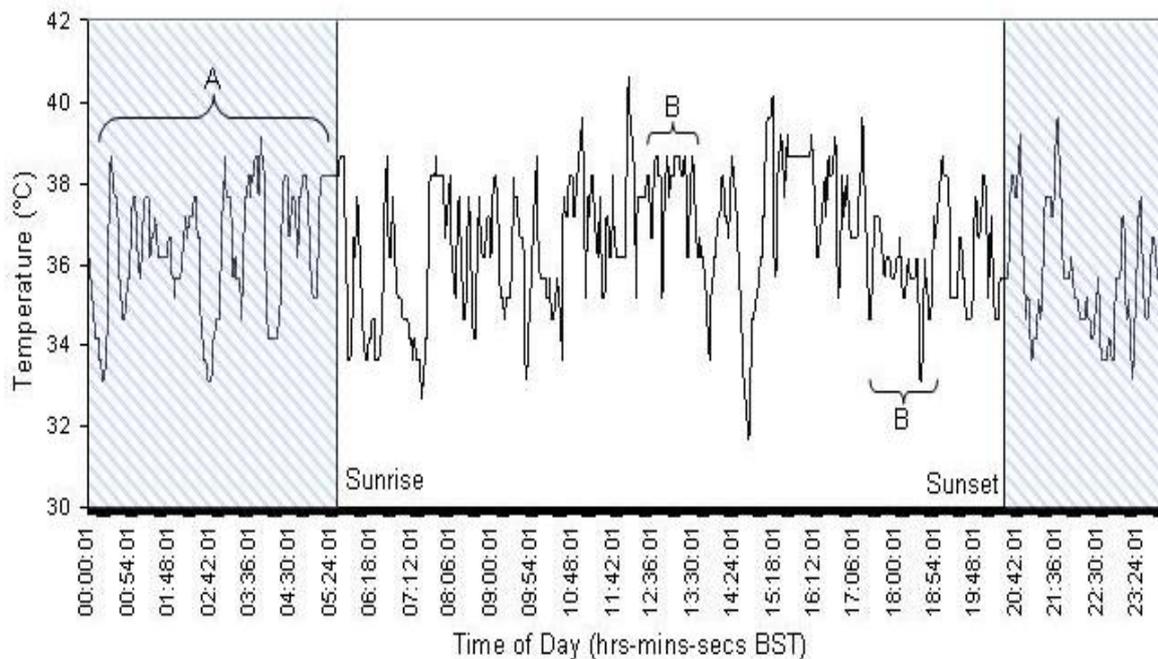
Figure 3.5. A 24-hr temperature trace from a nest logger placed in the nest of an incubating Blue Tit in Chaddesley Woods National Nature Reserve in 2007. The trace shows nest temperature with marked temperature fluctuations (A) throughout the night, and periods of more subtle temperature fluctuations (B) punctuating the trace throughout the day.

To quantify DIA (reflecting arrival and departures from the nest by the incubator and other subtle incubation behaviours), nest logger data were separated into 24-hr blocks

(00.00-23.59 hrs GMT) for each day of incubation and each block was then plotted as

a line plot in Excel and analysed.

I counted the number of times temperature shifted in direction (i.e. from

increasing to decreasing or *vice versa* – Fig. 3.6a), and the number of times
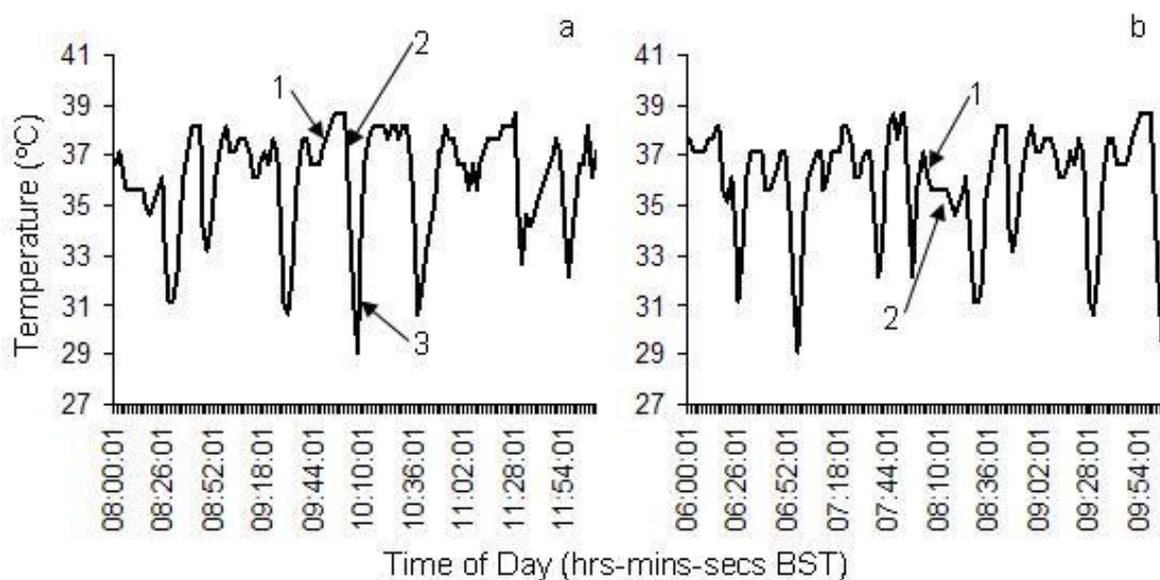
Figure 3.6. Temperature traces from a nest logger placed in the nest of an incubating Blue Tit in Chaddesley Woods National Nature Reserve in 2009 showing (**a**) shifts in temperature directions (1, 2 and 3), and (**b**) shifts in temperature to and from a period of constant temperature (1 and 2) (see text for details).

temperature shifted to and from a period of constant stable temperature (Fig. 3.6b). I

assumed that all temperature shifts reflected female movement associated with

incubation behaviour. Data were collected for all days of incubation, providing

repeated measures for each nestbox, apart from the partial days when nest loggers

were deployed and hatching occurred. In addition, if a bird appeared to 'abandon' the

nest for a period of >4 hrs, data from that day were discounted as it was assumed that

this did not represent routine incubation behaviour (Fig. 3.7).
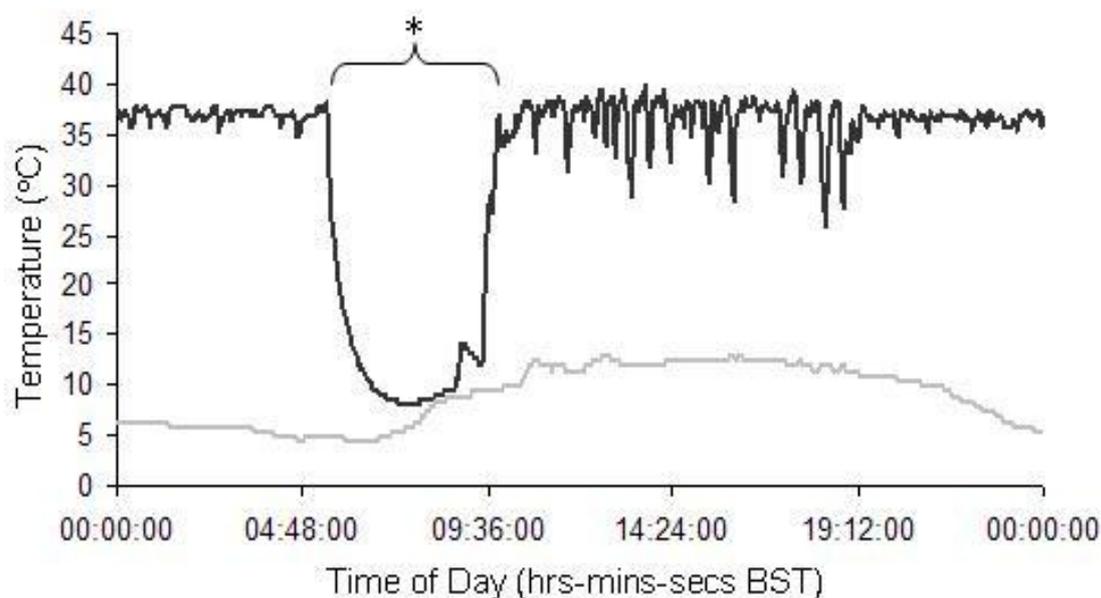
Figure 3.7. A 24-hr temperature trace from a nest logger (black line) and an ambient logger (grey line) placed in a nestbox of an incubating Blue Tit in Chaddesley Woods National Nature Reserve in 2009 showing a prolonged recess length or period of 'abandonment' (*).

3.3.8. Summary of data to be presented

- Incubation bout and recess lengths of Great Tits breeding in 2007-2009 collected using focal watches. Both bout and recess lengths were measured in secs (see Section 3.3.6)

- Daily Incubation Activity (DIA), reflecting arrival and departures from the nest of the incubator and subtle incubation behaviours (including egg turning, tremble-thrusting and bill pressing) of Blue Tits breeding in 2007-2009 collected using nest loggers. DIA was measured as temperature shifts per 24-hr period (see Section 3.3.7)

3.3.9. Statistical analysis

Due to small sample sizes in 2007 (focal watches: $n = 4$ per treatment block; nest loggers: $n = 5$ per treatment block), only data collected in 2008 and 2009 were considered in statistical analyses. Mixed models were constructed using PROC GLIMMIX in SAS version 9.2 (SAS Institute Inc. 2008) to test for the effects of supplementary feeding on individual response variables. Average bout and recess lengths were tested for normality using the Anderson-Darling Test and for homogeneity of variance using the F-test in Minitab 15 (Minitab 2007). Both were log transformed to normalise their distributions and then fitted with normal error distributions after examination of model fit (Generalised Chi-Square/DF = approx. 1). DIA was fitted with a Poisson error distribution.

In models where average bout or recess length was the response variable, treatment block and day of incubation of focal watch were specified as random factors due to the repeated measures treatment block and incubation day data. Ideally, nestbox nested in treatment block would have been included as a random factor too but there was insufficient power in either model to allow for this. For DIA as the response the variable nestbox nested in treatment block was specified as a random factor to account for repeated measures on nestboxes both within and between years. In all analyses year was included as a fixed factor to account for potential annual variation in breeding performance (Nager et al. 1997). Combined-year analysis was conducted if there was no significant interaction between dietary treatment and year (year $\times$ dietary treatment: $P > 0.05$) and no main effect of year (year: $P > 0.05$). Otherwise independent year analyses were performed.

For all analyses, clutch size and clutch completion date were included as covariates as they both may affect incubation behaviour (e.g. Haftorn and Reinertsen 1985, Moreno and Carlson 1989). Since ambient temperature may also influence

incubation behaviour (e.g. Conway and Martin 2000a,b), average daily ambient temperature was also included as a covariate. For DIA analyses ambient temperatures collected from each focal nestbox were used. However, to calculate average daily ambient temperatures for the days in which focal watches were conducted, I used data recorded by ambient loggers in nestboxes of Blue Tits. Average daily temperatures from all ambient loggers deployed throughout the study site were then combined and averaged for each day to reflect average daily ambient temperature across the study site. To determine if the effects of covariates were consistent between treatment blocks, covariate $\times$ dietary treatment interactions were tested independently of one another within each model. Where multiple covariate $\times$ dietary treatment interactions were significant they were both included in the model as long as each stayed significant (Grafen and Hails 2002). I performed backward model selections removing the least non-significant covariate interactions ($P > 0.05$) one-by-one from models ensuring that there was no significant change in deviance between models where applicable (Grafen and Hails 2002).

3.4. RESULTS

3.4.1. DIA (Blue Tits)

Combined-year analysis was conducted since there was no significant interaction between dietary treatment and year (year $\times$ dietary treatment: $P > 0.05$) and no main effect of year (year: $P > 0.05$) on DIA. Supplementary feeding significantly decreased DIA in Blue Tits ($F_{1,398} = 4.13$, $P = 0.04$; Fig. 3.8 and Table 3.2). Average daily temperature as a covariate further explained variation in DIA (DIA increased with increasing daily temperature $-$ $F_{1,398} = 4.04$, $P = 0.05$) as did a clutch size $\times$ dietary treatment interaction (DIA was positively correlated with clutch size in the

nonsupplemented block and negatively correlated with clutch size in the

supplementary fed block).
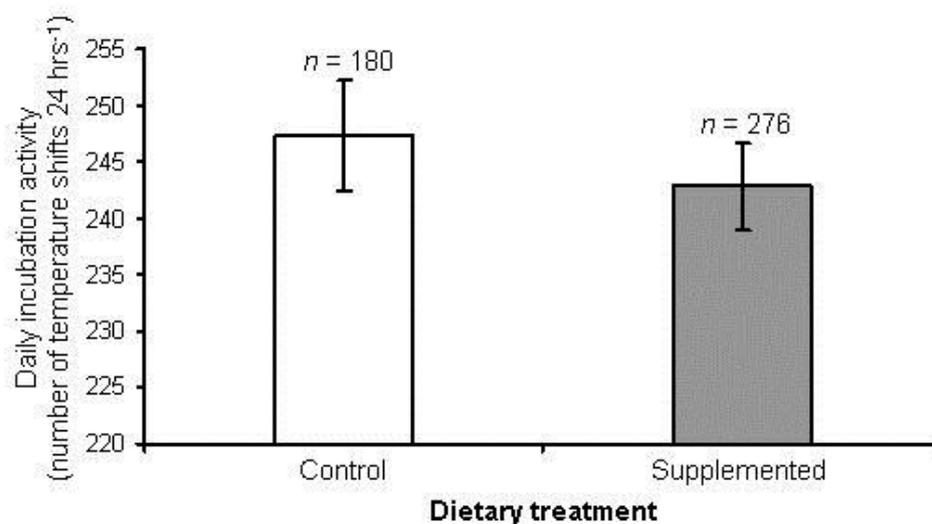
Figure 3.8. Daily incubation activity (mean ± 1 SE from statistical estimates) of control (nonsupplemented) and supplemented Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. The number of nests (*n*) is given above each bar.

3.4.2. Bout lengths (Great Tits)

Combined-year analysis was conducted since there was no significant interaction

between dietary treatment and year (year × dietary treatment: $P > 0.05$) and no main

effect of year (year: $P > 0.05$) on bout length. Supplementary feeding had no

significant effect on the average bout lengths of Great Tits ($F_{1,60} = 2.62$; $P = 0.11$; Fig.

3.9 and Table 3.2).

3.4.3. Recess lengths (Great Tits)

Recess length data were treated separately for each year of study following a

significant year × treatment effect ($F_{1,253} = 5.24$, $P = 0.03$; Table 3.2). In both 2008

and 2009 supplementary feeding had a significant effect on recess lengths of Great

Tits (2008: $F_{1,24} = 5.82$, $P = 0.02$; 2009: $F_{1,26} = 5.53$, $P = 0.03$; Table 3.2). However,

Table 3.2. Results from the final statistical models (see text for details) explaining measurements of incubation behaviour in control (nonsupplemented/N) and supplemented (S) Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. Significant main and interaction effects are given in bold. Directions of significant effects are given '+' and '-' denote significant positive and negative relationships, respectively, and 'NS' denotes nonsignficance ($P > 0.05$).

Response variable	Species	Year	Factor	F	df	P	Least square means (SE)		Direction
							Nonsupplemented	Supplemented	
Incubation bout length	GT	2008+2009	Dietary treatment	2.62	1,60	0.11	1299.27(109.04)	1524.05(87.71)	NS
			Year	1.28	1,60	0.26			NS
Incubation recess length	GT	2008+2009	Dietary treatment	0.16	1,53	0.92	457.09(79.94)	489.78(59.76)	NS
			Year	**4.67**	**1,53**	**0.04**			2008 = longest; 2009 = shortest
			Ambient temperature	**4.04**	**1,53**	**0.05**			+
			Year × dietary treatment	**5.14**	**1,53**	**0.03**			
	GT	2008	Dietary treatment	**5.82**	**1,24**	**0.02**	424.62(74.26)	691.83(84.42)	N = shortest; S = longest
	GT	2009	Dietary treatment	**5.53**	**1,26**	**0.03**	537.03(93.93)	338.84(32.69)	N = longest; S = shortest
			Clutch completion date	**7.21**	**1,29**	**0.01**			-
Daily incubation activity	BT	2008+2009	Dietary treatment	**4.13**	**1,398**	**0.04**	247.28(4.91)	242.82(3.89)	N = highest; S = lowest
			Year	1.49	1,398	0.23			NS
			Ambient temperature	**4.04**	**1,398**	**0.05**			-
			Clutch size	1.53	1,398	0.22			NS
			Clutch size × dietary treatment	**4.88**	**1,398**	**0.03**			N = +; S = -
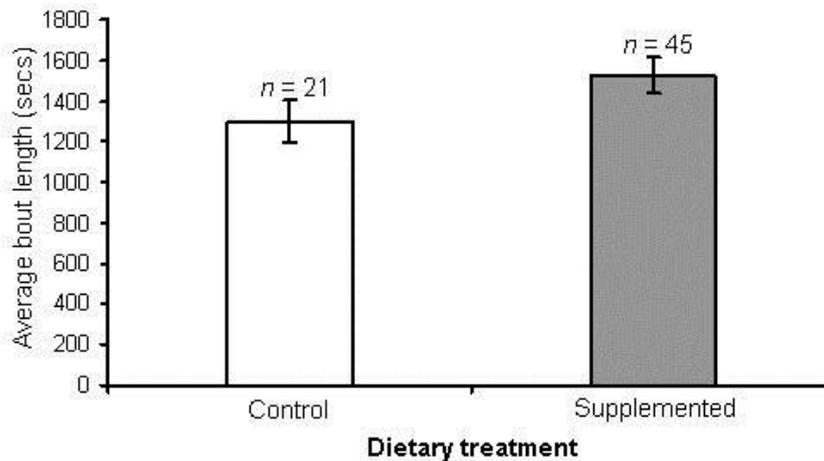
Figure 3.9. Average bout lengths (mean ± 1 SE from statistical estimates) of control (nonsupplemented) and supplemented Great Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. The number of nests (*n*) is given above each bar.

the effect of supplementary feeding on the recess lengths of Great Tits was opposite between years with recess lengths of supplemented Great Tits being shorter in 2008 but longer in 2009 (Fig. 3.10).
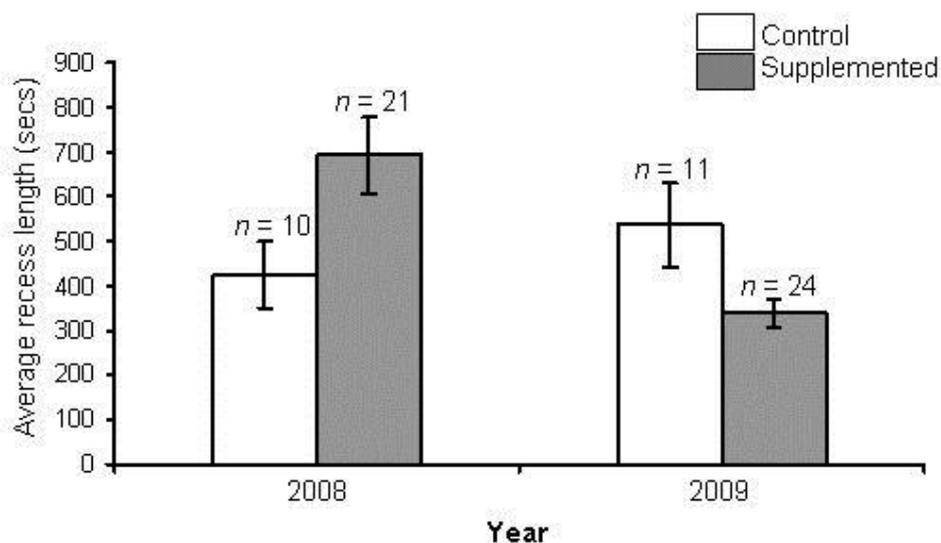
Figure 3.10. Average recess lengths (mean ± 1 SE from statistical estimates) of control (nonsupplemented) and supplemented Great Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. The number of nests (*n*) is given above each bar.

3.5. DISCUSSION

I hypothesised that supplementary feeding would partly alleviate the energetic and temporal constraints on foraging, allowing incubating females to invest more heavily in incubation and less time in self-maintenance. I only found partial support for this hypothesis. Contrary to it, supplemented Blue Tits had a lower DIA than nonsupplemented conspecifics while supplemented Great Tits in 2008 had longer recess lengths than nonsupplemented conspecifics. In support of the hypothesis, I found that supplemented Great Tits in 2009 had shorter recess lengths than nonsupplemented conspecifics but I found no effect on bout lengths of Great Tits in either 2008 or 2009.

3.5.1. Effect of supplementary feeding on DIA of Blue Tits

Supplementary feeding decreased (rather than increased) DIA of birds. DIA reflects arrival and departure of birds, tremble-thrusting, bill pressing and egg turning and, therefore, differences in any of these behaviours between treatments could have contributed to my overall measure of DIA. The primary function of tremble-thrusting during incubation is to increase the insulating properties of the nest by creating small air pockets (Haftorn 1994). Although I do not have data regarding the types of nest material or nest insulatory properties, it is possible that supplemented Blue Tits constructed nests with higher insulatory properties (e.g. down feathers rather than moss – Hilton et al. 2004) than nonsupplemented birds (see Section 2.4). Therefore, the amount of tremble-thrusting exhibited by supplemented birds might be decreased over that of nonsupplemented birds as a result of having more insulated nests.

In my study population supplemented Blue Tits lay smaller clutches than their control counterparts (Harrison et al. 2010). Although I do not have data reflecting the

extent of egg turning behaviour, it is possible that supplementary fed Blue Tits spent less time egg turning during incubation than their control counterparts as a direct consequence of having fewer eggs. If egg turning behaviour is reflected through the data loggers then such inconsistencies in egg turning behaviour between supplemented and nonsupplemented Blue Tits could account for the differences observed in DIA. However, caution should be taken when interpreting DIA since it is unclear exactly what behaviours are reflected in data from the nest loggers.

3.5.2. Effect of supplementary feeding on bout lengths of Great Tits

In support of my predication that supplemented birds would have longer bout lengths than nonsupplemented birds, a number of studies (see Table 3.1) have found that supplementary feeding enables incubators to increase incubation bout lengths and nest attentiveness suggesting that supplemented birds are less energetically constrained than nonsupplemented conspecifics. In contrast, I found no significant effect of supplementary feeding on incubation bout lengths in either 2008 or 2009. In my study supplementary food was available to both sexes but I only considered its effects on female behaviour. In both Blue and Great Tits males feed females during incubation, a behaviour known as incubation feeding (Nilsson and Smith 1988). When food is abundant, however, the time budget of a male may be adjusted if the energetic demands of the female are already satisfied through self-feeding. For example, male Bewick's Wrens (*Thryomanes bewickii*) decreased their rate of incubation feeding to females that were supplemented with mealworms during incubation suggesting that incubation feeding rates were regulated according to the nutritional state of the female (Pearse et al. 2004). In my study it is possible that incubation feeding rates of nonsupplemented males were higher than those of supplemented males in order to

satisfy the energetic demands of nonsupplemented females. While food

supplementation may have enabled females to increase bout lengths compared with

nonsupplemented birds, a disparity in incubation feeding rates between

nonsupplemented and supplemented males may have resulted in the nutritional and

energetic states of females in both dietary treatments being equivalent, resulting in

equivalent bout lengths.

3.5.3. Effect of supplementary feeding on recess lengths of Great Tits

In 2009 supplementary feeding resulted in shorter recess lengths of birds suggesting

that increased food availability alleviated the energetic and temporal costs of foraging,

thereby allowing them to reduce time away from the nest. These results support my

prediction that supplementary feeding would reduce recess lengths and are consistent

with previous studies that have demonstrated that supplementary food decreases

recess lengths (see Table 3.1). Birds that reduce time away from the nest are likely to

accrue fitness benefits through reductions in predation risk (Martin and Ghalambor

1999, Conway and Martin 2000a, Martin et al. 2000a) and sustained egg temperature

that prevents then from falling below physiological zero, the temperature at which

embryonic development suspends (Turner 2002). This reduces the potential of

associated adverse effects on embryonic development (e.g. Feast et al. 1998, Olsen et

al. 2008).

In contrast to findings in 2009, supplementary feeding in 2008 resulted in

longer recess lengths than those of nonsupplemented birds. A difference between

years in natural food availability and the time budget of males could partly explain the

inconsistency in findings across years. For example, during incubation males must

budget time and energy accordingly between activities (e.g. incubation feeding,

territoriality, partaking in EPC) to maximise fitness benefits (e.g. Sibley and

McFarland 1976, McFarland 1977). When food is abundant, time allocated towards

defending a feeding territory may be reduced since the costs of territorial behaviour

may exceed the associated benefits of gaining additional resources (Carpenter 1987).

Under these circumstances a male may be able to allocate more time and energy

towards incubation feeding (Nilsson and Smith 1988). In this study I do not have data

on the availability of natural food during incubation. However, if natural food

abundance during incubation was high during 2009, then it is possible that the

additional supplementary food enabled supplemented males to reduce the time spent

in territorial behaviour compared to nonsupplemented conspecifics and allocate more

towards incubation feeding. An increase in incubation feeding would partly alleviate

the energetic and temporal costs of foraging, thereby allowing females to reduce time

spent away from the nest to forage. In contrast, if natural food abundance was low

during 2008, then it is possible that the additional supplementary food resulted in

supplemented males allocating more time and energy towards territoriality than

nonsupplemented conspecifics since the benefits of gaining additional resources could

have exceeded the costs of territorial behaviour. A subsequent reduction in incubation

feeding by supplemented males may have resulted in supplemented females in 2008

having to spend more time and energy in satisfying their own nutritional and energetic

demands than nonsupplemented conspecifics resulting in longer recess lengths.

3.5.4. Mechanisms underlying incubation periods of my study population
Food supplementation results in truncation of the incubation period of both Blue and

Great Tits in my study population and this effect is consistent between years (Harrison

et al. 2010). Although changes in incubation behaviour have been shown to underlie

changes in incubation period, with increased incubation bouts and nest attentiveness

being associated with shorter incubation periods (Moreno 1989, Zimmerling and

Ankney 2005), mechanisms underlying such incubation patterns in my study

population remain unclear.

Supplemented females may be able to maintain egg temperature at the optimal

temperature for embryonic development (i.e. approx. 38°C – Deeming 2002a) more

successfully than nonsupplemented (control) females through meeting the energetic

demands of incubation more readily. Challenges to the incubating bird can include

declines in ambient temperature that can result in reduced rates of embryonic

development if the incubating bird does not invest more time and energy by

prolonging bout lengths (e.g. Booth 1987, Martin et al. 2007). Even marginal

reductions in ambient temperature (e.g. by 1-2°C) can prolong incubation by 1-2 days

(e.g. Domestic Fowl [*Gallus gallus*] – Deeming and Ferguson 1991).

3.5.5. Further considerations and directions

In this study I have discussed the effects of supplementary feeding on incubation

behaviour primarily in consideration of how supplementary feeding may affect

allocation of time and energy between behaviours associated with incubation and self-

maintenance (e.g. foraging). However, as incubation behaviour is likely to be state-

dependent, incubation rhythms may vary with the body condition of the incubator

(e.g. Erikstad 1986, Gorman and Nager 2003). For example, individual body

condition (i.e. residuals of body mass regressed on head-bill length) of female Tree

Swallows (*Tachycineta bicolor*) influenced incubation behaviour with individuals in

better body condition having higher incubation attentiveness (Ardia and Clotfelter

2006). Since food availability may influence body condition (e.g. Schoech 1996,

Cucco and Malacarne 1997), it is possible that differences in the body condition of incubating females between the dietary treatments underlie the results of my study. However, I did not collect body condition data of incubating females and, therefore, future research should consider this in investigations of incubation behaviour.

I have provided empirical evidence that food supplementation prior to and throughout incubation has marked effects on both recess lengths of Great Tits and on DIA of Blue Tits but not on bout length of Great Tits. However, my findings do not explain the truncation of the incubation period as a result of supplementary feeding as reported by Harrison et al. (2010) from the same population. Future studies should further consider subtle incubation behaviours through the use of telemetric eggs, for example, that accurately quantify egg turning rates (Boone and Mesecar 1989). Furthermore, direct measurements of egg temperature, in addition to energetic expenditure of incubating females, are likely to add to our knowledge of how supplementary feeding influences incubation behaviour.

3.5.6. The next chapter

Successful incubation results in egg hatching and, in altricial species, hatching initiates the onset of the nestling period in which the adults provide food, through brood provisioning, and warmth to the developing nestlings (Bennett and Owens 2002). While food availability is likely to limit reproduction and influence life-history traits (Martin 1987), the allocation of energy and nutrients from exogenous food sources is likely to vary across different reproductive stages (Martin 1987). With my findings showing a significant effect of supplementary feeding on incubation behaviour, I will now investigate the effect of supplementary feeding on brood provisioning behaviour in Chapter Four.

Chapter Four

THE EFFECTS OF SUPPLEMENTARY FEEDING ON BROOD PROVISIONING

BEHAVIOUR OF BLUE AND GREAT TITS

4.1. ABSTRACT

Brood provisioning behaviour is fundamental to the growth and survival of nestlings but is energetically costly. Therefore, parents must balance the time spent provisioning food to nestlings against that spent in self-maintenance to maximise energy delivered to nestlings without compromising their own energy balance. As food should alleviate energetic and temporal costs of self-maintenance, supplementary feeding provides a means of investigating provisioning behaviour and the trade-off between self- and alloprovisioning. I studied the effects of food supplementation on brood provisioning behaviour, nestling condition and post-fledging apparent survival of Blue and Great Tits between 2007 and 2009. Food supplementation had significant effects on both brood provisioning rates and post-fledging apparent survival but the direction of the effects was inconsistent between years. In contrast, supplementary feeding had no effect on nestling condition. As an index of natural food availability, the relative abundance of caterpillars (Winter Moth [*Operophtera brumata*] and *Geometridae* spp.) was determined throughout the study period. I use these data to suggest that the inconsistent effects of supplementary feeding on brood provisioning behaviour between years may partly result from inter-year differences in natural food availability.

4.2. INTRODUCTION

In Chapter Three I provided empirical evidence that food supplementation prior to and throughout incubation has marked effects on incubation behaviour. I showed significant effects on incubation recess length in Great Tits and DIA of Blue Tits but not on incubation bout length of Great Tits. Successful incubation results in egg hatching and in altricial species this initiates the nestling period in which the adults provide food (referred to hereafter as brood provisioning) and warmth to the developing nestlings (Bennett and Owens 2002). While food availability is likely to limit reproduction and influence life-history traits (Martin 1987), the allocation of energy and nutrients from exogenous food sources is likely to vary across different reproductive stages (Martin 1987). Therefore, with my findings showing a significant effect of supplementary feeding on incubation behaviour, in this chapter I investigate the effect of supplementary feeding on brood provisioning behaviour.

4.2.1. Brood provisioning and food availability

Nestlings of altricial species rely on parental food provisioning to meet their energetic and nutritional demands (O'Connor 1984). Food is critical for nestling growth, development and survival, and body mass at fledging in many species is directly related to the likelihood of survival to adulthood, and to subsequent breeding (e.g. Smith et al. 1989, Naef-Daenzer et al. 2001, Monrós and Barba 2002). Where parental feeding does not meet the food demands of the nestlings, constraints on nestling growth may be imposed and this can result in brood reduction, lower body masses at fledging, and a reduction in post-fledging survival (e.g. Nur 1984, Naef-Daenzer and Keller 1999, Ringsby et al. 2009).

Although brood provisioning is clearly fundamental to reproductive success of birds, such behaviour is costly to the parents. Repeated parental visits to the nest are likely to increase the risk of predation (Martin et al. 2000b) while foraging behaviour is energetically demanding (reviewed in Bryant and Tatner 1991). Therefore, during brood provisioning, the adult must provide food for not only its nestlings but also for self-maintenance to fuel its metabolism to sustain provisioning efforts (Ydenberg et al. 1994, Ydenberg 1998). Therefore, the provisioning adult must balance these two energetic demands (i.e. maximise energy delivered to nestlings without compromising its own energy balance – Nur 1984, Kacelnik and Cuthill 1990, Ydenberg et al. 1994). Brood provisioning and overall investment in current young, therefore, can be viewed as a trade-off between the potential fitness benefits of offspring production *versus* adult survival to breed again; they should be optimised to maximise life-time fitness benefits (Nur 1984, Kacelnik and Cuthill 1990).

As food should alleviate energetic and temporal costs of self-maintenance, it is likely to influence parental investment in brood provisioning and, subsequently, offspring survival. Therefore, when natural foods are available over a brief period, birds should time their breeding so that the peak energetic demand of nestlings coincides with the peak in natural food availability (e.g. van Noordwijk et al. 1995, Thomas et al. 2001). This will allow maximisation of investment in brood provisioning, resulting in high nestling growth rates and development (e.g. Goodbred and Holmes 1996, Naef-Daenzer and Keller 1999). For example, Blue and Great Tits time their breeding so that the peak energetic demand of nestlings coincides with the peak in the abundance of caterpillars (Winter Moth [*Operophtera brumata*] and other *Geometridae* spp. – Gibb 1950, van Noordwijk et al. 1995), the preferred foods for brood provisioning by both Blue and Great Tits (Perrins 1965, 1991, Wilkin et al.

2009b). When a mismatch between these peaks occurs there is likely to be an increase in the energetic costs of foraging for the adults (Tinbergen and Dietz 1994, Thomas et al. 2001) and a decrease in brood provisioning rates (Naef-Daenzer and Keller 1999, Naef-Daenzer et al. 2000). Such a mismatch may be driven by spring temperature that is likely to be the primary cue for caterpillar emergence (Visser and Holleman 2001). The timing of peak caterpillar abundance is earlier in years when mean spring temperature is higher (Visser et al. 1998). Tits initiate breeding in advance of caterpillar emergence but if temperatures are high then the peak in caterpillar abundance may occur before the peak food demand of the nestlings.

The effects of food availability on parental investment in brood provisioning can also be investigated by providing supplementary food which allows us to manipulate and control food availability and test specific hypotheses. Indeed, numerous supplementary feeding studies have provided empirical evidence that the availability of supplementary food can influence brood provisioning behaviour (Table 4.1). For example, Cucco and Malacarne (1997) provided Wax Moth (*Galleria mellonella*) caterpillars during the nestling period (14 days) to adult Black Redstarts (*Phoenicurus ochruros*) and investigated the time budgets of both parents. During the nestling period supplemented males spent more time singing, preening and being vigilant and less time feeding nestlings compared to nonsupplemented (control) birds. In contrast, supplemented females fed nestlings significantly more than control conspecifics to compensate for the reduction in male feeding rates. Similarly, Simons and Martin (1990) provided mealworms and caterpillars of *Pseudaletia* spp. and *Trichoplusia* spp. during the entire nestling period to Cactus Wrens (*Campylorhynchus brunneicapillus*) that then delivered more food to nestlings than

Table 4.1. Studies of parental investment during the nestling period in response to food availability including information about species, food type (S – supplementary fed; N – natural) and effects on rates of brood provisioning (↓ : decreased; = no change in response to food availability; ↑ : increased; – : data not available). Note: Common and scientific names of species follow Gill and Wright (2006).

Species common name	Species scientific name	Food type	Parental brood provisioning rate			Reference
			Female	Male	Male and female combined	
White-winged Cough	*Corcorax melanorhamphos*	S	–	–	↑	Boland et al. (1997)
Great Tit	*Parus major*	N	–	–	↑	Naef-Daenzer et al. (2000)
Blue Tit	*Cyanistes caeruleus*	S	–	–	↑	Grieco (2002b)
Cactus Wren	*Campylorhynchus brunneicapillus*	S	–	–	–	Simons and Martin (1990)
Black Redstart	*Phoenicurus ochruros*	S	↑	↓	=	Cucco and Malacarne (1997)
Palestine Sunbird	*Cinnyris osea*	S	↑	↑	↑	Markman et al. (2002)
Water Pipit	*Anthus spinoletta*	N	=	=	=	Rauter et al. (2000)
Red-winged Blackbird	*Agelaius phoeniceus*	N	↑	↓	–	Whittingham et al. (1994)

control conspecifics. Nestlings that were provisioned by supplemented parents had greater body masses than those provisioned by control parents. In another study Markman et al. (2002) provided supplementary food to Palestine Sunbirds (*Cinnyris osea*) to test the prediction that parents would invest more in brood provisioning as the energy content of their food increased. They provided adults with different concentrations of sucrose solution and showed that the rates at which the adults provisioned nestlings with arthropods were positively correlated with the sucrose solution concentration. This implied that adult provisioning effort was positively influenced by energy content of the supplementary food.

I investigate the effects of supplementary feeding on the brood provisioning behaviour of two small woodland passerines, Blue and Great Tits, by providing two dietary supplement treatments: (i). peanut cake *ad libitum* from approx. 7 weeks before hatching and throughout the nestling period; and (ii). peanut cake *ad libitum* from approx. 7 weeks before hatching and throughout the nestling period plus mealworms during the nestling period. I hypothesised that the provision of supplementary food for adults would reduce the energetic and temporal costs of foraging and would enable supplemented adults to invest more time and energy in provisioning nestlings. Therefore, I predicted that supplemented adult birds would have higher brood provisioning rates than nonsupplemented (control) birds. I also assumed that providing both peanut cake and mealworms in combination as supplementary foods would further enhance the investment of adults in their provisioning effort of nestlings compared with birds simply fed with peanut cake. I also predicted that nestlings in supplementary fed blocks would be in better body condition than those in the control block, and that survival of the fledglings from the supplementary fed blocks would be higher.

Prior to and during the nestling phase, Blue and Great Tits occupy different niches, the former feeding mainly in the canopy and the latter mainly on the ground (e.g. Minot 1981). At this time foraging niche breadth of 'generalist' Great Tits exceeds that of 'specialist' Blue Tits (Török 1986, Gosler and Clement 2007). However, where the dietary preferences of these species overlap, and there is inter-specific competition for food, Great Tits are dominant (Wilson 1992). Since I was studying both species at the same site, I hypothesised that Great Tits would exploit artificial feeders to a greater extent than Blue Tits both through having a more generalist diet and through out-competing Blue Tits at the feeders. I know that compared with Blue Tits food supplementation of Great Tits in my study population has more significant effects on some breeding parameters (e.g. clutch initiation date, incubation period length – Harrison et al. 2010). Thus, in comparison with Blue Tits, I predicted that any observed effects of supplementary feeding would be more pronounced in Great compared with Blue Tits.

4.3. METHODS

4.3.1. Study species

Blue and Great Tits are altricial and, thus, nestlings depend on food provided by adults to meet their energetic and nutritional demands (O'Connor 1984). In both species males and females provision the nestlings throughout the nestling period which lasts approx. 16-23 days (Perrins 1979, Gosler and Clement 2007). Early in the nestling phase, however, the female tends to brood the nestlings with the male providing most of the food to her (Gosler 1993), but by nestling day 6 both sexes provision at roughly equal rates (Kluijver 1950). Provisioning rates increase linearly with nestling age (e.g. Verhulst and Tinbergen 1997, Barba et al. 2009), levelling off

at around nestling day 10 to 12 (e.g. Eguchi 1980, Barba et al. 2009). Visits to the nest during the nestling phase usually result in nestling provisioning (Kluijver 1950). Therefore, I regarded all nest visits as provisioning events.

4.3.2. Study site and food supplementation

The overall experimental approach was consistent with that described in Section 2.3.2 but in the present study the peanut cake dietary treatment block was further equally divided into two blocks of 96 nestboxes following hatching. One of these blocks received live mealworms (a protein-rich food source known to be fed to nestlings by adults when provided as a food supplement – CJ Wildlife Ltd. pers. comm.). Thus, at the time of hatching, the woodland was split into three treatment blocks of equivalent numbers of nestboxes. They were Coalpit Coppice, Chaddesley Wood and Santry Hill Wood in which the treatments were rotated between the 3 years of the study in order to control for any habitat differences between blocks (Fig. 4.1).
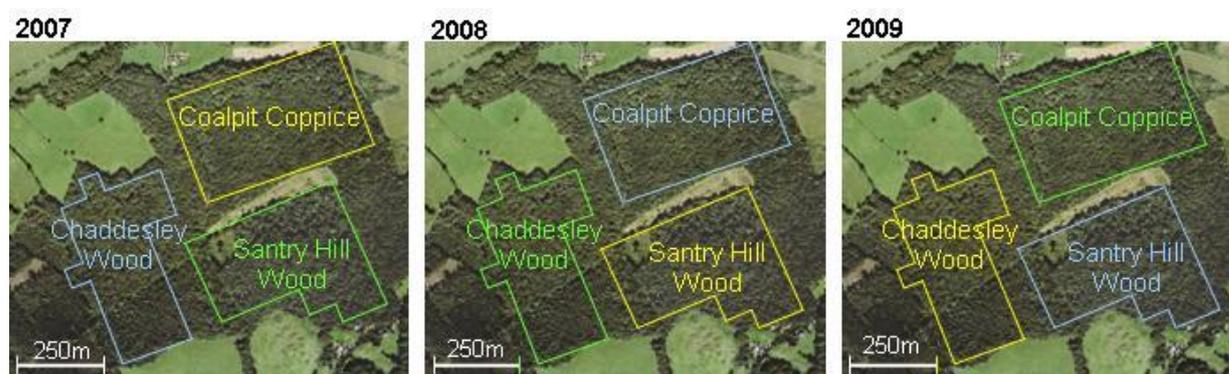
Figure 4.1. Dietary treatments (yellow: nonsupplemented [control]; blue: peanut cake; green: peanut cake + mealworms) in different blocks of Chaddesley Woods National Nature Reserve where Blue and Great Tits bred in 2007, 2008 and 2009. Treatments were rotated between the years of study so each woodland block received each dietary treatment (see text for details).

Live mealworms were provided between hatching and fledging in a pole-mounted feeder placed directly in front of, and between 5 and 8 m from, each focal nestbox. The quantity of mealworms provided was standardised for all nestboxes by using a film pot canister, providing approx. 15g of mealworms at each nestbox and feeders were checked and re-stocked every 2 days. Any dead mealworms were removed so that live food was always available.

4.3.3. Brood provisioning behaviour

Nestboxes were monitored to determine hatching date (defined as nestling day 0) (see Section 3.3.3). Provisioning rates are known to be highest for both species on nestling day 9 (e.g. Eguchi 1980, Gosler 1993, Verhulst and Tinbergen 1997, Barba et al. 2009). Therefore, on nestling day 9, brood provisioning rate at each nestbox was determined using either direct observation or filming using a Sony Handycam DCR-SR90E mounted on a tripod (see Section 3.3.4). Diurnal rhythms in provisioning behaviour have been documented in tits and these show that peak feeding activity occurs soon after dawn (Kluijver 1950, Gibb 1955, Eguchi 1980 – but see Cowie and Hinsley 1988b). Therefore, to account for possible diurnal variation in provisioning rates, all focal watches were conducted approx. 1 hr after sunrise. Focal watches or filming lasted for approx. 1 hr, and from these data the number of visits made by adults to nestboxes was determined and expressed as hourly feeding rates (number of visits per hr). To minimise disturbance during focal watches, I made myself inconspicuous by positioning myself as far away as possible from focal nestboxes using trees and shrubs as cover while still being able to observe the nestbox entrance hole. A settling period of approx. 10 mins was used before watches started although most birds started provisioning 1-5 mins after the observer arrived (pers. obs.).

Nestboxes were re-visited on nestling day 12 when nestlings were ringed under licence (BTO ringing permit no C/5418) using a uniquely numbered metal BTO ring.

4.3.4. Nestling body condition

To investigate the effects of supplementary feeding and brood provisioning on nestling body condition, three nestlings from each focal brood were randomly chosen during routine ringing (see above). For each nestling body mass was measured with an electronic balance (SA-500, SATRUE, Taiwan) and minimum tarsus length (Redfern and Clark 2001) was taken with a dial calliper (KWB, Switzerland). Because nestlings from the same nest are not statistically independent, average brood morphometrics from the three nestlings were calculated and average nestling body condition at each nestbox was expressed as a residual of average brood body mass regressed on average brood tarsus length (e.g. Christe et al. 1998, Ardia 2005). To increase sample sizes to gain a better understanding of how supplementary feeding affects nestling body condition morphometrics were also taken from nestlings from additional broods during routine nestling ringing.

4.3.5. Nestling survival

In the 3 years of study 2877 Blue Tit and 1904 Great Tit nestlings were ringed with every effort being made to ring all nestlings in all nestboxes. Nevertheless, each year a few broods (especially late ones) were not ringed as I was not always able to detect breeding attempts of either species late in the breeding season due to logistical problems. However, my nestling ringing efforts did allow me to investigate the effects of supplementary feeding and brood provisioning on the survival of fledglings. I carried out constant-effort mist-netting sessions in the autumn and winter in each year

of the study (see Harrison 2010 for full details). I determined the number of fledglings per brood that were recaptured in the same year as fledging and defined the probability of their recapture as their 'apparent survival' (*sensu* Harrison 2010). The term 'apparent' survival was used as apposed to 'absolute' survival since my study population is not an enclosed system. Therefore, my estimates of survival do not take into account individuals that have not been captured as a result of emigration, for example.

4.3.6. Caterpillar availability and local temperature records

To investigate if natural food availability influenced brood provisioning rates I monitored the availability of caterpillars (Winter Moth and *Geometridae* spp.) throughout the nestling period in each of the 3 years (see Appendix One for full methods). Since spring temperatures affect caterpillar abundance (Visser et al. 1998) and brood provisioning rates (Naef-Daenzer and Keller 1999, Naef-Daenzer et al. 2000), spring temperatures (from April and May) in 2007-2009 were obtained from the nearest UK Met Office recording station. This is situated at Coleshill, Warwickshire (52°48'N, 1°69'W) approx. 32 km from the study site (Met Office 2010).

4.3.7. Statistical analysis

I used both the number of provisions per brood per hr and the number of provisions per nestling per hr as measures of brood provisioning rate. To calculate provisioning rate per nestling I used the brood sizes at ringing on nestling day 12 which positively correlated with brood size on day 9 in both species (Blue: Pearson's rs = 0.98, P = 0.000; Great: Pearson's rs = 0.91, P = 0.000).

To test for the effects of supplementary feeding all response variables (brood provisioning rates per chick and per brood, nestling condition and apparent survival) were included in mixed models constructed using PROC GLIMMIX in SAS version 9.2 (SAS Institute Inc. 2008). Both brood provisioning rates per chick and provisioning rates per brood were fitted with Poisson error distributions, and apparent survival with binomial error distributions. Nestling body condition was tested for normality using the Anderson-Darling Test and for homogeneity of variance using the *F*-test using Minitab 15 (Minitab 2007) and was found to fit a normal distribution. Therefore, nestling body condition was fitted with a normal error distribution after examination of model fit (Generalised Chi-Square/DF = approx.1).

In all models treatment block was specified as a random factor due to repeated measures on treatment blocks. Ideally, nestbox nested in treatment block would have been included as a random factor but there was not sufficient power in either model to allow for this. Year was included as a fixed factor to account for potential annual variation in breeding performance (Nager et al. 1997). Combined-year analysis was conducted if there was no significant year $\times$ dietary treatment interaction and no significant main effect of year ($P > 0.05$). Otherwise, independent year analyses were performed.

Because rates of brood provisioning are positively associated with brood size (e.g. Nur 1984, Barba et al. 2009), and may vary temporally in relation to the availability of the main food items (i.e. caterpillars – Naef-Daenzer and Keller 1999), brood size and hatch date were both included as covariates in models where brood provisioning per chick and per brood were the dependent variables. Because nestling body mass significantly declines with brood size (Nur 1984) and is reduced in broods that hatch later in the season (Barba et al. 1995), both brood size and hatch date were

included as covariates in analyses of nestling body condition. To account for the

effects of hatch date and brood size on survival (earlier hatched broods and heavier

nestlings from smaller broods are more likely to survive – e.g. Norris 1993, Verboven

and Visser 1998, Monrós and Barba 2002), both were included as covariates in the

analysis of apparent survival. To determine if the effects of covariates were consistent

between treatment blocks, covariate × dietary treatment interactions were tested

independently of one another within each model. Where multiple covariate × dietary

treatment interactions were significant they were both included in the model as long

as each stayed significant (Grafen and Hails 2002). I performed backward model

selections removing the least non-significant covariate interactions ($P > 0.05$) one-by-

one from models ensuring that there was no significant change in deviance between

models where applicable (Grafen and Hails 2002). Tukey-Kramer *post hoc* tests were

conducted for pairwise comparisons of the least-squares means with results being

presented as adjusted *P* values.

4.4. RESULTS

4.4.1. Brood provisioning rates between years

For both Blue and Great Tits there was a significant year effect on provisioning rates

per brood (Blue: $F_{2,68} = 5.91$, $P < 0.01$; Great: $F_{2,61} = 11.32$, $P < 0.0001$; Table 4.2)

and per nestling (Blue: $F_{2,68} = 3.16$, $P = 0.05$; Great: $F_{2,61} = 14.14$, $P < 0.0001$; Table

4.2). Tukey Kramer *post hoc* tests were conducted to make pairwise comparisons

between years by pooling provisioning rates per brood, and per nestling, across

dietary treatment for each year (there was no main effect of dietary treatment on both

dependent variables in the combined-year model for either species; Table 4.2).

Table 4.2. Results from the final statistical models (see text for details) explaining measurements of brood provisioning behaviour, nestling condition and apparent survival in Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve between 2007 and 2009. Significant main and interaction effects are given in bold. Directions of significant effects are given '+' and '-' denote significant positive and negative relationships, respectively, and 'NS' denotes nonsignificance ($P > 0.05$).

							Least square means (SE) – Tukey-Kramer *post hoc* P values			
Breeding parameter	Species	Year	Factor	F	df	P	C *vs.* PC	C *vs.* PC+MW	PC *vs.* PC+MW	Direction
Provisioning rate per brood	BT	2007-9	Dietary treatment	0.13	2,68	0.88	47.87(2.70) *vs.* 46.39(3.00) $P = 0.93$	47.87(2.70) *vs.* 46.12(2.75) $P = 0.90$	46.39(3.00) *vs.* 46.12(2.75) $P = 1.00$	NS
			Year	**5.91**	**2,68**	**<.01**				2009 = highest; 2008 = lowest
			Brood size	**30.91**	**1,68**	**<0.0001**				+
		2007	Dietary treatment	**7.70**	**2,12**	**0.01**	41.91(2.70) *vs.* 43.44(2.99) $P = 0.92$	41.91(2.70) *vs.* 41.36(2.96) $P = 0.99$	43.44(2.99) *vs.* 41.36(2.96) $P = 0.88$	PC = highest; PC+MW = lowest
			Hatch date	**14.93**	**1,12**	**<0.01**				+
			Brood size	**47.94**	**1,12**	**<0.0001**				+
			Brood size × dietary treatment	**8.55**	**2,12**	**<0.01**				C = + ; PC = - ; PC +MW = +
		2008	Dietary treatment	**17.52**	**2,24**	**<0.0001**	36.68(2.12) *vs.* 36.55(1.94) $P = 1.00$	36.68(2.12) *vs.* 37.75(2.36) $P = 0.94$	36.55(1.94) *vs.* 37.75(2.36) $P = 0.92$	PC+MW = highest; PC = lowest
			Brood size	**43.65**	**1,24**	**<0.0001**				+
			Brood size × dietary treatment	**15.36**	**2,24**	**<0.0001**				C = + ; PC = + ; PC+MW = -
		2009	Dietary treatment	**36.41**	**2,20**	**<0.0001**	63.67(2.57) *vs.* 54.81(3.17) $P = 0.12$	63.67(2.57) *vs.* 46.84(2.46) $P < 0.001$	54.81(3.17) *vs.* 46.84(2.46) $P = 0.15$	C = highest; PC+MW = lowest
			Brood size	**11.04**	**1,20**	**<0.01**				+
			Hatch date	**30.17**	**1,20**	**<0.0001**				-
			Hatch date × dietary treatment	**37.40**	**2,20**	**<0.0001**				C = - ; PC = + ; PC+MW = -
	GT	2007-9	Dietary treatment	0.27	2,61	0.77	35.10(2.90) *vs.* 32.92(2.64) $P = 0.83$	35.10(2.90) *vs.* 32.60(2.73) $P = 0.78$	32.92(2.64) *vs.* 32.60(2.73) $P = 1.00$	NS
			Year	**11.32**	**2,61**	**<0.0001**				2009 = highest; 2007 = lowest

Table 4.2. continued

Response	Species	Year	Term	Statistic	df	P	Comparison 1	Comparison 2	Comparison 3	Direction
			Brood size	**15.22**	**1,61**	**<0.001**				+
		2007	Dietary treatment	**4.97**	**2,6**	**0.05**	41.04(4.53) *vs.* 29.41(2.79) $P = 0.13$	41.04(4.53) *vs.* 44.46(9.28) $P = 0.94$	29.41(2.79) *vs.* 44.46(9.28) $P = 0.25$	PC+MW = highest; PC = highest
			Hatch date	**6.29**	**1,6**	**0.05**				+
			Hatch date × dietary treatment	2,6	4.76	0.06				NS
		2008	Dietary treatment	**9.61**	**2,18**	**<0.01**	36.31(2.40) *vs.* 30.34(1.85) $P = 0.14$	36.31(2.40) *vs.* 19.59(2.96) $P < 0.01$	30.34(1.85) *vs.* 19.59(2.96) $P = 0.04$	C = highest; PC+MW = lowest
			Hatch date	**12.55**	**1,18**	**<0.01**				-
			Brood size	**20.95**	**1,18**	**<0.001**				+
			Brood size × dietary treatment	**9.69**	**2,18**	**<0.01**				C = weakly – ; PC = + ; PC+MW = +
			Hatch date × dietary treatment	**8.48**	**2,18**	**<0.01**				C = - ; PC = - ; PC+MW = +
		2009	Dietary treatment	**4.58**	**2,23**	**0.02**	51.95(4.62) *vs.* 42.29(1.99) $P = 0.12$	51.95(4.62) *vs.* 34.60(3.15) $P = 0.02$	42.29(1.99) *vs.* 34.60(3.15) $P = 0.16$	C = highest; PC+MW = lowest
			Hatch date	**15.85**	**1,23**	**<0.01**				-
			Brood size	**65.24**	**1,23**	**<0.0001**				+
			Hatch date × dietary treatment	**4.37**	**2,23**	**0.02**				C = + ; PC -; PC+MW = -
Provisioning rate per nestling	BT	2007-9	Dietary treatment	0.05	2,68	0.96	5.98(0.59) *vs.* 6.18(0.63) $P = 0.95$	5.98(0.59) *vs.* 6.09(0.61) $P = 0.98$	6.18(0.63) *vs.* 6.09(0.61) $P = 0.99$	NS
			Year	**3.16**	**2,68**	**0.05**				2009 = highest; 2008 = lowest
			Brood size	**25.24**	**1,68**	**<0.0001**				-
		2007	Dietary treatment	**4.88**	**2,12**	**0.03**	4.81(0.88) *vs.* 5.17(1.09) $P = 0.96$	4.81(0.88) *vs.* 5.18(0.97) $P = 0.96$	5.17(1.09) *vs.* 5.18(0.97) $P = 1.00$	PC+MW = highest; control = lowest
			Hatch date	**4.52**	**1,12**	**0.05**				+
			Brood size	1.26	1,12	0.28				NS
			Brood size × dietary treatment	**4.52**	**2,12**	**0.03**				C = - ; PC = - ; PC+MW = +
		2008	Dietary treatment	**5.73**	**2,24**	**0.01**	5.48(0.75) *vs.* 5.27(0.74) $P = 0.98$	5.48(0.75) *vs.* 4.92(0.97) $P = 0.90$	5.27(0.74) *vs.* 4.92(0.97) $P = 0.96$	Control = highest; PC+MW = lowest
			Brood size	**8.49**	**1,24**	**0.01**				-

Table 4.2. continued

Response	Species	Year	Effect	Stat	df	P	Comparison 1	Comparison 2	Comparison 3	Summary
			Brood size × dietary treatment	**4.27**	**2,24**	**0.03**				C = - ; PC = - ; PC+MW = strongly -
		2009	Dietary treatment	**3.68**	**2,20**	**0.04**	6.87(0.85) *vs.* 6.16(1.06) $P = 0.87$	6.87(0.85) *vs.* 5.21(0.80) $P = 0.36$	6.16(1.06) *vs.* 5.21(0.80) $P = 0.76$	C = highest; PC+MW = lowest
			Brood size	**4.22**	**1,20**	**0.05**				-
			Hatch date	3.66	1,20	0.07				NS
			Hatch date × dietary treatment	**3.94**	**2,20**	**0.04**				C = - ; PC = - ; PC+MW = strongly -
	GT	2007-9	Dietary treatment	0.66	2,61	0.52	5.39(0.42) *vs.* 4.85(0.37) $P = 0.57$	5.39(0.42) *vs.* 4.85(0.39) $P = 0.59$	4.85(0.37) *vs.* 4.85(0.39) $P = 1.00$	C = highest; PC+MW = lowest
			Year	**14.14**	**2,61**	**<0.0001**				2009 = highest; 2007 = lowest
			Brood size	**12.26**	**1,61**	**<0.01**				-
		2007	Dietary treatment	0.55	2,9	0.59	4.89(1.11) *vs.* 3.96(1.0) $P = 0.81$	4.89(1.11) *vs.* 3.39(0.92) $P = 0.58$	3.96(1.0) *vs.* 3.39(0.92) $P = 0.91$	NS
		2008	Dietary treatment	0.03	2,24	0.97	4.38(0.66) *vs.* 4.50(0.71) $P = 0.99$	4.38(0.66) *vs.* 4.24(0.73) $P = 0.99$	4.50(0.71) *vs.* 4.24(0.73) $P = 0.97$	NS
		2009	Dietary treatment	0.19	2,26	0.83	7.22(0.96) *vs.* 6.54(0.76) $P = 0.84$	7.22(0.96) *vs.* 7.13(0.87) $P = 1.0$	6.54(0.76) *vs.* 7.13(0.87) $P = 0.87$	NS
			Brood size	**5.95**	**1,26**	**0.02**				-
Nestling condition	BT	2007-9	Dietary treatment	0.00	2,135	1.00	$161 \times 10^{-17}(0.14)$ *vs.* $11 \times 10^{-16}(0.15)$ $P = 1.0$	$161 \times 10^{-17}(0.14)$ *vs.* $131 \times 10^{-17}(0.15)$ $P = 1.0$	$11 \times 10^{-16}(0.15)$ *vs.* $131 \times 10^{-17}(0.15)$ $P = 1.0$	NS
			Year	0.00	2,135	1.00				NS
	GT	2007-9	Dietary treatment	0.01	2,111	0.99	-0.0004(0.16) *vs.* -0.02(0.16) $P = 1.00$	-0.0004(0.16) *vs.* 0.02(0.17) $P = 1.00$	-0.02(0.16) *vs.* 0.02(0.17) $P = 0.99$	NS
			Year	0.00	2,111	1.00				NS
Apparent survival	BT	2007-9	Dietary treatment	1.94	2,107	0.15	0.33(0.01) *vs.* 0.05(0.01) $P = 0.23$	0.33(0.006) *vs.* 0.05(0.01) $P = 0.15$	0.05(0.01) *vs.* 0.05(0.01) $P = 0.97$	NS
			Year	**3.49**	**2,107**	**0.03**				2009 = highest; 2007 = lowest
			Hatch date	**17.79**	**1,107**	**<0.0001**				+

Table 4.2. continued

		Year × dietary treatment	**6.97**	**4,107**	**<0.0001**				
	2007	Dietary treatment	**3.53**	**2,91**	**0.03**	0.02(0.01) *vs.* 0.05(0.02)	0.02(0.01) *vs.* 0.03(0.01)	0.05(0.02) *vs.* 0.03(0.01)	PC = highest; C = lowest
						$P = 0.03$	$P = 0.39$	$P = 0.31$	
		Hatch date	**10.37**	**1,91**	**0.02**				-
	2008	Dietary treatment	**6.60**	**2,109**	**<0.01**	0.02(0.01) *vs.* 0.03(0.01)	0.02(0.01) *vs.* 0.08(0.02)	0.03(0.01) *vs.* 0.08(0.02)	PC+MW = highest; C = lowest
						$P = 0.75$	$P = 0.01$	$P = 0.02$	
		Hatch date	**8.97**	**1,109**	**<0.01**				-
	2009	Dietary treatment	2.61	2,112	0.08	0.09(0.02) *vs.* 0.06(0.01)	0.09(0.02) *vs.* 0.05(0.01)	0.06(0.01) *vs.* 0.05(0.01)	NS
						$P = 0.32$	$P = 0.08$	$P = 0.82$	
GT	2007-9	Dietary treatment	**3.45**	**2,84**	**0.04**	0.05(0.01) *vs.* 0.06(0.01)	0.05(0.01) *vs.* 0.09(0.01)	0.06(0.01) *vs.* 0.09(0.01)	PC+MW = highest; C = lowest
						$P = 0.92$	$P = 0.06$	$P = 0.11$	
		Year	0.09	2,84	0.92				NS
		Hatch date	**11.28**	**1,84**	**<0.01**				-
		Year × dietary treatment	**2.67**	**4,84**	**0.04**				
	2007	Dietary treatment	**8.92**	**2,54**	**<0.001**	0.01(0.01) *vs.* 0.09(0.03)	0.01(0.01) *vs.* 0.05(0.03)	0.09(0.03) *vs.* 0.05(0.03)	PC = highest; C = lowest
						$P = 0.02$	$P = 0.26$	$P = 0.59$	
		Hatch date	**17.86**	**1,54**	**<0.0001**				-
		Hatch date × dietary treatment	**9.64**	**2,54**	**<0.001**				C = - ; PC = weakly - ; PC+MW = -
	2008	Dietary treatment	2.63	2,96	0.08	0.04(0.02) *vs.* 0.02(0.01)	0.04(0.02) *vs.* 0.14(0.02)	0.02(0.01) *vs.* 0.14(0.02)	NS
						$P = 0.43$	$P = 0.01$	$P < 0.01$	
		Hatch date	**15.52**	**1,96**	**<0.01**				-
		Hatch date × dietary treatment	**3.85**	**2,96**	**0.02**				C = strongly - ; PC = - ; PC+MW = weakly -
	2009	Dietary treatment	**3.41**	**2,100**	**0.04**	0.11(0.03) *vs.* 0.05(0.02)	0.11(0.03) *vs.* 0.07(0.02)	0.05(0.02) *vs.* 0.07(0.02)	C = highest; P= lowest
						$P = 0.19$	$P = 0.38)$	$P = 0.88$	
		Hatch date	**5.71**	**1,100**	**0.02**				-
		Brood size	**5.15**	**1,100**	**0.03**				+
		Brood size × dietary treatment	**3.60**	**2,100**	**0.03**				C = - ; PC = + ; PC+MW = +

For both Blue and Great Tits provisioning rates per brood in 2009 were significantly higher than in both 2007 (Blue: Tukey-Kramer $P = 0.04$; Great: Tukey-Kramer $P < 0.01$; Fig. 4.2) and 2008 (Blue: Tukey-Kramer $P = 0.01$; Great: Tukey-Kramer $P < 0.001$; Fig. 4.2). However, there was no significant difference in provisioning rates per brood between 2007 and 2008 for either Blue (Tukey-Kramer $P = 0.90$; Fig 4.2) or Great (Tukey-Kramer $P = 0.90$; Fig. 4.2) Tits.
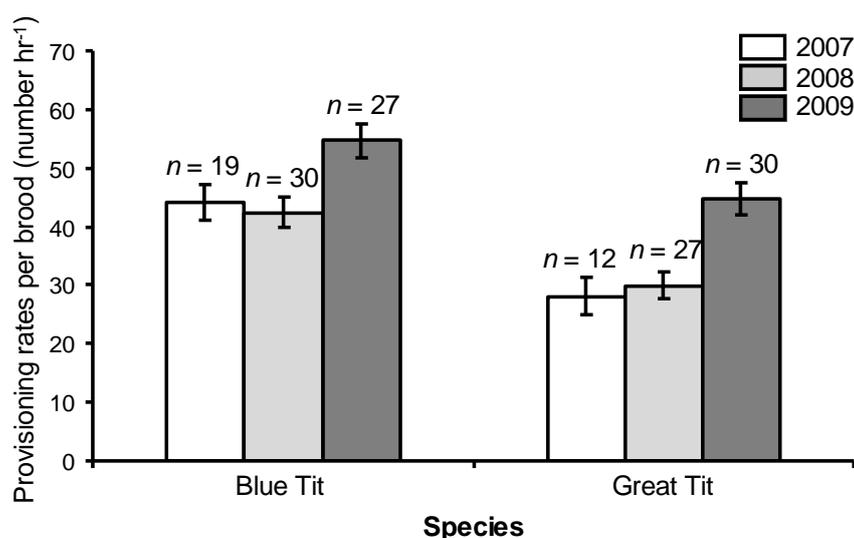
Figure 4.2. Provisioning rates per brood (mean $\pm$ 1 SE from statistical estimates) of day 9 broods by adult Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve from 2007 to 2009. The number of broods (n) is given above each bar.

For both Blue and Great Tits provisioning rates per nestling in 2009 were significantly higher than in 2008 (Blue: Tukey-Kramer $P = 0.04$; Great: Tukey-Kramer $P < 0.0001$; Fig. 4.3) while they were significantly higher than in 2007 for Great (Tukey-Kramer $P < 0.01$; Fig. 4.3), but not for Blue, (Tukey-Kramer $P = 0.40$; Fig. 4.3) Tits. However, there was no significant difference in provisioning rates per nestling between 2007 and 2008 for either Blue (Tukey-Kramer $P = 0.51$; Fig 4.3) or Great (Tukey-Kramer $P = 1.0$; Fig. 4.3) Tits. Since there was a significant year effect on both brood provisioning rates per brood and per nestling, separate analyses for

each year were performed to investigate the effects of supplementary feeding on both

dependent variables.
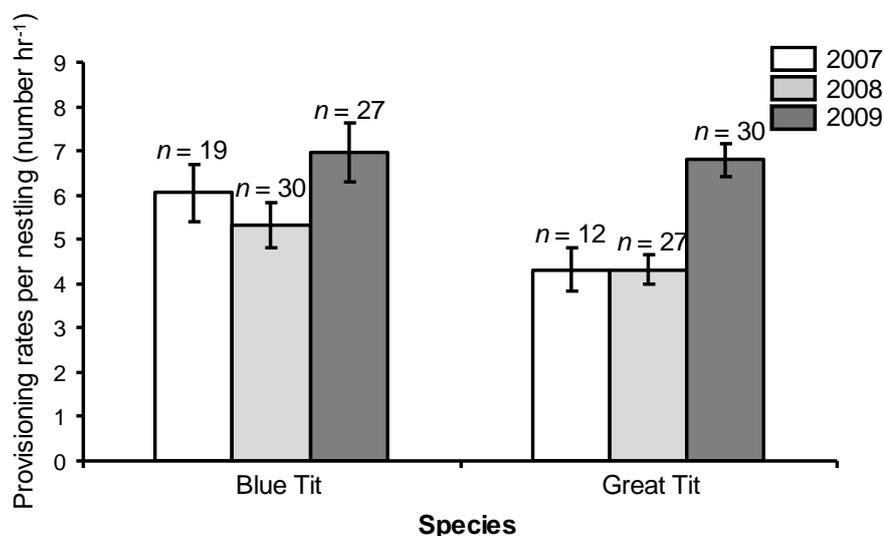
Figure 4.3. Provisioning rates per nestling (mean ± 1 SE from statistical estimates) of day 9 broods by adult Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve from 2007 to 2009. The number of broods (*n*) is given above each bar.

4.4.2. Effects of supplementary feeding on brood provisioning rates per brood

When independent year analyses were performed there was a significant effect of

dietary treatment on provisioning rates per brood in Blue Tits in all years of the study

(2007: $F_{2,12} = 7.70$, $P = 0.01$; 2008: $F_{2,24} = 17.52$, $P < 0.0001$; 2009: $F_{2,20} = 36.41$, $P <$

0.0001; Fig. 4.4 and Table 4.2). In 2009 Tukey-Kramer *post hoc* tests (testing for

main effects of dietary treatment) indicated that Blue Tits breeding in the control

block had significantly higher brood provisioning rates than Blue Tits breeding in the

peanut cake + mealworms block (Tukey-Kramer $P < 0.001$; Fig. 4.4 and Table 4.2).

In contrast, there was no significant difference in provisioning rates per brood

between Blue Tits breeding in the control and peanut cake block (Tukey-Kramer $P =$

0.12; Fig. 4.4 and Table 4.2) and between Blue Tits breeding in the peanut cake and

peanut cake + mealworms block (Tukey-Kramer $P = 0.15$; Fig. 4.4 and Table 4.2).

However, in 2007 and 2008 Tukey-Kramer *post hoc* tests (testing for main effects of

dietary treatment) indicated that there was no significant main effect of dietary

treatment on provisioning rates per brood between pairs of treatment blocks (see

Table 4.2 for full details of statistical models).
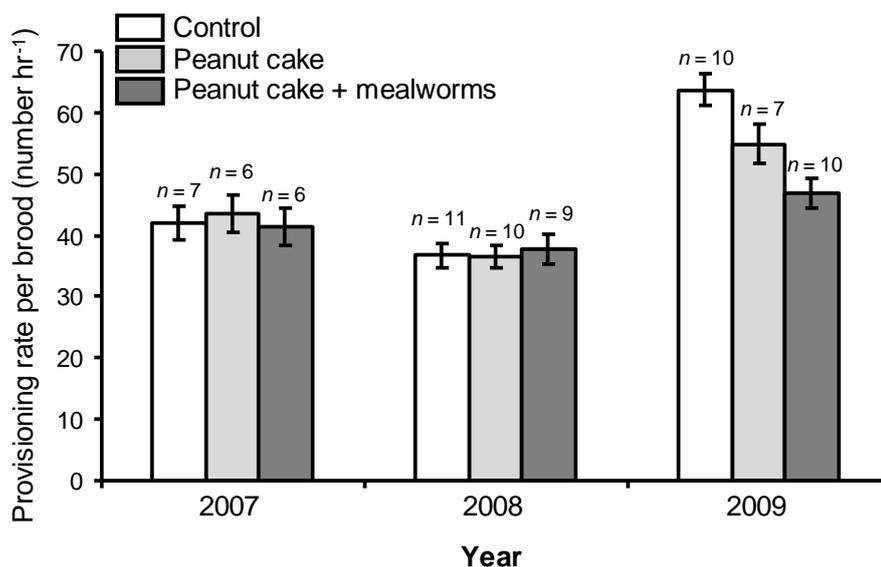
Figure 4.4. Provisioning rates per brood (mean ± 1 SE from statistical estimates) of day 9 broods by adult Blue Tits breeding in Chaddesley Woods National Nature Reserve from 2007 to 2009. The number of broods (n) is given above each bar.

In Great Tits there was a significant effect of dietary treatment on provisioning rates per brood in all years of the study (2007: $F_{2,6} = 4.97$, $P = 0.05$; 2008: $F_{2,18} = 9.61$, $P < 0.01$; 2009: $F_{2,23} = 4.58$, $P = 0.02$; Fig. 4.5 and Table 4.2). In 2008 and 2009 Tukey-Kramer *post hoc* tests (testing for main effects of dietary treatment) indicated that Great Tits breeding in the control block had significantly higher brood provisioning rates than Great Tits breeding in the peanut cake + mealworms block (2008: Tukey-Kramer $P < 0.01$; 2009: Tukey-Kramer $P = 0.02$; Fig. 4.5 and Table 4.2). Furthermore, in 2008 Great Tits breeding in the peanut cake block had significantly higher provisioning rates per brood than those breeding in the peanut cake + mealworms block (Tukey-Kramer $P = 0.04$; Fig. 4.5 and Table 4.2). In contrast, there was no significant difference in provisioning rates per brood between Great Tits

breeding in the control and peanut cake block in both 2008 and 2009 (2008: Tukey-

Kramer $P = 0.14$; 2009: Tukey-Kramer $P = 0.12$; Fig. 4.5 and Table 4.2) and between
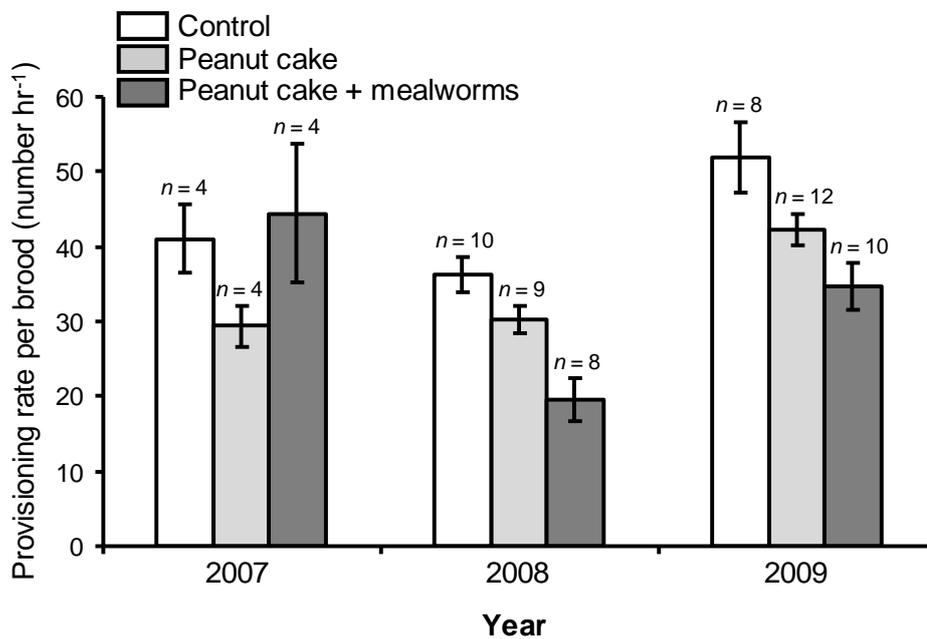
Figure 4.5. Provisioning rates per brood (mean $\pm$ 1 SE from statistical estimates) of day 9 broods by adult Great Tits breeding in Chaddesley Woods National Nature Reserve from 2007 to 2009. The number of broods (n) is given above each bar.

Great Tits breeding in the peanut cake and peanut cake + mealworms block in 2009

(Tukey-Kramer $P = 0.16$; Fig. 4.5 and Table 4.2). In 2007, however, Tukey-Kramer

post hoc tests (testing for main effects of dietary treatment) indicated that there was

no significant main effect of dietary treatment on provisioning rates per brood

between pairs of treatment blocks (see Table 4.2 for full details of statistical models).

4.4.3. Effects of supplementary feeding on brood provisioning rates per nestling

In Blue Tits there was a significant effect of dietary treatment on provisioning rates

per nestling in all years of the study (2007: $F_{2,12} = 4.88$, $P = 0.03$; 2008: $F_{2,24} = 5.73$,

$P = 0.01$; 2009: $F_{2,20} = 3.68$, $P = 0.04$; Fig. 4.6 and Table 4.2). However, in all years,

Tukey-Kramer *post hoc* tests (testing for main effects of dietary treatment) indicated
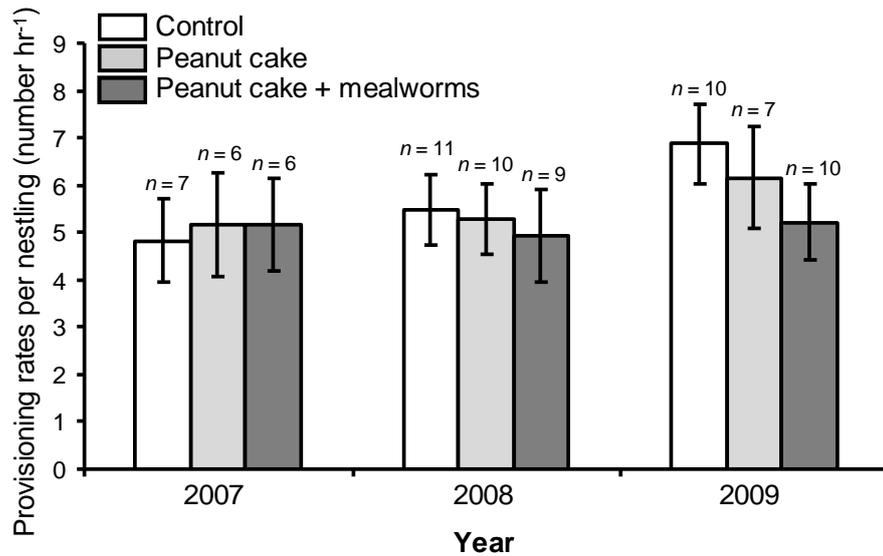
Figure 4.6. Provisioning rates per nestling (mean ± 1 SE from statistical estimates) of day 9 broods by adult Blue Tits breeding in Chaddesley Woods National Nature Reserve from 2007 to 2009. The number of broods (n) is given above each bar.
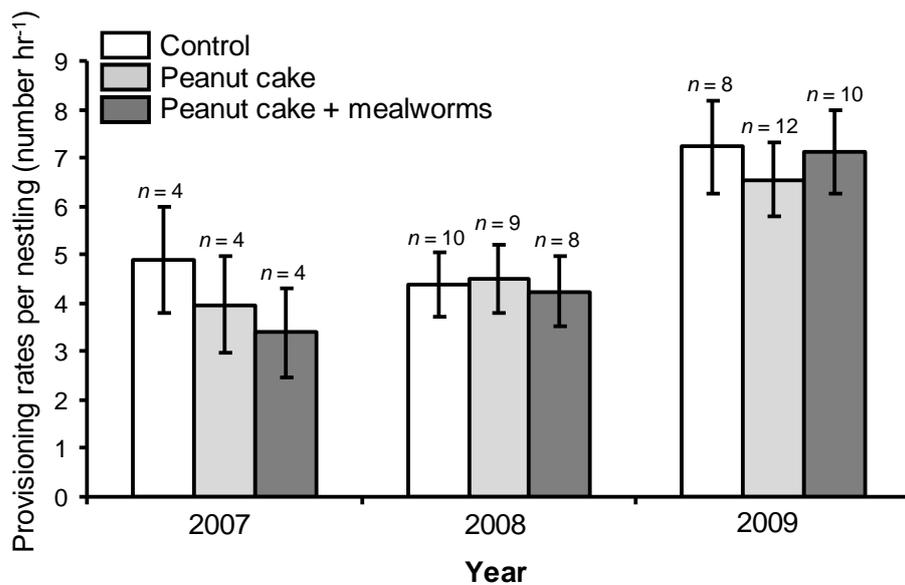
Figure 4.7. Provisioning rates per nestling (mean ± 1 SE from statistical estimates) of day 9 broods by adult Great Tits breeding in Chaddesley Woods National Nature Reserve from 2007 to 2009. The number of broods (n) is given above each bar.

that there was no significant main effect of dietary treatment on brood provisioning rates per nestling between pairs of treatment blocks (Table 4.2). In contrast, dietary treatment did not have a significant effect on provisioning rates per nestling for Great

Tits in all years of the study (2007: $F_{2,9} = 0.55$, $P = 0.59$; 2008: $F_{2,24} = 0.03$, $P = 0.97$; 2009: $F_{2,26} = 0.19$, $P = 0.83$; Fig. 4.7 and Table 4.2).

4.4.4. Nestling condition

In both species nestling body condition was consistent between years (Blue: $F_{2,135} = 0.00$, $P = 1.00$; Great: $F_{2,111} = 0.66$, $P = 1.00$). Furthermore, supplementary feeding had no significant effect on nestling body condition in either species (Blue: $F_{2,135} = 0.00$, $P = 1.00$; Great: $F_{2,111} = 0.01$, $P = 1.00$).

4.4.5. Apparent survival

A significant year $\times$ dietary interaction indicated that the effect of dietary treatment on apparent survival of fledglings of both species was inconsistent between years (Blue: $F_{4,107} = 6.97$, $P < 0.0001$; Great: $F_{4,84} = 2.67$, $P = 0.04$; Table 4.2). Therefore, separate analyses for each year were conducted. Dietary treatment had significant effects on apparent survival of fledglings in each year of study. In 2007 fledglings from supplementary fed territories rather than from control territories showed higher apparent survival (Blue: $F_{2,91} = 3.53$, $P = 0.03$; Great: $F_{2,54} = 8.92$, $P < 0.001$; Fig. 4.8, Fig. 4.9 and Table 4.2). Food supplementation had a similar, if statistically stronger, effect in both species in 2008 (Blue: $F_{2,109} = 6.60$, $P < 0.01$; Great: $F_{2,96} = 15.52$, $P < 0.001$; Fig. 4.8, Fig. 4.9 and Table 4.2) with fledglings that fledged from the peanut cake + mealworm dietary treatment block surviving better than fledglings that fledged from either peanut cake-supplemented or control territories (Table 4.2). In 2009 dietary treatment did not appear to have a significant effect on apparent survival of Blue Tits ($F_{2,112} = 2.61$, $P = 0.08$; Fig. 4.8), but it did for Great Tit fledglings ($F_{2,100} = 3.41$, $P = 0.04$; Fig. 4.9).
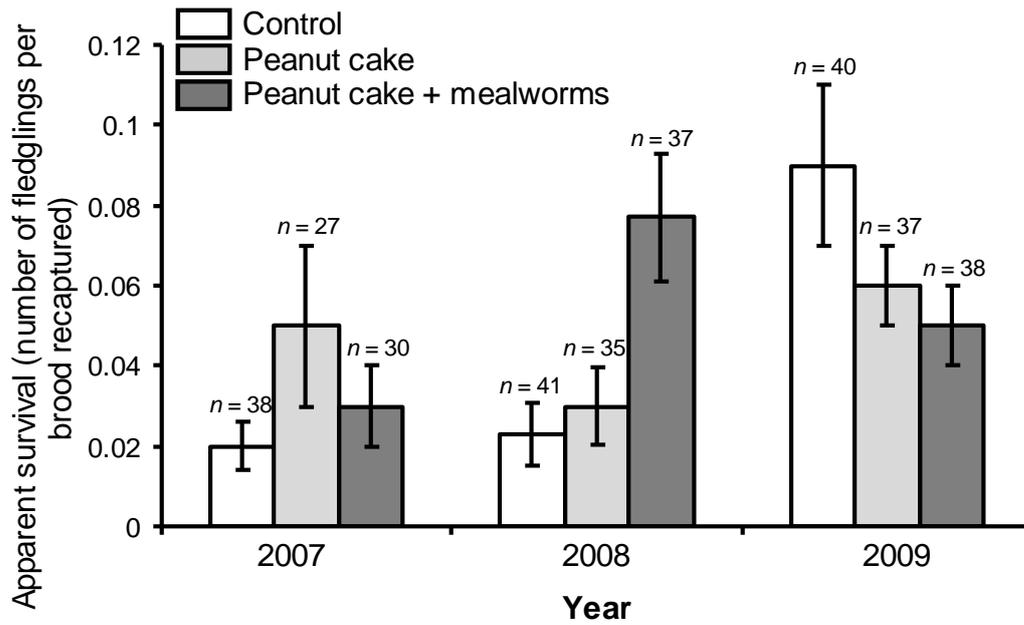
Figure 4.8. Apparent survival (mean recapture rate ± 1 SE from statistical estimates) of Blue Tits fledged in Chaddesley Woods National Nature Reserve between 2007 and 2009. The number of broods (*n*) is given above each bar.
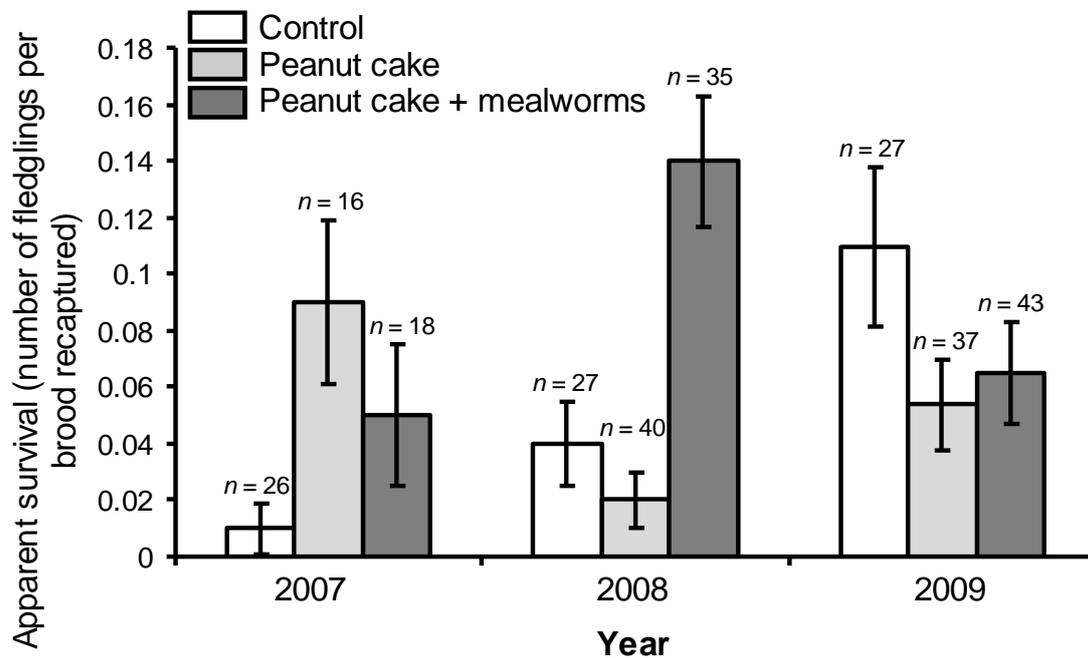
Figure 4.9. Apparent survival (mean recapture rate ± 1 SE from statistical estimates) of Great Tits and fledged in Chaddesley Woods National Nature Reserve between 2007 and 2009. The number of broods (*n*) is given above each bar.

4.4.6. Caterpillar availability and local temperature

Due to unforeseen circumstances, caterpillar data from 2009 were unavailable. In

2007 and 2008 both the timing of the peak and the relative abundance of caterpillars
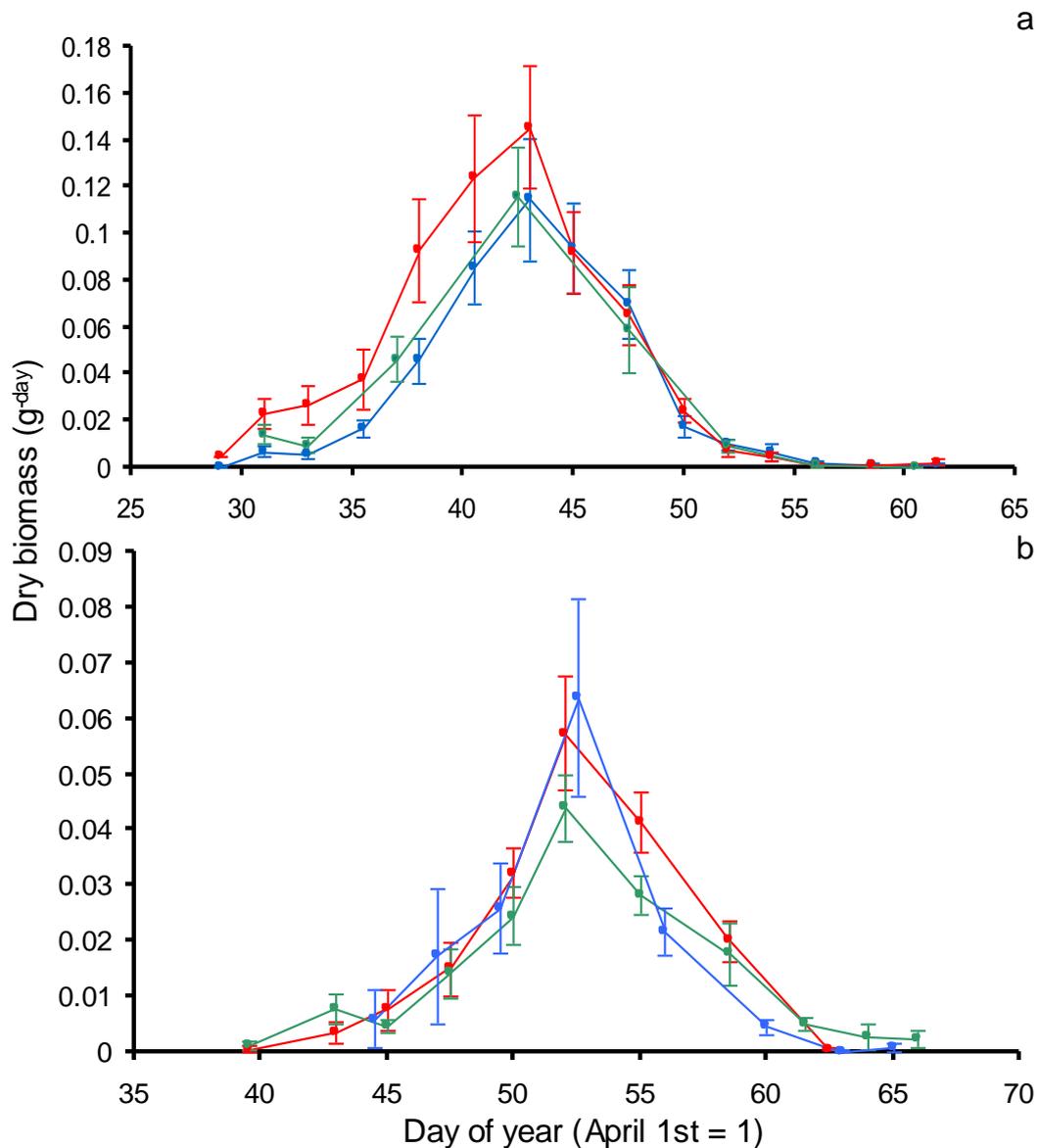
Figure 4.10. Relative *Geometridae* spp. and Winter Moth caterpillar abundance
(mean ± 1 SE) and timing of their emergence in Chaddesley Wood National Nature
Reserve in (**a**) 2007 and (**b**) 2008. Colours indicate the experimental blocks: Blue –
control; red – peanut cake; green – peanut cake + mealworms.

were consistent between dietary treatment blocks within each year (Fig 4.10a and b).

In 2007 the peak in caterpillar abundance occurred approx. 10 days earlier than in

2008 (Fig 4.10) while the relative abundance of caterpillar biomass was lower in 2008

than in 2007 (Fig 4.10). In 2007-2009 average daily spring temperatures fluctuated

between 5°C and 23°C and increased as the season progressed (Fig. 4.11). Spring

temperatures were consistent between years (ANOVA: $F_{2,182} = 1.65$, $P = 0.20$; mean

temperatures ± 1SE: 2007 – 14.00 ± 0.32°C; 2008 – 13.08 ± 0.50°C; 2009: 13.91 ±

0.32°C – Met Office 2010).
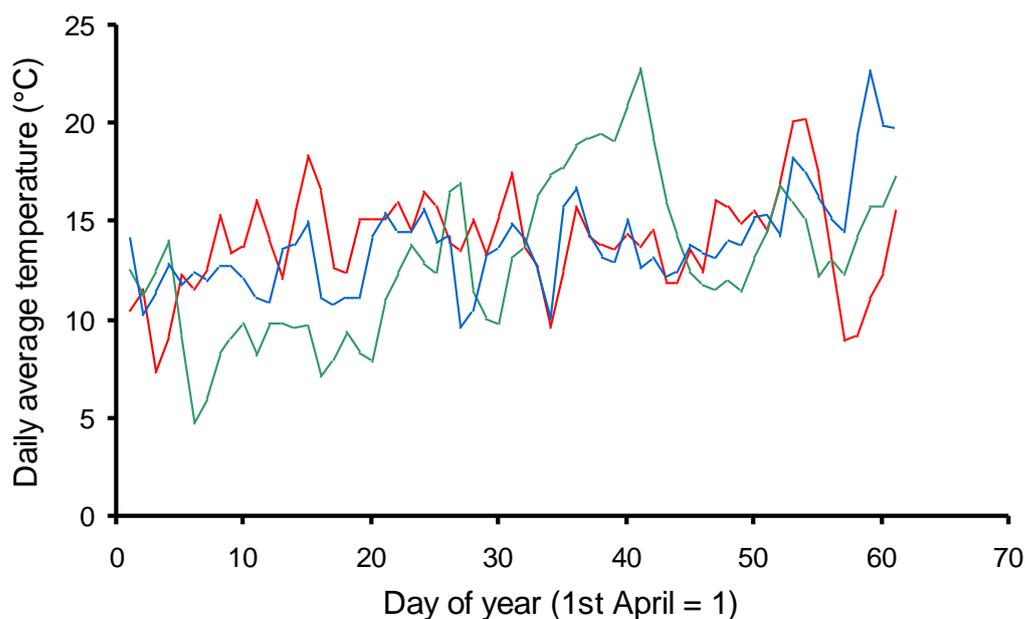
Figure 4.11. Average daily spring time temperatures in 2007 (red), 2008 (green) and
2009 (blue) obtained from Coleshill Met Office Land Surface Station in Warwickshire
(52°48'N, 1°69'W), approx. 32 km from Chaddesley Woods National Nature Reserve
(Met Office 2010).

4.5. DISCUSSION

Supplementary feeding should reduce the energetic and temporal costs of foraging

consequently allowing supplemented adults to invest more time and energy in

provisioning nestlings. Therefore, I predicted that food supplementation would result

in higher brood provisioning rates and that adult birds provided with peanut cake and

mealworms would have higher brood provisioning rates than birds fed with only

peanut cake. However, although supplementary feeding had marked effects on the

rates of nestling provisioning across all 3 years of this study for both Blue and Great Tits, my results do not fully support my predictions. For Blue Tits the effects of supplementary feeding on brood provisioning varied between years and were more pronounced in 2009 than in both 2007 and 2008 when the effects were more subtle. In 2009 supplementary feeding resulted in a reduction of brood provisioning rates per brood for Blue Tits with birds supplemented with both peanut cake and mealworms having the lowest brood provisioning rates. In 2008 and 2009 provisioning rates per brood were highest for Great Tits in the control block and lowest for birds supplemented with peanut cake and mealworms. In 2007, however, provisioning rates per brood were highest for Great Tits supplemented with peanut cake and mealworms. I also predicted that nestlings in supplementary fed blocks would be in better body condition than those in the control block, and that apparent survival of the fledglings from the supplementary fed blocks would be higher. In contrast to my prediction, food supplementation had no effect on nestling body condition for either species. However, food supplementation enhanced apparent survival of fledglings of both species across the 3 years of study although the effects were inconsistent between years.

4.5.1. Effects of supplementary feeding on provisioning rate

In 2008 and 2009 for Great Tits, and 2009 for Blue Tits, provisioning rates per brood for both species, and provisioning rates per nestling for Blue Tits, were higher in the control block than in the supplementary fed blocks. This is similar to the findings of Grieco (2002a) who showed that Blue Tits supplemented with mealworms and Wax Moth larvae fed their nestlings less frequently than control conspecifics but, instead, delivered larger food items than control birds. The authors suggested that the reduction in foraging constraints experienced by supplemented birds may have

resulted in a decrease in brood provisioning rates in two ways (Grieco 2002a). First,

supplementary food may have enabled birds to travel further afield to exploit feeding

sites which were less food-depleted and contained larger, more profitable food items

(Naef-Daenzer 2000), thereby increasing time spent away from the nest. Secondly,

supplemented birds may have been able to allocate more time in prey selectivity,

foraging for larger, more profitable food items while rejecting smaller, less profitable

food items (Grieco 2001, 2002b). Since selectivity determines the average searching

time (Grieco 2001, 2002b), greater selectivity would have reduced brood provisioning

rates. Increasing selectivity, and decreasing brood provisioning rates, are likely to be

beneficial since they result in a reduction in the cost of repeat visitations to the nest

(e.g. cost of flight – Bryant and Tatner 1991; predation risk – Martin et al. 2000b). In

my study providing supplementary food may have enabled adults to satisfy their

energetic and nutritional needs through a reduction in time spent foraging compared

with the time needed for foraging by control birds. Although I have no data on the

size of food items delivered to nestlings, it is possible that supplemented birds became

more selective in their prey choice for nestlings. Such a response to supplementary

feeding might explain the decrease in provisioning rates across dietary treatments.

In contrast to the effects of supplementary feeding on brood provisioning in

2009 for Blue Tits, and 2008 and 2009 for Great Tits, its effects on brood

provisioning in 2007 for Great Tits and 2007 and 2008 for Blue Tits were more

subtle. In a previous study investigating the provisioning behaviour of Water Pipits

(*Anthus spinoletta*) between sites which differed in abundance of natural foods, food

availability was found to have no significant effect on provisioning rates (Rauter et al.

2000). They suggested that food was likely to be super-abundant throughout the

foraging sites and, thus, food availability did not limit reproduction. While I only have

data that reflect relative natural food abundance (i.e. caterpillars) in 2007 and 2008, it is possible that differences in natural food availability between years accounted for the inconsistent effects of supplementary feeding on brood provisioning observed in my study. For example, if natural food availability was lower in 2009 than in 2007 and 2008, it is possible that supplementary food had a more marked effect on brood provisioning rates as a result of greater energetic constraints of foraging when food was limited in 2009 (Tinbergen and Dietz 1994, Thomas et al. 2001).

Below, I discuss the mechanisms that may lead to differences in natural food availability (i.e. caterpillars) during the nestling period, and how natural food availability may explain the inconsistent effects of supplementary feeding on brood provisioning behaviour between the years of study.

4.5.2. Interactive effects of supplementary and natural food on brood provisioning behaviour

Peak availability of caterpillars, the preferred food of Blue and Great Tits during the breeding season, occurs over a brief period in temperate habitats (Perrins 1965, 1991, Wilkin et al. 2009b). Breeding is timed with the peak energetic demands of nestlings coinciding with the peak in natural food availability (Gibb 1950, van Noordwijk et al. 1995). A mismatch between these peaks may occur either too early or too late (e.g. Visser et al. 1998, Naef-Daenzer and Keller 1999) resulting in reduced foraging efficiency of adults and search times for food items increasing. Sometimes, birds switch foraging preferences, resulting in decreased provisioning rates or reduced quality of food items (e.g. Naef-Daenzer and Keller 1999, Tremblay et al. 2005). The results from 2007 and 2008 for Blue Tits support this explanation since the timing between the peak energetic demand of nestlings (approx. nestling day 9 – e.g. Eguchi

1980, Gosler 1993, Verhulst and Tinbergen 1997, Barba et al. 2009) and the peak in

caterpillar abundance was similar as were provisioning rates (compare Fig. 4.12 with
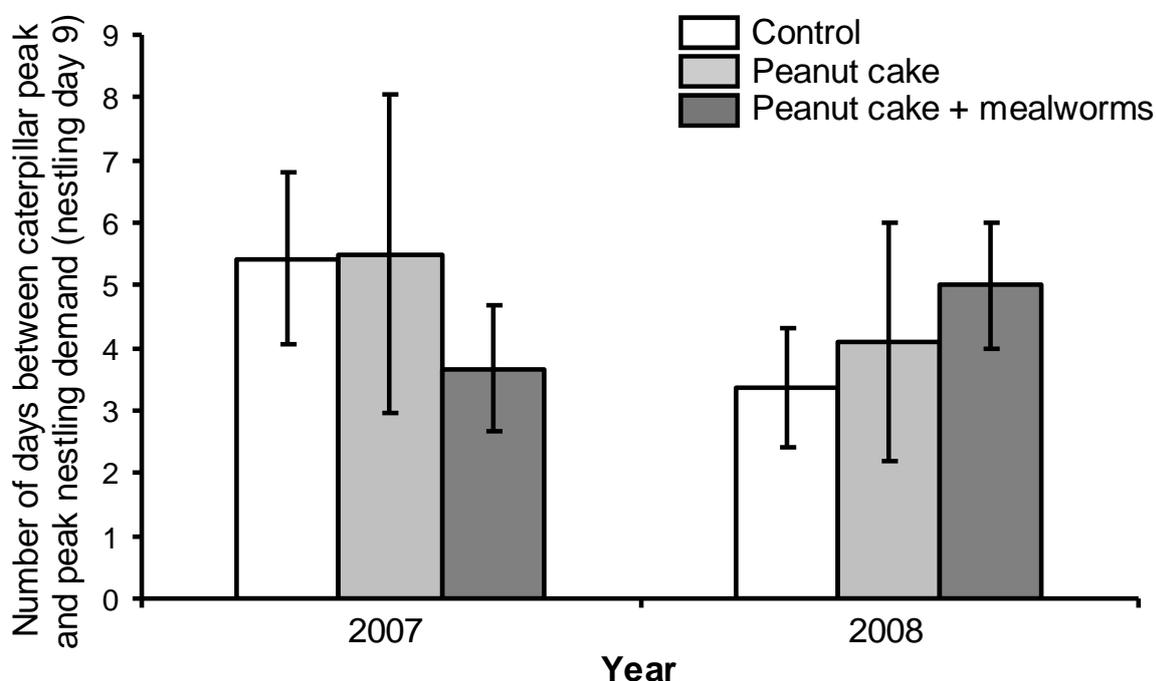
Figure 4.12. Timing of breeding of supplemented and control (nonsupplemented) Blue Tits in relation to the peak in caterpillar (Winter Moth and *Geometridae* spp.) abundance (mean ± 1 SE) in Chaddesley Woods National Nature Reserve in 2007 and 2008.

Figs 4.2 and 4.3). Moreover, between 2007 and 2008, the effects of supplementary

feeding on brood provisioning were consistent suggesting that the energetic costs of

foraging were equivalent. However, although Great Tits breeding in 2007 and 2008

had equivalent provisioning rates, in 2008 they bred less synchronously with the

emergence of caterpillars than in 2007 (compare Fig. 4.13 with Figs 4.2 and 4.3).

Since Great Tits are generalist feeders compared to Blue Tits (e.g. Török 1986), it

seems likely that Great Tits in 2008 were able to forage for other foods in addition to

caterpillars and, therefore, maintained equivalent provisioning rates to Great Tits in

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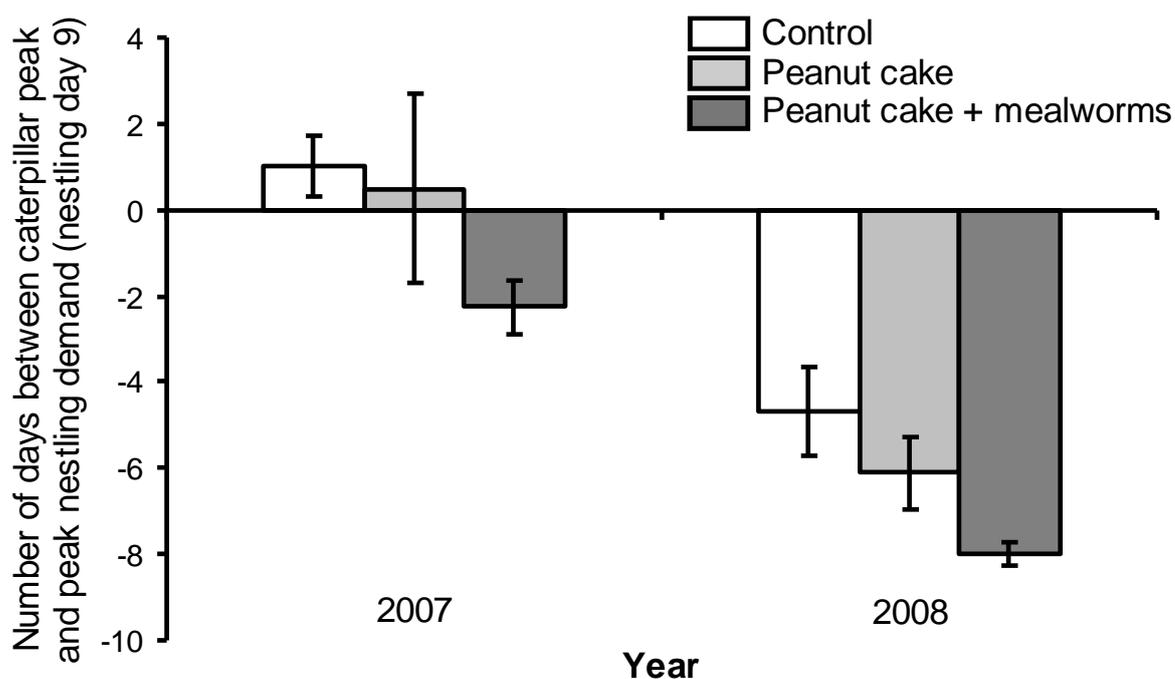
Figure 4.13. Timing of breeding of supplemented and control (nonsupplemented) Great Tits in relation to the peak in caterpillar (Winter Moth and *Geometridae* spp.) abundance (mean ± 1 SE) in Chaddesley Woods National Nature Reserve in 2007 and 2008.

2007. However, these results should be treated with caution due to small sample sizes.

Temperature is likely to be the primary cue for caterpillar emergence (Visser and Holleman 2001) with the timing of peak caterpillar abundance being earlier in years when mean spring temperatures are higher (Visser et al. 1998). Tits initiate breeding in advance of caterpillar emergence. If subsequent temperatures are high, the peak in caterpillar abundance may occur before the peak food demand of the nestlings (Visser et al. 1998). Therefore, knowledge of spring temperatures should enable us to predict whether breeding birds will mismatch with the peak in caterpillar availability. In 2009, average daily temperatures between April and May recorded at the Coleshill Met Office Land Surface Station were not significantly different from those in 2007 and 2008 (see Section 4.4.6). Therefore, the degree of synchrony between peak

caterpillar abundance and nestling food demand between years is likely to have been similar. It is unlikely, therefore, that inter-year variation in timing of caterpillar emergence in relation to timing of breeding accounts for the inconsistent effects of supplementary feeding on brood provisioning between the years of my study.

However, the size of the caterpillar abundance peak between years varies (Perrins 1965, Visser et al. 1998). Annual fluctuations in Winter Moth caterpillar population densities are driven by cyclic variations in insect parasitoids, predators, disease, inter-specific competitors, and thermal conditions during development (e.g. Varley and Gradwell 1960, Berryman 1996, Hunter 1998). Numerous studies have shown that caterpillar abundance can have pronounced effects on the foraging performance of both Blue and Great Tits (Naef-Daenzer 2000, Stauss et al. 2005a, Tremblay et al. 2005). For example, in poor habitats (of low caterpillar abundance), mean foraging distances of Blue Tits were significantly longer than those in rich habitats (of higher caterpillar abundance) (39.8 ± 10.8 m vs. 22.6 ± 6.2 m, respectively − Stauss et al. 2005a; 53.2 ± 22.9 m vs. 25.2 ± 12.3 m respectively − Tremblay et al. 2005). Adults in the poor habitat were unable to match feeding rates to brood demand compared with those in the rich habitat. Therefore, although it seems likely that the timing of breeding in relation to the peak in caterpillar availability may have been consistent between years (but I do not have data on the relative abundance of caterpillars in 2009), it is possible that variation in the size of the caterpillar peak may account for inter-year differences in the effects of supplementary feeding on provisioning rates by adults.

However, since differences in relative caterpillar abundance between 2007 and 2008 did not subsequently result in differences in provisioning rates in either Blue or Great Tits (compare Figs 4.2, 4.3 and 4.10), it seems unlikely that caterpillar

availability can independently explain inter-year differences in the effects of supplementary feeding on brood provisioning behaviour. Additional factors that may affect foraging efficiency include rainfall (Radford et al. 2001) and availability of other sources of food consumed by both Blue and Great Tits during the breeding season (e.g. spiders, diptera, coleoptera – Betts 1955). Although I do not have data reflecting the availability of other food sources or weather variables, it is plausible that year differences in these factors may have resulted in the observed differences in effects of supplementary food on provisioning rates between the years of my study.

4.5.3. Effects of supplementary feeding on nestling condition and apparent survival

Results from previous studies suggest that low food availability during the nestling phase can lower reproductive success through reduced provisioning rates and, subsequently, reduce nestling body condition (e.g. Simons and Martin 1990, Naef-Daenzer and Keller 1999) and reduce nestling survival (e.g. Verhulst 1994, Ringsby et al. 2009). Although I found significant effects of supplementary feeding on provisioning behaviour, I did not find an effect on nestling body condition. However, supplementary feeding appeared to promote apparent survival of fledglings but the effects were inconsistent between years. These results are partly consistent with those of other studies. For example, Reynolds et al. (2003b) found that food supplementation had little effect on nestling growth of Florida Scrub-Jays (*Aphelocoma coerulescens*) although the survival of fledglings from food-supplemented territories was enhanced over that of fledglings from control territories. In this species supplemented females lay significantly heavier third eggs than control conspecifics (Reynolds et al. 2003a). Since the chances of survival of fledglings hatched from large eggs exceed those of nestlings hatched from smaller eggs (e.g.

Parsons 1970, Hegyi 1996), it was suggested that food supplementation during pre-laying may have improved egg quality and, subsequently, influenced nestling survival. In my study population supplemented Great Tits lay heavier eggs than control conspecifics (Harrison 2010). However, since the effects of supplementary feeding on apparent survival were inconsistent between years, it remains unclear if egg quality influenced survival in my population. Moreover, since supplementary food was provided both throughout the nestling period and post-fledgling, separating pre- and post-fledging effects on survival (i.e. mediated through egg quality, brood provisioning, post-fledging foraging) remains challenging.

The inconsistency of effects of dietary treatment on apparent survival between years could be attributed to the locations of the three mist-netting sites during the autumn recapture sessions (Fig. 4.14). Such inter-year inconsistencies mirror the rotation of
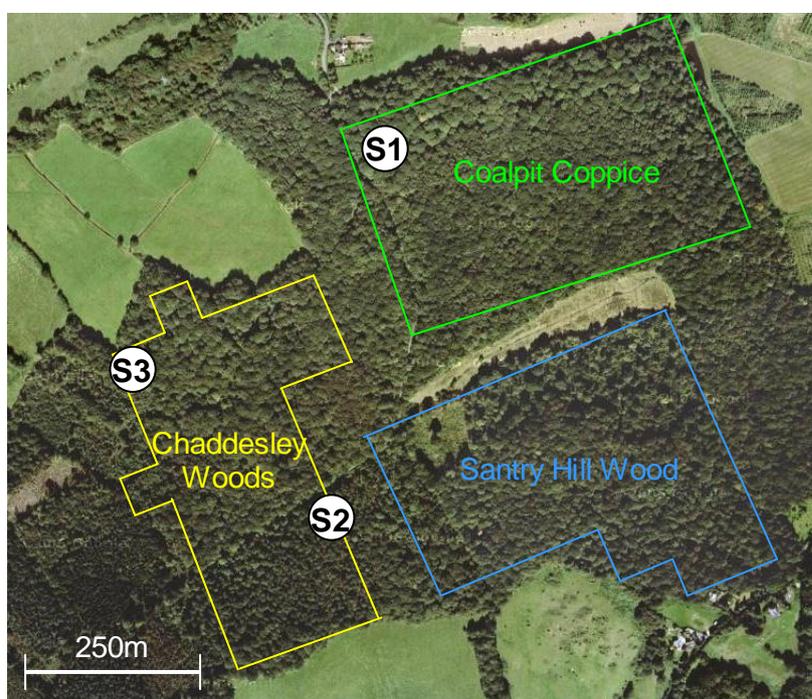
Figure 4.14. Location of mist-netting sites in Chaddesley Woods National Nature Reserve between 2007 and 2009. S1 – site 1; S2 – site 2; S3 – site 3.

the dietary treatments (i.e. recapture rate of fledglings in each year was always highest from the dietary treatment which was allotted to the Chaddesley Wood block – compare Figs 4.8 and 4.9 with 4.14). This provides insights into post-fledging behaviour demonstrating that fledglings may remain relatively close to where they fledged at least through their first autumn and winter until the time that we mist-netted. However, measurements of apparent survival should be taken with caution since the methods used to calculate apparent survival (i.e. mist-netting) are unlikely to capture all surviving individuals in the population and, furthermore, will not capture those which have emigrated.

4.5.4. Summary and future directions

Throughout this study I have discussed my findings assuming that the effect of supplementary feeding is indirect and could result from the consumption of the food by the parent which, in turn, results in a reduction of time needed to forage. Subsequently, this could result in an increase in the amount of time and energy available for brood provisioning (Martin 1987, Grieco2002a). The effects of supplementary feeding on brood provisioning behaviour could also be direct whereby the parent feeds the nestlings with the additional food, resulting in reduced begging intensity by nestlings and a decreased provisioning rate by adults (e.g. Quillfeldt and Masello 2004, Hamer et al. 2006). Direct effects of supplementary feeding have been observed in a number of supplementary feeding studies. For example, Cucco and Malacarne (1997) found that supplementary food was almost always provided to the nestlings by adult Black Redstarts; adults were rarely observed consuming it. In my study it is unclear if the effects of supplementary feeding were direct or indirect.

Therefore future research should determine the mechanisms by which supplementary

food acts on brood provisioning.

In a biparental mating system, such as that of Blue and Great Tits, parental

investment during the nestling phase may be influenced by food availability and differ

between the sexes when each sex differentially allocates time towards other activities

such as preening, singing, vigilance and anti-predator behaviours (e.g. Ward and

Kennedy 1996, Cucco and Malacarne 1997, Rastogi et al. 2006). Supplemented male

Black Redstarts spent more time singing, preening and being vigilant and less time

feeding nestlings compared with unfed birds while females increased provisioning

rates to offset reduced male provisioning rates (Cucco and Malacarne 1997). Since

Great and Blue Tits are polygamous, it is possible that supplementary feeding

differentially affected the behaviour of males and females. However, this can only be

investigated by monitoring the provisioning efforts of both sexes perhaps using PIT

tag technology that allows the automatic identification of individual birds as they

enter a nestbox (Freitag et al. 2001).

I have shown that the combined provisioning efforts of breeding pairs of Great

and Blue Tits at day 9 of the nestling period were significantly influenced by food

supplementation whether it be food that is consumed by adults (i.e. peanut cake) or

fed directly to chicks (i.e. mealworms). Further data on sex-specific brood investment

by adults, natural food abundance, foraging distances and prey size would provide

further insights in this regard.

Nestling provisioning rates have important consequences for nestling growth

rate, fledging body mass (Naef-Daenzer and Keller 1999) and, as a consequence,

survival and fitness. Future work should focus on the downstream effects of nestling

provisioning such as on nestling growth rates and nestling recruitment into the

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breeding population. Results from my study and future work may assist in understanding the life-history trade-offs between self-maintenance and investment in current and future broods. This will increase our understanding of population and community dynamics in response to food availability. Finally, since feeding garden birds is a widespread activity amongst homeowners internationally (Jones and Reynolds 2008, Davies et al. 2009), the results of this and future studies may have important implications for recommended best practice in the timing, nature and extent of feeding wild birds during the breeding period.

4.5.5 The next chapter

Parental investment during the nestling phase may be influenced by food availability and differ between the sexes through changes in the potential for polygamy (Emlen and Oring 1977). For example, when reproductive success is more variable in males than in females and food abundance enables females to provide full parental care, it may be advantageous for males to allocate time and energy to seeking out EPCs (Emlen and Oring 1977). With my findings showing a significant effect of supplementary feeding on brood provisioning behaviour, I will now investigate the effect of supplementary feeding on the rate of EPP in Chapter Five.

Chapter Five

THE EFFECT OF FOOD AVAILABILITY ON EXTRA-PAIR PATERNITY OF BLUE TITS

5.1. ABSTRACT

Extra-pair paternity (EPP) can provide both direct benefits and indirect benefits. However, since extra-pair copulations (EPCs) may be both energetically and temporally costly, the incidence of EPP is likely to vary in relation to food availability. Furthermore, since within-pair young (WPY) and extra-pair young (EPY) experience differential mortality, the availability of food may further influence the incidence of EPP within a brood post-hatching. During 2008 and 2009 I studied the effects of food supplementation on EPP in Blue Tits by providing supplementary food (peanut cake) during the fertile period, and by providing a further food supplement (mealworms) intended for chick consumption during the nestling period. Supplementary feeding had a significant effect on the proportion of EPY per brood with 14.75%, 28.35% and 28.64 % of nestlings being EPY in control, peanut cake, and peanut cake and mealworms treatments, respectively. This study provides empirical evidence of food availability influencing the mating behaviour of a common woodland passerine. Therefore, further study of the behavioural mechanisms that underlie mating strategies of birds is important in elucidating effects of supplementary food on EPP and the genetic structure of bird populations.

5.2. INTRODUCTION

In Chapter Four I provided evidence that the supplementation of food prior to, and throughout, the nestling period had marked effects on brood provisioning behaviour of both Blue and Great Tits. Food availability during the nestling period may differentially influence male and female investment in brood provisioning through changes in the potential for polygamy (Emlen and Oring 1977). In this chapter, therefore, I investigate the effects of supplementary feeding on the incidence of EPP in Blue Tits.

5.2.1. EPP and food availability

The mating systems of birds are diverse and vary across species in respect to the number of social partners (i.e. partners who share responsibilities of parental care and/or territory defence) and the number of sexual partners (i.e. partners who copulate but do not invest in parental care). While 85% of species practise social monogamy (Lack 1968) (i.e. do not form social bonds with other birds outside of the social pair), more than 75% of socially monogamous bird species are sexually polygamous (Griffith et al. 2002) and partake in EPCs (i.e. where the female copulates with a male who is not her social mate – Bennett and Owens 2002). Engaging in EPCs, which may result in EPP (Bennett and Owens 2002), can provide both direct benefits and indirect benefits. For example, a female engaging in EPP can directly benefit by gaining additional food through increased foraging opportunities on an extra-pair male's territory (Gray 1997); she may also gain indirect genetic benefits such as an increased likelihood of 'good genes' within her offspring (e.g. Hasselquist et al. 1996, Kempenaers et al. 1997). Several non-mutually exclusive hypotheses framed within ecological and genetic contexts have been proposed to explain the function of EPCs

(reviewed in Petrie and Kempenaers 1998, Griffith et al. 2002). Despite the debate

still raging about the finer points of mechanics and functionality of EPC (reviewed in

Petrie and Kempenaers 1998, Griffith et al. 2002), it is generally accepted that EPC

enables males to increase the number of offspring they father and females to increase

the quality of their offspring (Birkhead and Møller 1992).

Despite such benefits to both sexes, undertaking EPC is likely to be costly. For

example, a female participating in EPCs may experience a reduction in paternal care

from her social mate who may adjust his reproductive investment in relation to his

level of perceived cuckoldry (e.g. Dixon et al. 1994, Weatherhead et al. 1994).

Seeking EPCs is likely to be costly for a male too as he reduces the time spent mate-

guarding and, therefore, increases the probability of being cuckolded (e.g. Komdeur et

al. 1999, Chuang-Dobbs et al. 2001). For both sexes mating with multiple partners

increases exposure to sexually-transmitted diseases and, because searching for EPCs

reduces the amount of time for foraging, it is likely to be both energetically and

temporally costly (reviewed in Birkhead and Møller 1992, Petrie and Kempenaers

1998).

Since the energetic and temporal constraints of foraging are likely to be

alleviated when food is abundant, food availability may also influence the rate of EPP.

However, to date, although some studies suggest that food availability may at least

partially determine rates of EPP (e.g. Dunn and Hannon 1992, Charmantier and

Blondel 2003, Rubenstein 2007), to the best of my knowledge only three food

supplementation studies have examined the role of food availability in this regard in

any detail. Westneat (1994) provided supplementary food (a mixture of cracked corn,

sunflower seeds and mealworms) to Red-winged Blackbirds from the onset of nest

construction until the third egg was laid to investigate how foraging behaviour

influenced paternity. Food-supplemented males sired significantly more offspring (88%) produced on their territories than control (nonsupplemented) males (69%) and made significantly fewer foraging trips (1.0 trips per hr) off their territories than did controls (1.7 trips per hr). Westneat (1994) concluded that the difference in paternity between the supplemented and control birds was the result of supplemented males investing more in mate-guarding and, therefore, safeguarding paternity.

Supplementary feeding of commercially available seed mix prior to egg laying also led to a decrease in EPP in broods of House Sparrows with the proportion of EPY in broods being five times lower than in broods at sites where supplementary food was not available (Václav et al. 2003). This may have resulted from a change in female behaviour (supplemented females, but not males, increased time spent at the nest) which enabled them to avoid EPCs with neighbouring males (Václav et al. 2003). In contrast, Hoi-Leitner et al. (1999) manipulated food availability by providing food plants (*Diplotaxis* spp.) during the fertile period of female European Serins and showed that the number of broods containing EPP was significantly higher on territories with high food availability.

Females that participate in EPCs may face a reduction in paternal care from social mates who may adjust their reproductive investment in relation to the level of perceived cuckoldry (e.g. Dixon et al. 1994, Weatherhead et al. 1994). Hoi-Leitner et al. (1999) suggested that the costs of having help withdrawn from males were less for females in territories with high food availability than for those in poorer ones. Therefore, the costs of producing EPY for supplemented females would be reduced over those for the control females, thereby providing support for the 'constrained female hypothesis' (Gowaty 1996).

Although previous supplementary feeding studies have shown food availability influences the incidence of EPP (e.g. Westneat 1994, Hoi-Leitner et al. 1999, Václav et al. 2003), whether extended supplementary feeding throughout the spring and summer, as recommended by organisations such as the BTO and RSPB (see Section 1.1.2), influences the mating systems of common garden birds has not been investigated. Furthermore, few studies have considered the effects of supplementary feeding during the nestling phase on the survival to fledging of within-pair young (WPY) and EPY. This may be important to consider since WPY and EPY may experience differential mortality in the nest with the latter having a higher chance of fledging (e.g. Kempenaers et al. 1997, O'Brien and Dawson 2007).

In this chapter I investigate the effects of supplementary food on the incidence of EPP both between and within broods of Blue Tits, a small passerine species of woodlands which readily feeds on garden bird feeders and nests in nestboxes (Gosler and Clement 2007). Although Blue Tits are socially monogamous (Cramp and Perrins 1993), EPCs (resulting in EPY) are common in this species (e.g. Kempenaers et al. 1992, Krokene et al. 1998) during the female's fertile period between 5 days before the first egg is laid and the day on which the penultimate egg is laid (Møller 1987). I hypothesised that supplementary food (peanut cake) provided prior to, and throughout, the egg laying period would partly alleviate the energetic and temporal constraints of foraging allowing male Blue Tits to increase time spent mate-guarding during the fertile period of female Blue Tits. I also hypothesised that feeders containing the supplementary food would act as 'food hotspots' resulting in a reduction in the roaming behaviour of foraging female Blue Tits allowing them to be closely mate-guarded by male Blue Tits. Therefore, I predicted that the supplementation of peanut cake would result in fewer nests containing EPY and that

broods of supplemented birds would have a lower proportion of EPY than broods of control (nonsupplemented) birds. In addition, I hypothesised that an additional food supplement (mealworms) intended for nestlings, and provided to birds already receiving peanut cake, would result in increased nestling survival and a lessening of differential mortality between WPY and EPY compared with only peanut cake-supplemented birds. Therefore, I predicted that the additional supplementation of food intended for nestlings would result in a lower proportion of EPY within nests compared to that in nests of birds only supplemented with peanut cake.

5.3. METHODS

5.3.1. Study site and supplementation

Please see full details that are provided in Section 4.3.1.

5.3.2. Field methods

Nestboxes were checked every other day to determine clutch initiation date, onset of incubation and hatch date (see Section 3.3.2 for further details). In both 2008 and 2009 a subset of nestboxes were chosen (approx. 15 from each dietary treatment) to investigate EPP. As availability of opportunities for EPCs may vary seasonally (e.g. Thusius et al. 2001, Stewart et al. 2010), nestboxes were selected in accordance with lay date to ensure that I sampled birds with hatch dates distributed throughout the entire breeding season. Furthermore, focal nestboxes were distributed as evenly as possible throughout each woodland block so as to control for potential localised habitat effects. In 2008, both adults and all nestlings between 10 and 14 days after hatching at each focal nestbox were caught under Natural England licence (20080377) with adults captured using spring-loaded nestbox traps (Amber Electronics Ltd.,

Daventry, UK) and nestlings removed from nestboxes. All birds were tissue sampled

under Home Office licence (PPL 40/2926). One to three contour feathers were

plucked from adults and nestlings to obtain adequate amounts of DNA for paternity

analysis (e.g. Taberlet and Bouvet 1991, Harvey et al. 2006) and feather tips were cut

from them using dissecting scissors and stored in 1 ml of absolute ethanol. As a result

of low yields of DNA from feather pulp in 2008, in 2009 I took 25-50 µl blood

samples from each adult and nestling by venipuncture with a 27G hypodermic needle

(Becton Dickinson U.K. Ltd., UK), drawing blood into glass heparinised capillary

tubes (Bilbate, Daventry, UK) and flushing into 1 ml of absolute ethanol. Tissue

samples were stored in a domestic fridge at 3°C pending molecular analysis. All birds

had either been ringed previously or were ringed under BTO licence (under ringing

permit: C/5418) at the time of tissue sampling using standard BTO metal rings for

individual recognition. Both adults and three randomly chosen nestlings within each

brood had tarsus length (minimum tarsus length – Redfern and Clark 2001) measured

to the nearest 0.1 mm using a dial calliper (KWB, Switzerland) and body mass to the

nearest 0.1 g using an electronic balance (SA-500, SATRUE, Taiwan). I took all

morphometric measurements to avoid repeatability issues with multiple experimenters

(Gosler et al. 1998).

5.3.3. Molecular methods

Genomic DNA was extracted using the Ammonium acetate method of Nicholls et al.

(2000) from a total of 151 adults and 730 nestlings from 83 broods sampled in 2008

and 2009. Extracted DNA was quantified by measuring the optimal density of

samples at 250 nm using a Fluostar Optima fluorimeter (BMG Labtech, Offenburg,

Germany). I used 14 autosomal polymorphic microsatellite loci in 4 multiplex primer

sets (see Appendix Two for details of development of multiplex primer sets) to

determine paternity. Loci were amplified by polymerase chain reaction (PCR). Each

2-µl PCR involved approx. 15 ng of genomic DNA, 0.5 µl of each primer (0.2 µM)

and 1.0 µl of QIAGEN Multiplex PCR Master Mix (Kenta et al. 2008). PCR

amplification was performed using a DNA Engine Tetrad PTC-225 Peltier thermal

cycler (MJ Research, Bio-Rad, Hemel Hempstead, UK). A touchdown PCR was used

(TD65-55) with the following profile: 95ºC for 15 min, then 10 cycles of 94ºC for 30

sec, 65ºC for 90 sec (dropping 1.0ºC per cycle), 72ºC for 1 min, followed by 25 cycles

of 94ºC for 30 sec, 55ºC for 90 sec, 72ºC for 1 min. PCR products were diluted using

high grade water (1 in 400 dilution). One µl of each diluted sample was then mixed

with 9.5 µl of formamide/size standard mix (made up of 1 ml of formamide and 3 µl

of ABI ROX 500 internal size standard [Applied Biosystems, California, USA]).

Samples were then denatured for 5 min at 95ºC and quenched on ice prior to being

separated on an ABI 3730 DNA Analyser (Applied Biosystems, California, USA).

Genotypes were scored relative to the 500 ROX internal size standard (Applied

Biosystems, California, USA) using GENEMAPPER software v3.7 (Applied

Biosystems, California, USA).

5.3.4. Confirming suitability of loci for paternity analysis

To confirm that each locus was suitable for paternity analysis, loci characteristics

were measured (see Appendix Two for full details of measuring loci characteristics).

For each locus, observed and expected heterozygosities (i.e. measurements of genetic

diversity – Weir 1990), estimated null allele frequencies and non-exclusion

probabilities were calculated using CERVUS v3.0 (Marshall et al. 1998) for each year

independently using the genotypes from all unrelated individuals. Tests for departures

from Hardy-Weinberg (see Russell 1996 for overview of the Hardy-Weinberg

equilibrium) and linkage disequilibrium (the association between alleles at different

loci) (Amos et al. 1992) were also conducted using a Markov-chain method

implemented in GENEPOP v.4.0 (Rousset 2008) for each year independently using

the genotypes from all unrelated individuals. Independent year analysis was applied as

initial inspections of genotypes suggested that more loci failed to amplify during PCR

in DNA from the 2008 feather samples than in DNA from the 2009 blood samples.

Therefore, it was likely that estimations of genetic variability (i.e. observed and

expected heterozygosities, estimated null allele frequencies and non-exclusion

probabilities) would not be consistent between years (Hoffman and Amos 2005).

5.3.5. Paternity analysis

Paternity was assigned using COLONY version 2.0.1.1 (Wang 2004). COLONY uses

a maximum-likelihood approach to assign paternity whilst accounting for genotyping

errors and allelic dropout.

      To estimate genotyping errors and instances of allelic dropout the number of

allelic mismatches (i.e. when an offspring and a mother do not share a common allele

at a locus) between putative mothers and nestlings at each locus was initially

estimated using all genotyped individuals independently for each year. In addition to

allelic mismatches occurring as a result of genotyping error and allelic dropout, allelic

mismatches between offspring and putative mothers also occur when offspring

originate from dumped eggs (i.e. intra-brood parasitism) (e.g. Meek et al. 1994, Reyer

et al. 1997). Therefore, the presence of nestlings originating from dumped eggs may

result in an overestimation of genotyping error and allelic dropout rates. To avoid

overestimation of genotyping error and allelic dropout rates, I plotted the distribution

of allelic mismatches between putative mothers and nestlings for each year. This assisted in determining which allelic mismatches were due to genotyping error/allelic dropout as opposed to egg dumping and allowed me to omit the latter in calculations of genotyping error and allelic dropout.

During paternity assignment COLONY version 2.0.1.1 (Wang 2004) accounts for genotyping errors and allelic dropout separately. To calculate allelic dropout rate using allelic mismatches, I identified allelic mismatches which resulted from one individual having a homozygous profile. These mismatches were assumed to result from the failure of one allele to amplify (i.e. allelic dropout) (Fig. 5.1). All other

Mother genotype: **A / B**   Father genotype: **C / D**

'Real' offspring genotype: **A / C**

PCR amplification in which the A allele from the mother does not amplify due to allelic dropout

'False' offspring genotype: **C / C**

Offspring now appears to have a homozygous profile resulting in an allelic mismatch between the mother and offspring
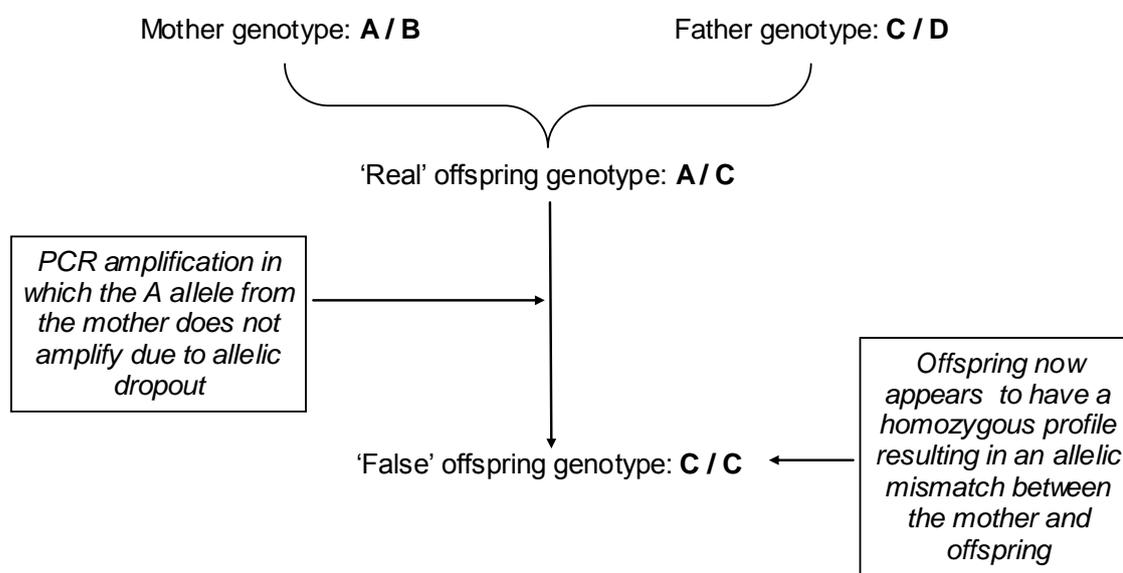
Figure 5.1. Allelic mismatch between the mother and offspring resulting from allelic dropout (i.e. where the allele from the mother failed to amplify). The 'real' offspring genotype (A/C) consists of one allele from the mother (A) and one from the father (C). However, the offspring genotype determined through genetic analysis is 'false' (C/C) as a result of 'A' failing to amplify.

mismatches (i.e. where both individuals were heterozygous) were assumed to be a result of other genotyping errors (i.e. allele scoring issues – Marshall et al. 1998, Hoffman and Amos 2005).

5.3.6. Statistical analyses

Statistical analyses were performed using data from all nestlings apart from those hatching from dumped eggs. I used both the proportion of nests containing EPY (model 1) and the proportion of EPY within a nest (model 2) as measures of EPP. Mixed models were constructed using PROC GLIMMIX in SAS version 9.2 (SAS Institute Inc. 2008) and fitted with binomial error distributions to test for the effects of supplementary feeding on both of the response variables independently. In both models, treatment block was specified as a random factor, due to repeated measures on treatment blocks between years, and year was included as a fixed factor to account for potential annual variation in breeding performance (Nager et al. 1997). Combined-year analysis was conducted if there was no significant interaction between year and dietary treatment (year $\times$ dietary treatment: $P > 0.05$) and no main effect of year (year: $P > 0.05$). Otherwise independent year analyses were performed.

Clutch size was included as a covariate in both models because the probability of having an EPY may depend on clutch size (Brommer et al. 2007). Hatch date was also included as a covariate in both models to control for temporal changes in availability of extra-pair matings resulting from seasonal changes in breeding synchrony and breeding densities (e.g. Thusius et al. 2001, Stewart et al. 2010). Male body condition (residuals from the regression of body mass on tarsus length) was also included as a covariate in all models because females mated to low quality (i.e. body condition) males are more likely to seek out EPCs than those paired to high quality males (e.g. Kempenaers et al. 1992, 1997). Finally, male age was included as a covariate in all models because older males are less likely to be cuckolded than younger males (e.g. Lubjuhn et al. 2007, Moreno et al. 2010). To determine if the effects of covariates were consistent between treatment blocks, covariate $\times$ dietary

treatment interactions were tested independently of one another within each model. Where multiple covariate × dietary treatment interactions were significant they were both included in the model as long as each stayed significant (Grafen and Hails 2002). I performed backward model selections removing the least non-significant covariate interactions ($P > 0.05$) one-by-one from models ensuring that there was no significant change in deviance between models where applicable (Grafen and Hails 2002). Tukey-Kramer *post hoc* tests were conducted for pairwise comparisons of the least-square means with results being presented as adjusted *P* values.

5.4. RESULTS

5.4.1. Suitability of loci for paternity analysis

The results from the tests used to investigate loci characteristics revealed that one locus, BT22D06, in both 2008 and 2009 was significantly out of Hardy-Weinberg equilibrium ($P < 0.00001$ – 2008 and 2009; Tables 5.1 and 5.2) and also possessed a high estimated null allele frequency in 2009 (0.118 [11.8% estimated as being null]; Table 5). Furthermore, following a Bonferroni correction for multiple comparisons (Rice 1989), tests for linkage disequilibrium between pairs of loci revealed that one pair of loci were in linkage disequilibrium in 2008 (BT19F01 *vs.* BT22G10, $P = 0.00$). The locus BT22D06 was, therefore, excluded from genetic parentage analysis (see Appendix Two for details of loci characteristics and consequences for paternity assignment).

Table 5.1. Details of polymorphic microsatellite loci used in the paternity analysis of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008. Note: N is the number of birds tested; H_O the observed heterozygosity; H_E the expected heterozygosity; HWE the Hardy-Weinberg Equilibrium from which the P value is generated (values in bold indicate a departure from Hardy-Weinberg equilibrium where $P < 0.05$); F(null) is the expected null allele frequency as a proportion (values in bold indicate where proportion of null allele frequencies for the loci are > 0.1); NE-1P is the non-exclusion probability (first parent); EMBL accession number is the unique identifier given to each primer once submitted to the EMBL nucleotide sequence database - http://www.ebi.ac.uk/embl/; and Reference is the place of publication.

Locus	N	Number of Alleles	H_O	H_E	HWE P-value	F(Null)	NE-1P	EMBL accession number	Reference
BT19F01	55	15	0.818	0.847	0.164	+0.011	0.480	FM878046	*Unpubl.*
BT21C07	48	6	0.792	0.731	0.207	-0.043	0.692	FM878058	*Unpubl.*
BT22D06	52	25	0.846	0.945	**0.000**	+0.050	0.229	FM878073	*Unpubl.*
BT19G06	62	13	0.871	0.867	0.190	-0.008	0.436	FM878047	*Unpubl.*
BT20C03	56	11	0.857	0.790	0.909	-0.048	0.582	FM878052	*Unpubl.*
BT22G10	58	8	0.845	0.828	0.598	-0.017	0.530	FM878081	*Unpubl.*
BT23E05	55	16	0.818	0.823	0.591	-0.001	0.516	FM878091	*Unpubl.*
BT19H11	59	13	0.864	0.875	0.234	+0.004	0.413	FM878051	*Unpubl.*
BT23F02	62	15	0.984	0.920	0.769	-0.038	0.301	FM878092	*Unpubl.*
Pca3	57	20	0.877	0.927	0.317	+0.024	0.281	AJ279805	Dawson et al. (2000)
Pca4	56	10	0.911	0.814	0.153	-0.063	0.554	AJ279806	Dawson et al. (2000)
Pca7	56	11	0.893	0.859	0.962	-0.024	0.457	AJ279809	Dawson et al. (2000)
Pca9	61	11	0.787	0.872	0.318	+0.048	0.426	AJ279811	Dawson et al. (2000)
POCC1	26	13	0.885	0.870	0.912	-0.025	0.443	U59113	Bensch et al. (1997)

Table 5.2. Details of polymorphic microsatellite loci used in the paternity analysis of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2009. Note: N is the number of birds tested; H_O the observed heterozygosity; H_E the expected heterozygosity; HWE the Hardy-Weinberg Equilibrium from which the P value is generated (values in bold indicate a departure from Hardy-Weinberg equilibrium where $P < 0.05$); F(null) is the expected null allele frequency as a proportion (values in bold indicate where proportion of null allele frequencies for the loci are > 0.1); NE-1P is the non-exclusion probability (first parent); EMBL accession number is the unique identifier given to each primer once submitted to the EMBL nucleotide sequence database - http://www.ebi.ac.uk/embl/; and Reference is the place of publication.

Locus	N	Number of Alleles	H_O	H_E	HWE P-value	F(Null)	NE-1P	EMBL accession number	Reference
BT19F01	82	18	0.878	0.846	0.112	-0.024	0.475	FM878046	*Unpubl.*
BT21C07	81	6	0.728	0.716	0.866	-0.012	0.705	FM878058	*Unpubl.*
BT22D06	82	26	0.732	0.932	**0.000**	**0.118**	0.258	FM878073	*Unpubl.*
BT19G06	81	14	0.914	0.897	0.781	-0.012	0.362	FM878047	*Unpubl.*
BT20C03	80	10	0.85	0.824	0.951	-0.019	0.534	FM878052	*Unpubl.*
BT22G10	79	8	0.835	0.84	0.731	0.001	0.502	FM878081	*Unpubl.*
BT23E05	81	16	0.84	0.786	0.142	-0.041	0.582	FM878091	*Unpubl.*
BT19H11	81	12	0.914	0.86	0.509	-0.033	0.445	FM878051	*Unpubl.*
BT23F02	81	18	0.926	0.906	0.581	-0.015	0.331	FM878092	*Unpubl.*
Pca3	82	26	0.939	0.921	0.838	-0.014	0.287	AJ279805	Dawson et al. (2000)
Pca4	82	15	0.878	0.827	0.175	-0.039	0.523	AJ279806	Dawson et al. (2000)
Pca7	82	14	0.829	0.879	0.434	0.026	0.405	AJ279809	Dawson et al. (2000)
Pca9	82	12	0.866	0.84	0.946	-0.019	0.485	AJ279811	Dawson et al. (2000)
POCC1	77	12	0.948	0.896	0.948	-0.033	0.367	U59113	Bensch et al. (1997)

5.4.2. Genotyping errors and instances of allelic dropout

To allow genotyping error and allelic dropout rates to be estimated for use in paternity analysis, the number of allelic mismatches was calculated. In 2008, 34 nestlings had allelic mismatches at one or more loci with their putative mothers compared with 17 in 2009. The distribution of the number of allelic mismatches between nestlings and putative mothers was bimodal with a group of nestlings and their putative mothers having one or two allelic mismatches and a group having over four allelic mismatches (Fig 5.2a and b). I assumed that where the number of allelic mismatches between a nestling and its putative mother was less than three the allelic mismatches could be explained by genotypic error/allelic dropout. Alternatively, I assumed that where the number of allelic mismatches between a nestling and its putative mother was greater than four the allelic mismatches could be explained by egg dumping (see Meek et al. 1994, Reyer et al. 1997 for separating EPY and young originating from intra-specific brood parasitism).

5.4.3. Occurrence of EPP in the study population

Thirty-two males and 38 females in 2008 and 41 males and 41 females in 2009 from 41 and 42 nests, respectively, were genotyped and none was found to have bred in both years of the study. Out of 348 nestlings genotyped in 2008 and 381 in 2009, I successfully assigned paternity to 233 (67.0%) and 306 (80.3%), respectively. Eighty nestlings (23.0%) in 2008 and 67 nestlings (17.6%) in 2009 were EPY. Furthermore, 25 of 41 nests (61.0%) in 2008 and 19 of 42 nests (45.2%) in 2009 contained EPY. In addition, two nests in 2008 and three nests in 2009 contained nestlings that hatched from dumped eggs.

Of 25 nests in 2008 and 19 nests in 2009 containing EPY, 10 and four
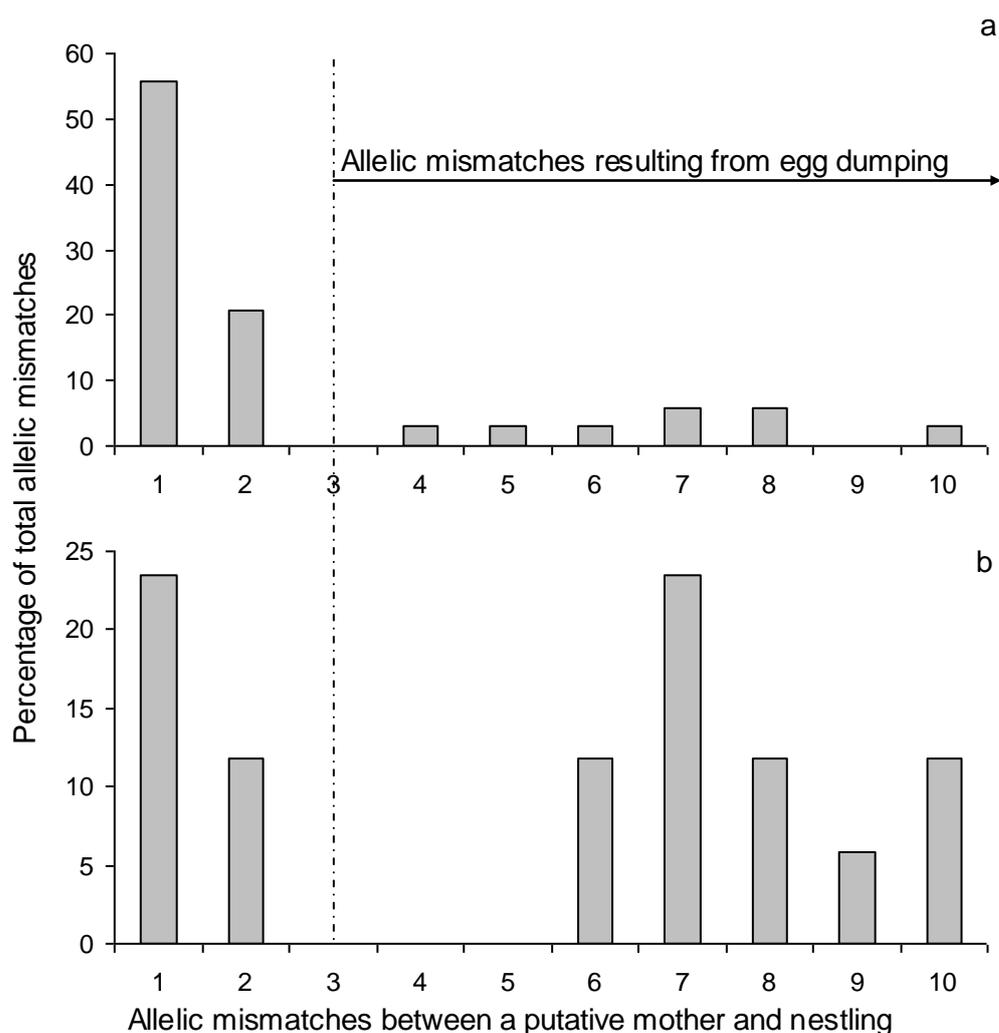
Figure 5.2. Distribution of allelic mismatches between genotyped putative mother and nestling Blue Tits sampled in (**a**) 2008 and (**b**) 2009 in Chaddesley Woods National Nature Reserve. Three or less mismatches between a nestling and a putative mother result from either genotyping error or from allelic dropout whereas four or more such mismatches result from incidents of egg dumping.

respectively, contained nestlings that were sired by two or more extra-pair fathers while the remainder contained nestlings sired by a single extra-pair father (Fig. 5.3). In four incidences of EPP at nests in 2008, and two in 2009, the extra-pair father was caught at another nest (i.e. at the nest of his social mate) which was never more than two nestboxes away (i.e. within 80 m). However, as the dietary treatment blocks were separated by approx. 70 m, this highlighted the potential for EPCs to be elicited in a
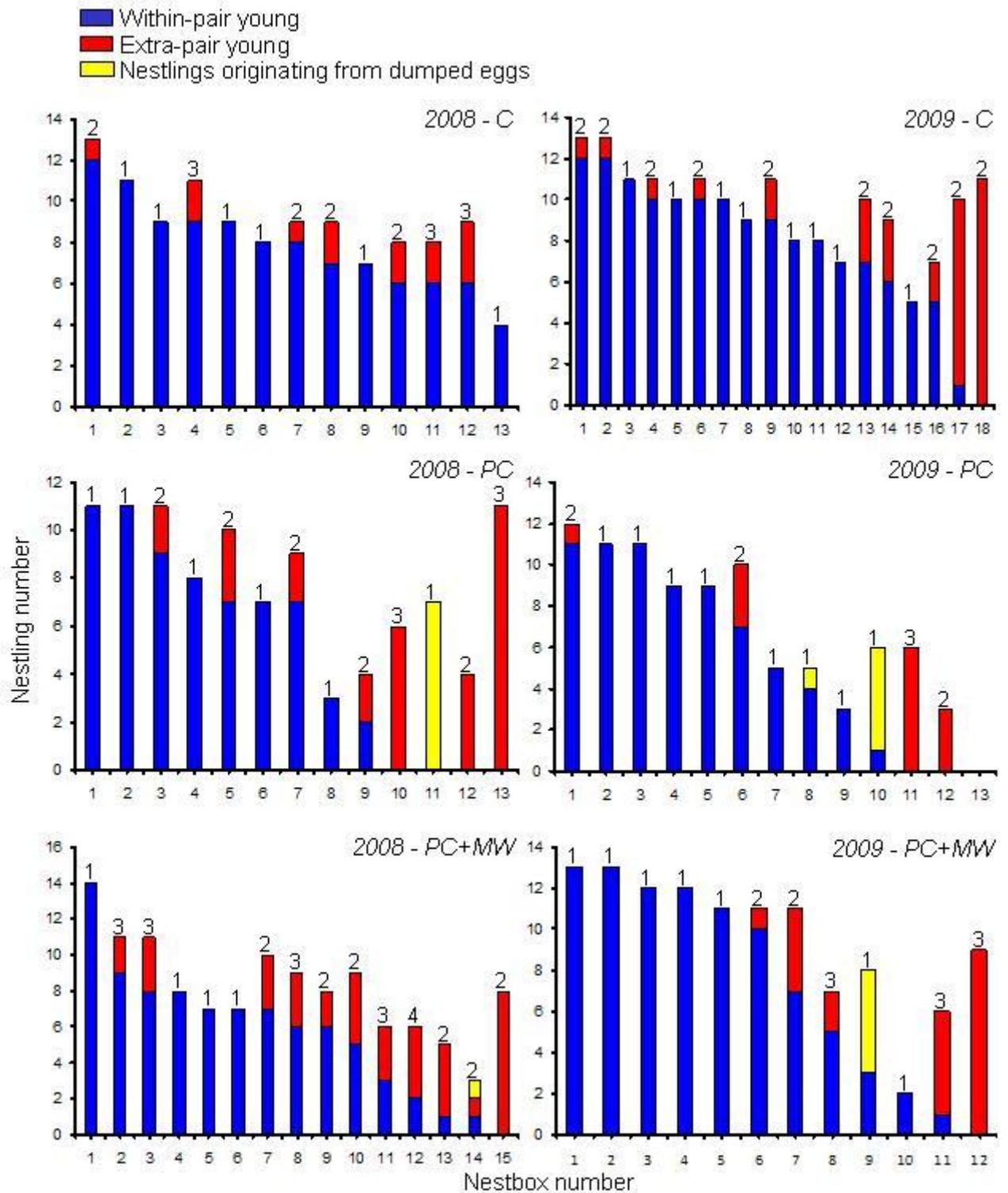
Figure 5.3. Paternity of young (within-pair, extra-pair or from dumped eggs) in Blue
Tit broods sampled in Chaddesley Woods National Nature Reserve in 2008 and 2009
where birds were fed peanut cake (PC), peanut cake + mealworms (PC+MW), and no
supplement (control - C). Numbers above bars indicate the number of fathers
represented in each brood.

neighbouring dietary treatment block. Indeed, in one incidence in 2009, I found that one male nesting on the edge of one dietary treatment block was found to have fathered nestlings in a nestbox on the edge of a neighbouring dietary treatment block (Fig. 5.4).

5.4.4. Effects of supplementary feeding on EPP

There was no significant effect of year on EPP in terms of either proportion of broods ($F_{2,48} = 1.72$, $P = 0.12$) or proportion of EPY within broods ($F_{1,72} = 0.24$, $P = 0.62$). Supplementary feeding significantly affected the proportion of broods containing EPY ($F_{2,72} = 3.91$, $P = 0.02$; Fig. 5.5) with a higher proportion of broods in both the control and peanut cake + mealworm treatment blocks containing EPY compared to those in the peanut cake treatment block (Fig. 5.5).

Supplementary feeding also significantly influenced the proportion of EPY within a brood ($F_{2,72} = 4.74$, $P = 0.01$; Fig. 5.6) with those in both of the supplementary fed treatment blocks containing a higher proportion of EPY than control broods (Fig. 5.6).

Tukey-Kramer *post hoc* tests (testing for main effects of dietary treatment) indicated that there was no significant main effect of dietary treatment on either proportion of broods containing EPY or on the proportion of EPY within broods (see Table 5.3 for full details of statistical models). In both models there was a significant clutch size $\times$ dietary treatment interaction (model 1: $F_{2,72} = 3.93$, $P = 0.02$; model 2: $F_{2,72} = 4.48$, $P = 0.01$; Fig 5.7) indicating that in both food-supplemented blocks, the proportion of EPY within a brood decreased as clutch size increased. In contrast, the proportion of EPY within a brood increased positively with clutch size in the control treatment block.

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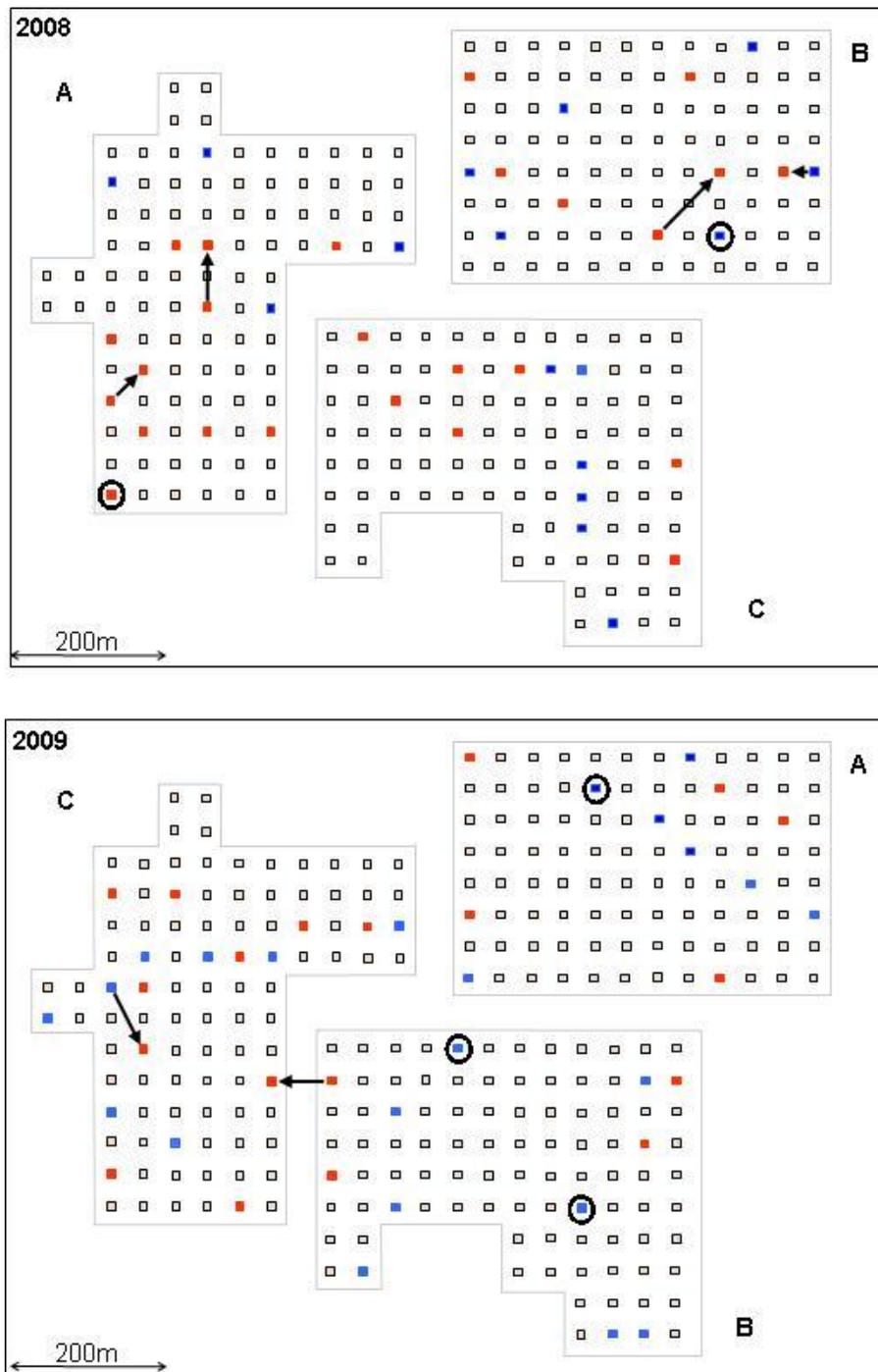
Figure 5.4. Occurrence and location of nestboxes (smallest squares) where extra-pair Blue Tit young were either present (red) or absent (blue) in Chaddesley Woods National Nature Reserve in 2008 and 2009. Each outlined block represents a dietary treatment block (A: peanut cake + mealworms; B: peanut cake; and C: control) with open nestboxes not sampled. Circled nestboxes contained dumped eggs and arrows indicate distances between 'extra-pair nests' and 'social-pair nests' and movements of social mates that fathered EPY.

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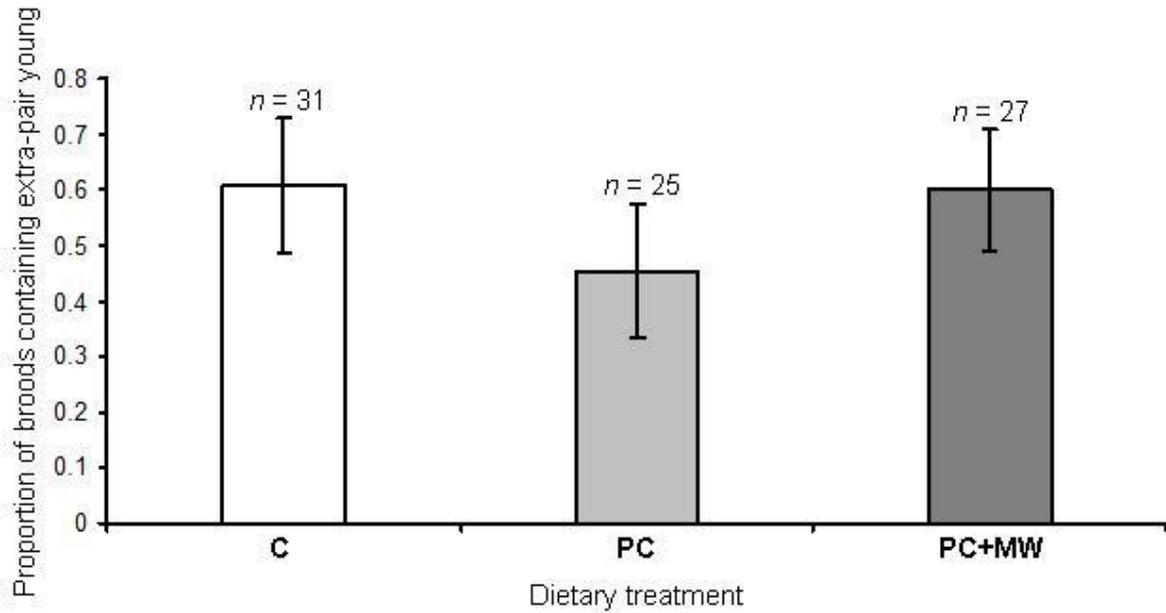
Figure 5.5. Proportion of Blue Tits broods containing EPY (mean ± 1 SE from statistical estimates) in each dietary treatment (C: control; PC: peanut cake; and PC+MW: peanut cake + mealworms) in Chaddesley Woods National Nature Reserve in 2008 and 2009. The number of nests (*n*) is given above each bar.
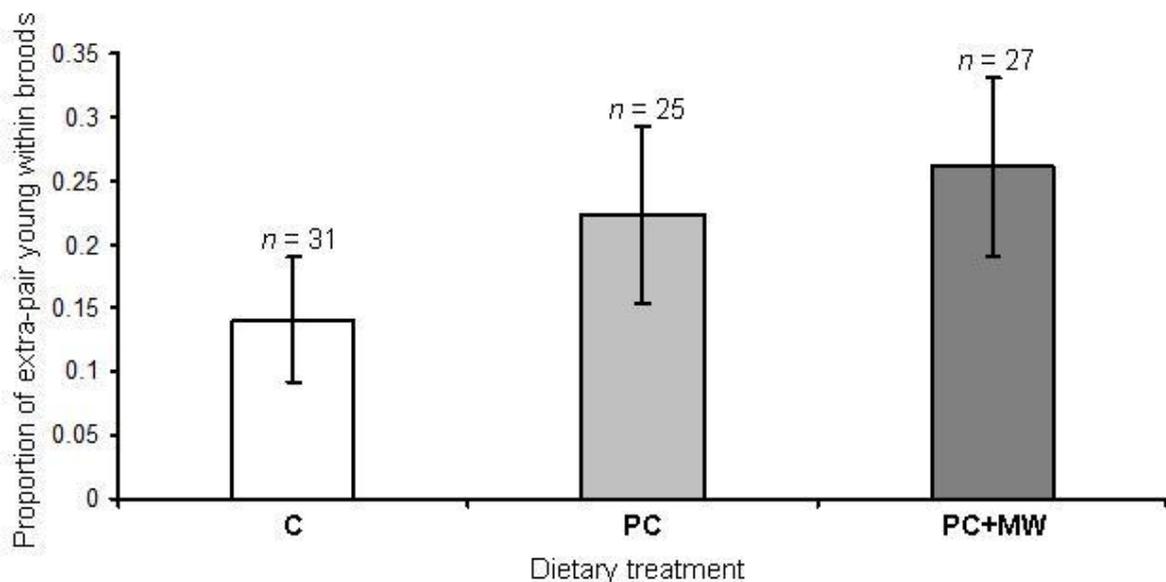
Figure 5.6. Proportion of EPY per brood (mean ± 1 SE from statistical estimates) in each dietary treatment (C: control; PC: peanut cake; and PC+MW: peanut cake + mealworms) in Chaddesley Woods National Nature reserve in 2008 and 2009. The number of nests (*n*) is given above each bar.

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Table 5.3. Results from the final statistical models (see text for details) explaining measurements of EPP in Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. Significant main and interaction effects are given in bold. Dietary treatments: C – control; PC – peanut cake; and PC+MW – peanut cake + mealworms. Directions of significant effects are given '+' and '-' denote significant positive and negative relationships, respectively, and 'NS' denotes nonsignificance ($P > 0.05$).

Measure of paternity	Factor	F	df	P	Least square means (SE) – Tukey-Kramer *post hoc* P values			Direction
					C *vs.* PC	C *vs.* PC+MW	PC *vs.* PC+MW	
Proportion of broods containing EPY	Dietary treatment	**3.91**	**2,72**	**0.02**	0.61(0.12) *vs.* 0.45(0.12) $P = 0.66$	0.61(0.12) *vs.* 0.60(0.11) $P = 1.00$	0.45(0.12) *vs.* 0.60(0.11) $P = 0.65$	C = highest; PC = lowest
	Year	2.48	1,72	0.12				NS
	Clutch size	1.20	1,72	0.28				NS
	Clutch size × dietary treatment	**3.93**	**2,72**	**0.02**				C = + ; PC = - ; PC+MW = -
Proportion of EPY within a brood	Dietary treatment	**4.74**	**2,72**	**0.01**	0.14(0.05) *vs.* 0.22(0.07) $P = 0.58$	0.14(0.05) *vs.* 0.26(0.07) $P = 0.30$	0.22(0.07) *vs.* 0.26(0.07) $P = 0.91$	PC+MW = highest; C = lowest
	Year	0.24	1,72	0.62				NS
	Clutch size	2.71	1,72	0.10				NS
	Clutch size × dietary treatment	**4.48**	**2,72**	**0.01**				C = + ; PC = - ; PC+MW = -
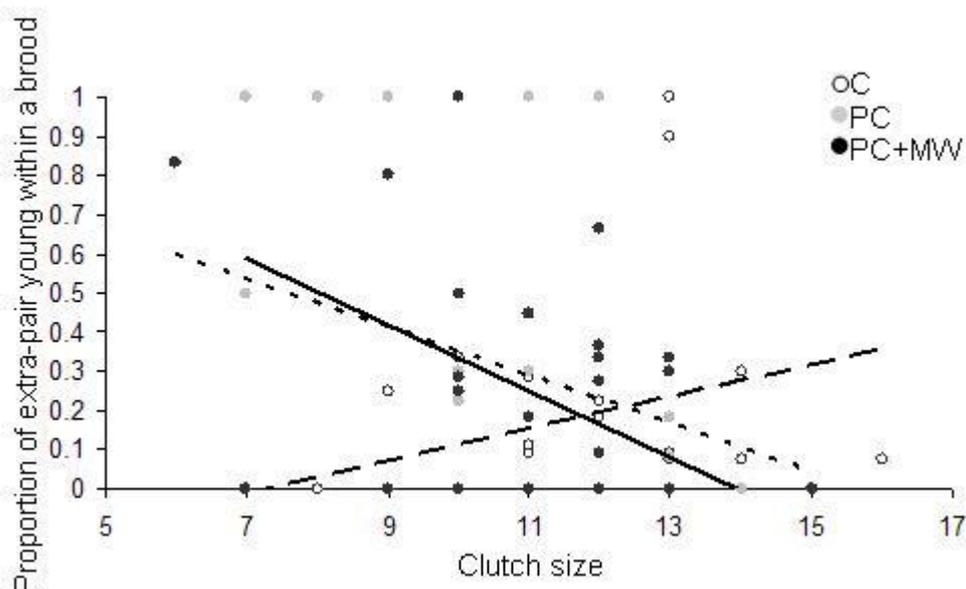
Figure 5.7. Proportion of EPY within a brood in relation to clutch size of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. Regression lines are for the peanut cake – PC) (solid: Spearman's $r = 0.32$, $d.f. = 21$, $P > 0.05$), peanut cake + mealworms – PC+MW (dotted: Spearman's $r = -0.32$, $d.f. = 26$, $P > 0.05$); and control – C (unfed) (dashed: Spearman's $r = 0.44$, $d.f. = 29$, $P < 0.05$).

5.5. DISCUSSION

Supplementary feeding should reduce a bird's time and effort spent foraging and, consequently, it might allow social-pair males to increase time spent mate-guarding. Furthermore, feeders containing supplementary food may act as 'food hotspots' allowing females to reduce the amount of time spent roaming whilst foraging for food making them easier to mate-guard. Therefore, I predicted that the supplementation of food intended for adult consumption (peanut cake) from approx. 4 weeks prior to the egg laying period and throughout the nestling period would result in both a lower proportion of broods containing EPY and a lower proportion of young within a brood being EPY. Additional supplementary feeding during the nestling period should increase nestling survival and reduce differential mortality between WPY and EPY.

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Therefore, I predicted that additional supplementary feeding during the nestling period intended for chicks (mealworms) would result in a lower proportion of young within a brood being EPY compared to broods of birds only receiving supplementary food intended for adult consumption. The results, however, do not support my predictions. Blue Tits supplemented with peanut cake were less likely to have a brood containing EPY than birds in either the peanut cake + mealworms or in the control treatment blocks. Furthermore, Blue Tits supplemented with either peanut cake or peanut cake + mealworms had a higher proportion of EPY in their broods than control birds, and the proportion of EPY within broods in the peanut cake + mealworms treatment block exceeded that of broods in the peanut cake treatment.

5.5.1. Effects of supplementary feeding on the incidence of EPP: female behaviour

The similarity between proportions of broods containing EPY in control and peanut cake + mealworms treatment blocks suggests that the number of females partaking in EPCs is consistent in at least two-thirds of my study population regardless of food availability at least in the 2 years of study. In contrast, supplementary feeding affected the proportion of EPY in broods with those in the supplementary fed treatment blocks containing higher proportions of EPY compared with broods in the control block (Fig. 5.6). This is consistent with the findings of Hoi-Leitner et al. (1999) who demonstrated that European Serins provided with additional food plants had a higher likelihood of having EPY. They also found that provisioning rates of cuckolded males decreased as EPP increased, indicating that females on territories with high food abundance and incidence of EPY were not constrained by a reliance on male assistance. Previous studies have found a cost of EPP for females in the form of withdrawal of paternal investment (e.g. Dixon et al. 1994, Weatherhead et al. 1994).

The 'constrained female hypothesis' of Gowaty (1996) states that females in poor environments (i.e. those of low food availability) are more vulnerable to help from a cuckolded male being withdrawn. It also predicts that females in good environments (i.e. those of high food availability) are able to make up for any withdrawal in male assistance and, therefore, are likely to have higher rates of EPP (Gowaty 1996). However, since it is unclear whether supplementary feeding has an effect on male brood provisioning rates in my study population (see Section 4.4) it is unclear whether my results lend support to the 'constrained female hypothesis'.

I found that the number of fathers siring offspring per nest was variable throughout the woodland with single extra-pair fathers accounting for EPY in 30 of 44 (68.2%) broods and multiple extra-pair fathers accounting for EPY in 14 of 44 (31.8%) broods. Moreover, the proportion of broods containing EPY sired by two or more extra-pair fathers was inconsistent between treatments with the highest in the peanut cake + mealworms dietary treatment block (8 of 16 or 50%) and the lowest in the control treatment block (3 of 17 or 17.6%) (Fig. 5.3). In Blue Tits EPP is thought to be largely under the control of females (Kempenaers et al. 1995) who actively seek out EPCs by visiting territories of neighbouring males (Kempenaers et al. 1992). The 'genetic diversity hypothesis' proposes that EPP maximises genetic diversity within a brood (Williams 1975, Westneat et al. 1990), increasing the likelihood that at least some offspring will survive under unpredictable environmental conditions. This assumes that certain genotypes are more favourable than others under certain environmental conditions. Thus, in accordance with the 'genetic diversity hypothesis', females should seek EPCs with multiple males to maximise genetic diversity within the brood, and also to obtain associated fitness benefits. In support of this hypothesis my results suggest that a reduction in the energetic and temporal constraints of

foraging through food supplementation appeared to allow females to partake in EPCs with multiple males, possibly through undertaking more off-territory forays, thereby increasing both the proportion of EPY and the genetic diversity of offspring within broods. Alternatively, feeders may have acted as 'food hotspots' attracting multiple males allowing females to obtain multiple EPCs when they visit the feeder to feed.

5.5.2. Effects of supplementary feeding on the incidence of EPP: male behaviour

Many bird species actively defend feeding territories (e.g. Gill and Wolf 1975, Ford 1981). However, territorial behaviour requires significant energetic and temporal investments and, therefore, it is likely to vary in relation to food availability and time needed to forage (e.g. Carpenter and MacMillen 1976, Carpenter 1978). Ydenberg (1984) demonstrated that food supplementation resulted in Great Tits spending more time mounting a territorial response to an intruder (a stuffed Great Tit mounted on a pole). Although I do not have data reflecting investment in mate guarding, supplemented male birds in my study might have similarly invested more resources in territorial behaviour and, consequently, less in paternity guarding. A reduction in mate-guarding can lead to an increase in EPCs (e.g. Møller 1987, Westneat 1994) and this might explain why supplemented birds had higher proportions of EPY within broods than controls. Alternatively, supplementary fed males may have allocated more time and energy towards defending a feeder and, by doing so, gained more EPCs with females visiting the feeder to feed.

When females copulate with multiple males in succession, sperm will compete to fertilise ova, a phenomenon referred to as sperm competition (Parker 1970). The timing of copulations is an important mechanism underlying the outcome of sperm competition across avian taxa (Birkhead 1998), with the last male to copulate often

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achieving paternity, an effect referred to as 'last male sperm precedence' (Birkhead and Møller 1992). A social mate, therefore, may intensely mate-guard and/or perform frequent within-pair copulations to maximise the likelihood of his sperm fertilising ova, a strategy to guard against loss of paternity (e.g. Birkhead 1988, Møller and Birkhead 1991). However, mate-guarding behaviour may be relaxed when ecological constraints are imposed such as intense intra-specific competition for nest sites (Møller and Birkhead 1991) or when low food availability results in a male having to spend more time foraging (Rubenstein 2007). Under such constraints, when the risk of being cuckolded is high, the frequency of within-pair copulations increases ('sperm competition hypothesis' – Birkhead 1988) as mate-guarding becomes ineffective (reviewed in Petrie and Kempenaers 1998). In my study, control males may have been more constrained by food availability than those in supplemented treatment blocks. Therefore, while the latter may have mate-guarded more intensely than control males, lower mate-guarding may have been compensated for by higher rates of within-pair copulations by control males. Despite this, mate-guarding behaviour in Blue Tits has not always been demonstrated to be an effective guard of paternity (Kempenaers et al. 1995). Therefore, while the strategies outlined above may apply to Blue Tits in my study, in terms of male mate-guarding behaviour lowering the incidence of EPCs, and thus the proportion of EPY within broods in the control treatment block, much further research is needed to understand fully the role of mate-guarding, and within-pair copulation behaviours of birds in determining the genetic outcomes of breeding attempts.

5.5.3. Effects of additional supplementary feeding during the nestling period on the incidence of EPP

There was a slightly higher proportion of EPY within broods in the peanut cake + mealworm treatment block compared with the peanut cake treatment when nestlings were sampled on approx. nestling day 12 (Fig. 5.6). I assume that the proportions of EPY at hatching in the two supplementary fed blocks were equivalent because the dietary treatments were equivalent during the fertile period of females and, therefore, it appears that the additional mealworm supplement had a slight effect on the proportion of EPY within broods post-hatching. Previous studies have shown differential mortality in the nest may exist between WPY and EPY in Blue Tits with the latter having a higher chance of surviving (e.g. Kempenaers et al. 1997, Magrath et al. 2009). I found slightly higher proportions of EPY within broods in the peanut cake + mealworms treatment block compared with just the peanut cake treatment block suggesting perhaps that nestlings in the former may have experienced higher mortality rates, with survivors being biased towards EPY. However, I do not have data reflecting differential mortality between EPY and WPY and, therefore, whether the additional supplementary feeding of mealworms during the nestling period had an effect on the incidence of EPY remains unclear.

5.5.4. Effect of clutch size on EPP in Blue Tits between dietary treatments

The significant interaction between dietary treatment and clutch size in both models indicated that clutch size had a different effect on the rate of EPP between the dietary treatments. In control broods the proportion of EPY significantly increased with clutch size. In contrast, the proportion of EPY within a brood in both supplemented treatment blocks decreased as clutch size increased (although not significantly) (Fig. 5.7.). Since control females were more likely to be both energetically and temporally constrained by foraging, they may have allocated fewer resources to seeking out EPCs

than supplemented females. This may have restricted control females to opportunistic EPCs with extra-pair males that encroached on their territories. Under this scenario where females are not actively choosing extra-pair males and EPCs are the result of unplanned encounters, the chance of EPP is random and, therefore, the probability of producing EPY increases with clutch size (Brommer et al. 2007). Thus, this appears to explain why larger control clutches contain more EPY. In contrast, supplemented females were probably less constrained by available resources and, therefore, could undertake more off-territory forays to seek out EPCs resulting in more EPY within their broods. As female Blue Tits preferentially mate with extra-pair males that are in better body condition than their social mates (e.g. Kempenaers et al. 1992, Kempenaers et al. 1997 – but see Leech et al. 2001), EPY are often in better body condition than WPY and have a higher likelihood of survival. Since the energetic costs of reproduction increase with brood size, supplemented birds may adopt a strategy to maximise fitness benefits by producing smaller clutches containing higher quality offspring when broods contain EPY.

5.5.5. Future directions: other mechanisms driving EPP

Paternity in birds has been shown to vary with song (e.g. Hasselquist et al. 1996, Byers 2007) which can act as an honest signal of male quality because it can be energetically and temporally costly to produce (reviewed in Gil and Gahr 2002). For example, male Dusky Warblers (*Phylloscopus fuscatus*) that maintained a high song amplitude during singing (a measure of song quality in this species) were more likely to father EPY (Forstmeier et al. 2002). As singing performance was related to both EPP and survival, they concluded that females chose to mate with males with high song quality in order to obtain indirect genetic benefits. When food is abundant, male

birds can allocate more resources to song production through a reduction in the time and energy needed to forage (e.g. Berg et al. 2005, Barnett and Briskie 2007). It is possible that supplemented males in my study, therefore, may have been able to allocate more towards singing than control males resulting in an increase in EPCs and in the proportion of EPYs within broods. However, this can only be investigated through further study of male song characteristics.

5.5.6. Conclusions and future considerations

I have provided evidence that food supplementation (peanut cake) of adult Blue Tits during the fertile period, and potential food supplementation of nestlings (mealworms), has marked effects on both the likelihood of a brood containing EPY and on the proportion of EPY within a brood. However, I investigated the effects of food supplementation on EPP between days 10 and 14 of the nestling period, but did not take into account either dead nestlings (i.e. brood reduction) or unhatched eggs. Although my approach allows me to investigate the post-hatching effects of food supplementation on EPP, tissue sampling unhatched eggs and dead nestlings would have further elucidated the effects of food supplementation on mating behaviour during the female's fertile period.

While I have demonstrated an effect of supplementary feeding on EPP, the effects of supplementary feeding on the behavioural mechanisms which drive EPP in this study population remain unknown. Therefore, further studies are necessary to determine the effects of supplementary feeding on behaviours such as female foraying, mate-guarding, within-pair copulation and singing performance. Since food supplementation in my study was protracted and used commercially available food supplements, it mimicked feeding patterns of garden bird feeding by the public (Toms

and Sterry 2008). Therefore, my findings may have implications for a wide range of species of birds that feed in towns and cities (Jones and Reynolds 2008). My results create new concerns about the extensive feeding of birds, especially with respect to its potential influence on the genetic structure of avian populations.

5.5.7. The next chapter

Since EPY often have greater fitness benefits than WPY (e.g. Kempenaers et al. 1992, Charmantier et al. 2004), sex allocation theory predicts that it is adaptive for females to bias the sex of EPY towards the sex which provides the greatest fitness benefits (e.g. Sheldon and Ellegren 1996, Johnson et al. 2009). With my findings showing a significant effect of supplementary feeding on EPP, I will now investigate the effect of supplementary feeding on brood sex ratio in Chapter Six.

Chapter Six

THE EFFECTS OF SUPPLEMENTARY FEEDING ON THE SECONDARY SEX RATIO OF BLUE TITS

6.1. ABSTRACT

According to sex allocation theory when food is abundant a female should bias brood sex ratio towards the sex which has the greatest reproductive variance in order to maximise fitness. In Blue Tits reproductive variance is greater for males than for females and high quality males have higher reproductive success than high quality females due to increased opportunities for extra-pair paternity. In 2008 and 2009 I tested the prediction that supplementary feeding results in male-biased secondary brood sex ratio in Blue Tits by providing supplementary food to breeding birds prior to and during egg-laying. Since food availability is also likely to affect brood sex ratio post-hatching in this species through differential mortality between the sexes, I also investigated the effects of additional supplementary feeding on secondary brood sex ratio by providing a supplement to be consumed by nestlings. In 2008 the effects of supplementary feeding on secondary brood sex ratio could not be elucidated due to poor DNA amplification during PCR. However, in 2009, supplementary feeding had a significant effect on secondary brood sex ratio of Blue Tits with those receiving supplementary food intended for both adult and nestling consumption having broods which were more male-biased than both control birds and those receiving supplementary food only prior to and during egg-laying. Since I measured secondary sex ratio, I suggest that a combination of both pre- and post-hatching manipulation of sex ratio account for the findings of this study.

6.2. INTRODUCTION

Female birds should alter their behaviour and life-history strategies relative to the prevailing environmental conditions to maximise fitness (e.g. McGinley et al. 1987, Morris 1987). For example, maximum fitness returns may be achieved through adjustment in parental investment during the nestling period (see Chapter Four and references therein) and in mating behaviour (see Chapter Five and references therein). Furthermore, according to sex allocation theory if parents can predict the relative reproductive value of producing males and females, and are able to control the sex of their offspring, they should adjust the sex ratio accordingly to maximise fitness (Trivers and Willard 1973).

Although there is controversy over the mechanism involved (reviewed in Krakow 1995, Komdeur and Pen 2002, Alonso-Alvarez 2006), the numbers of studies suggesting that female birds are able to manipulate and control brood sex ratio adaptively during the pre-laying period, resulting in biased primary brood sex ratios (i.e. sex ratios at laying – Burley 1986), are increasing (e.g. Kilner 1998, Nager et al. 1999). In birds the female determines the sex of offspring since she is the heterogametic sex and is able to adjust primary brood sex ratio (e.g. Svensson and Nilsson 1996, Komdeur et al. 2002). Primary brood sex ratio may result from non-random segregation during meiosis (e.g. Svensson and Nilsson 1996, Komdeur et al. 2002), from sex-specific reabsorption of the ova in the oviduct (Emlen 1997), from differential survival between male and female zygotes and/or embryos (Cichoń et al. 2005), or from the influence of circulating hormones such as corticosterone (e.g. Pike and Petrie 2005, 2006). Further adjustment to sex ratio at post-laying stages (so-called secondary manipulation resulting in secondary sex ratio – Burley 1986) result from interactions between primary sex ratio, parental behaviour and environmental factors

(e.g. Cooch et al. 1997, Korpimäki et al. 2000). One such example was documented by Clotfelter (1996) who found that secondary sex ratio in broods of Tree Swallows differed from parity as a consequence of sex-biased laying sequences followed by brood reduction during the nestling phase.

In support of sex allocation theory (Trivers and Willard 1973) there is increasing evidence to suggest that birds are able to adjust brood sex ratio to maximise fitness when the relative fitness benefits of males and females differ (e.g. Komdeur 1996, Kilner 1998). This occurs in relation to a number of factors including sexually-selected traits (e.g. Sheldon et al. 1999, Korsten et al. 2006), paternal survival prospects (Svensson and Nilsson 1996) and local resource competition (Gowaty 1993). Furthermore, resource availability (e.g. food) during the nestling period can differentially affect the potential fitness benefits of offspring and numerous studies have provided evidence that females bias primary brood sex ratio in relation to local food abundance to maximise fitness benefits (e.g. Clout et al. 2002, Rutstein et al. 2004). The fitness consequences of biasing primary brood sex ratio in relation to food are likely to vary between species. Factors affecting adaptive sex allocation include the sex-related likelihood to help in co-operatively breeding species (e.g. Seychelles Warbler [*Acrocephalus sechellensis*] – Komdeur 1996), differential nestling survival between the sexes (e.g. Nager et al. 1999, Kalmbach et al. 2001) and fecundity (e.g. Kilner 1998, Rutstein et al. 2004). Komdeur (1996) demonstrated that helpers in the co-operatively breeding Seychelles Warbler are usually female and only increase their parents' fitness when on territories with high food availability. Pairs breeding on high-quality territories (as measured by food abundance) produced an excess of female offspring. Conversely, on poor quality territories, offspring were

male-biased as producing female helpers under such conditions reduces reproductive success (Komdeur 1996).

The effect of food availability on maternal condition may also influence primary brood sex ratio when maternal condition influences sex-specific nestling survival (Nager et al. 1999, Kalmbach et al. 2001). Nager et al. (1999) demonstrated that primary brood sex ratio varies with maternal condition in the Lesser Black-backed Gull (*Larus fuscus*) acting through egg quality (Bolton et al. 1992) by directly influencing chick survival (Bolton 1991). Males are more susceptible to starvation than females (Griffiths 1992) since they are the larger sex (Cramp 1985). In accordance with sex allocation theory (Trivers and Willard 1973), Nager et al. (1999) manipulated female condition at the time of egg laying by continuous egg removal and supplementary feeding, and demonstrated that females in poor condition produced lower quality eggs, thereby skewing sex ratio towards females.

When food availability is limited it is adaptive for a female to bias primary brood sex ratio when nutritional conditions during the nestling period differentially affect the potential reproductive success of male and female offspring. For example, a number of studies have found that for Zebra Finches (*Taeniopygia guttata*) in which the reproductive success of males and females is differentially affected by nutritional conditions during the nestling phase (Kilner 1998), brood sex ratio is sensitive to food availability (e.g. Kilner 1998, Rutstein et al. 2004). Female fecundity is strongly influenced by food resources received during the nestling period (Haywood and Perrins 1992) and, therefore, female nestlings are expected to be more vulnerable to nutritional stress than their male siblings (Kilner 1998). As a result, when food is restricted, Zebra Finches produce male-biased broods (Kilner 1998, Rutstein et al. 2004 – but see Bradbury and Blakey 1998, Arnold et al. 2003).

Food availability may further influence brood sex ratio through differential mortality resulting in a biased secondary brood sex ratio. For example, in sexually dimorphic species in which the larger sex is more susceptible to starvation, food shortages during the nestling period may lead to biased mortality of the larger sex resulting in a biased secondary sex ratio (e.g. Great-tailed Grackle [*Quicalus mexicanus*] – Teather and Weatherhead 1989; Blue-footed Booby [*Sula nebouxii*] – Torres and Drummond 1997). In contrast, Hipkiss et al. (2002) showed that nestling mortality of Tengmalm's Owls (*Aegolius funereus*) was biased towards the smaller sex (males – Cramp 1985) suggesting that the larger females had a competitive advantage over their smaller siblings and were able to monopolise food items. The authors suggested that the counteractive effect of food on Tengmalm's Owls compared to other species (e.g. Great-tailed Grackle – Teather and Weatherhead 1989; Blue-footed Booby – Torres and Drummond 1997) could be explained by a sex-biased hatching sequence in which the males hatch last. Youngest brood members are often more susceptible to mortality than their older and larger siblings that are able to out-compete them (e.g. Nuechterlein 1981, Malacarne et al. 1994).

Although there is extensive literature relating food availability to primary brood sex ratio, experimental studies have failed to investigate how supplementary feeding might influence secondary brood sex ratio. This may be fundamental to our understanding of how food availability in urban habitats influences avian breeding biology, especially since feeding wild birds is a widespread activity in towns and cities of the UK (Jones and Reynolds 2008) but we understand relatively little about its influence on the genetic structure of avian populations. In this study, therefore, I investigated the effects of two commercially available garden bird food supplements

on the secondary brood sex ratios of Blue Tits fed between pre-laying to post-hatching inclusive.

Blue Tits are small socially monogamous passerines that readily utilise bird feeders, nest in nestboxes and lay large clutches (7-13 eggs – Gosler and Clement 2007). Reproductive variance in Blue Tits is greater for males than for females and high quality males have higher reproductive success than high quality females due to EPP (Dreiss et al. 2006). Furthermore, Blue Tits exhibit slight size dimorphism with males being approx. 6% larger than females (Råberg et al. 2005). Therefore, males are likely to be more susceptible to starvation and mortality than their smaller female conspecifics, making it a good study species to investigate secondary brood sex ratio in relation to food availability.

I hypothesised that the supplementation of peanut cake intended for adult consumption during the fertile period would increase female condition enabling supplemented females to produce higher quality nestlings. Since higher quality male Blue Tits have higher reproductive success than high quality females (Dreiss et al. 2006), I predicted that broods in blocks supplemented with peanut cake would be more male-biased than in the control (nonsupplemented) block. In addition, I hypothesised that the additional supplementation of mealworms intended for nestling consumption would reduce male-biased mortality. Therefore, I predicted that broods in the treatment block supplemented with both peanut cake and mealworms would be more male-biased than broods in both the peanut cake-supplemented and control blocks.

6.3. METHODS

6.3.1. Study site and supplementation

Please see Section 4.3.1 for full details.

6.3.2. Field methods

Please see Section 5.2.1 for full details.

6.3.3. Molecular methods

In birds the female is heterogametic with two different sex chromosomes (ZW) while the male is homogametic with two sex chromosomes that are homologous (ZZ) (see Ellegren 2000 for review of avian sex determination). Located on each avian sex chromosome is the CHD (Chromo-helicase-DNA-binding) gene that is present in two forms: the CHD-W gene located on the W chromosome and, therefore, occurring only in females (ZW); and the CHD-Z gene located on the Z chromosome and occurring in both sexes (Griffiths and Tiwari 1995). Two primers, P2 and P8, have been developed (Griffiths et al. 1998) that can be used to amplify homologous fragments of the CHD genes by PCR. The homologous fragments of the CHD-W and CHD-Z genes that are amplified during PCR differ in size as a result of different intron lengths between the fragments. Therefore, the PCR products differ in size (Griffiths et al. 1998) and can be separated and visualised through gel electrophoresis (Alberts et al. 1983) with one band being visible for a male (ZZ) and two for a female (ZW) (Griffith et al. 1998).

The sex of all nestlings and adults from 2008 and 2009 was determined by PCR amplification of the CHD genes using the P2 and P8 primers (Griffiths et al. 1998) using DNA extracted for paternity analysis (as described in Section 5.3.3). In addition, two sex-linked loci linked to the Z chromosome (Z037B and Z040 –

Dawson 2007) were amplified using PCR to increase the reliability of allocating sex to individuals. Please see Appendix One for full details of sex-linked loci.

Each 2-µl PCR contained approx. 15 ng of genomic DNA, 0.5 µl of each primer (0.2 µM) and 1.0 µl of QIAGEN Multiplex PCR Master Mix (Kenta et al. 2008). PCR amplification was performed using a DNA Engine Tetrad PTC-225 Peltier thermal cycler (MJ Research, Bio-Rad, Hemel Hempsted, UK). A PCR was used with the following temperature and duration profile: 95ºC for 15 min, 34 cycles of 94ºC for 30 sec, 57ºC for 90 sec, 72ºC for 1 min, and 72ºC for 1 min. PCR products were separated on an ABI 3730 DNA Analyser (Applied Biosystems, California, USA) and genotypes were scored using GENEMAPPER software v3.7 (Applied Biosystems, California, USA).

Observed and expected heterozygosities (i.e. measurements of genetic diversity – Weir 1990), estimated null allele frequencies (Dakin and Avise 2004) and non-exclusion probabilities (i.e. probability of not being able to exclude a male from paternity – Jones and Ardren 2003) for both of the Z-linked loci were calculated using CERVUS v3.0 (Marshall et al. 1998) for each year independently (see Section 5.3.3) using genotypes from all unrelated males. Female genotypes were excluded from analysis since females are always homozygous for Z-linked loci and, therefore, including them would result in incorrect estimations of heterozygosity. Tests for departures from Hardy-Weinberg and linkage equilibria were then conducted using a Markov-chain method implemented in GENEPOP v.4.0 (Rousset 2008). Tests revealed that both loci did not deviate significantly from the Hardy-Weinberg equilibrium in either year (Table 6.1) and that they did not deviate from linkage equilibrium following a Bonferroni correction for multiple comparisons (Rice

Table 6.1. Details of polymorphic microsatellite loci used in the brood sex ratio analysis of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. Note: N is the number of birds tested; H_O is the observed heterozygosity; H_E is the expected heterozygosity; HWE is the Hardy-Weinberg Equilibrium from which the P value is generated; NE-1P is the non-exclusion probability for the first parent; F(Null) is the expected null allele frequency as a proportion; and Reference is the place of publication.

Locus	Year	N	Number of alleles	H_O	H_E	HWE P-value	NE-1P	F(Null)	Reference
Z040	2008	29	6	0.621	0.648	0.316	0.764	-0.010	Dawson (2007)
	2009	41	5	0.659	0.742	0.610	0.687	+0.055	
Z037B	2008	30	3	0.367	0.362	1.000	0.937	-0.030	Dawson (2007)
	2009	42	3	0.262	0.301	0.417	0.956	+0.060	

1989). Furthermore, neither locus had high estimated null allele frequencies (i.e. 1-6%; Table 6.1) for either year.

6.3.4. Sexing analysis

The amplified PCR products from the CHD-W and CHD-Z genes were found to be different in size by a magnitude of 65 base pairs, the larger 384 base pair product being from the female CHD-W gene. Therefore, females were separated from males during genotype scoring by the presence of two clearly separated peaks compared to a single peak in the genotype profiles of males (Fig. 6.1)
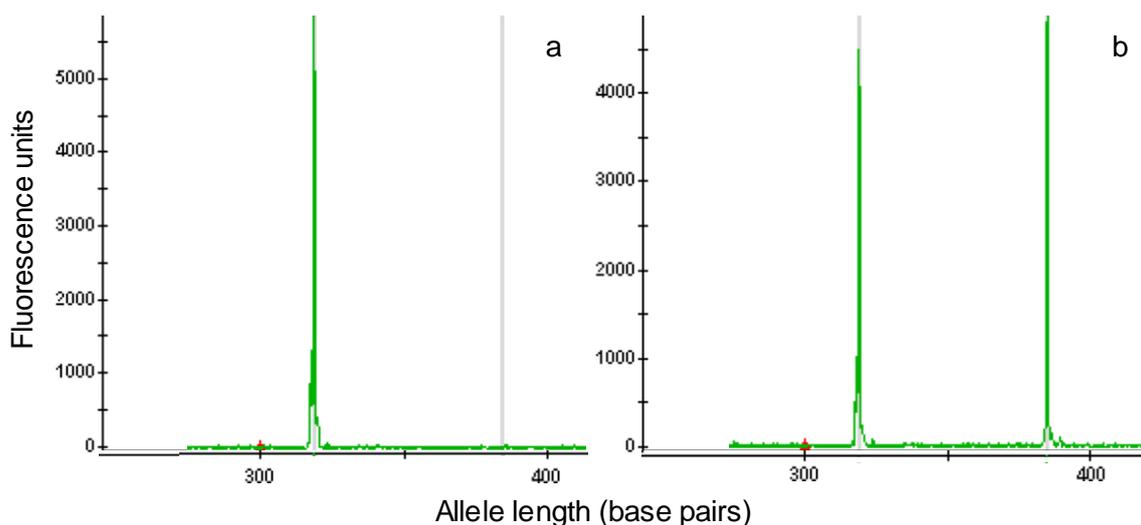
Figure 6.1. Two different genotypes for the CHD locus from Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009 with the smaller allele from the CHD-Z gene and the larger one from the CHD-W gene. (**a**) One peak is present in the electropherogram for a male (ZZ) and (**b**) two peaks for a female (ZW).

6.3.5. Statistical analyses

Statistical analyses were performed using data from all nestlings apart from those hatching from dumped eggs ($n = 11$). Mixed models were constructed using PROC

GLIMMIX in SAS version 9.2 (SAS Institute Inc. 2008) and fitted with binomial error distributions to test for the effects of supplementary feeding on brood sex ratio (expressed as the number of males per brood).

To account for seasonal variation in brood sex ratio (e.g. Cordero et al. 2001, Krebs et al. 2002), and for the potential effects of both maternal (e.g. Bradbury and Blakey 1998, Whittingham and Dunn 2000) and paternal (e.g. Kölliker et al. 1999, Stauss et al. 2005b) condition on sex allocation, hatch date, and male and female body condition (residuals from the regression of body mass on tarsus length) were included as covariates. In addition, since larger clutches have been shown to be more female-biased than smaller clutches (Lessells et al. 1996, Griffith et al. 2003), clutch size was also included as a covariate. Finally, since EPY often have greater fitness benefits than WPY (e.g. Kempenaers et al. 1992, Charmantier et al. 2004), the proportion of EPY within the nest was included as a covariate. To determine if the effects of covariates were consistent between treatment blocks, covariate $\times$ dietary treatment interactions were tested independently of one another. Where multiple covariate $\times$ dietary treatment interactions were significant, they were both included in the model as long as each remained significant (Grafen and Hails 2002). I performed backward model selections removing the least non-significant covariate interactions ($P > 0.05$) one-by-one from models ensuring that there was no significant change in deviance between models where applicable (Grafen and Hails 2002). Tukey-Kramer *post hoc* tests were conducted for pairwise comparisons of the least-squares means with results presented as adjusted *P* values.

6.4. RESULTS

6.4.1. Nestling sex ratio

In 2009 the sexes of 366 out of 369 chicks from 41 broods were determined with 179 (or 49%) sexed as male and 187 (or 51%) sexed as female. The sex of the remaining three chicks was not determined since both Z-linked loci and the CHD genes did not amplify during PCR. In addition, the sexes of deceased chicks and unhatched eggs were not determined as a result of both often being removed from the nest prior to collection, possibly by the parental bird (see Kirkpatrick et al. 2009).

6.4.2. Effects of supplementary feeding on brood sex ratio

Supplementary feeding had a significant effect on brood sex ratio of Blue Tits (expressed as the proportion of males in each brood) ($F_{2,34} = 4.23$, $P = 0.02$) with a higher proportion of males within broods in the peanut cake + mealworm treatment block compared to those in the peanut cake treatment and control blocks (Fig. 6.2,.
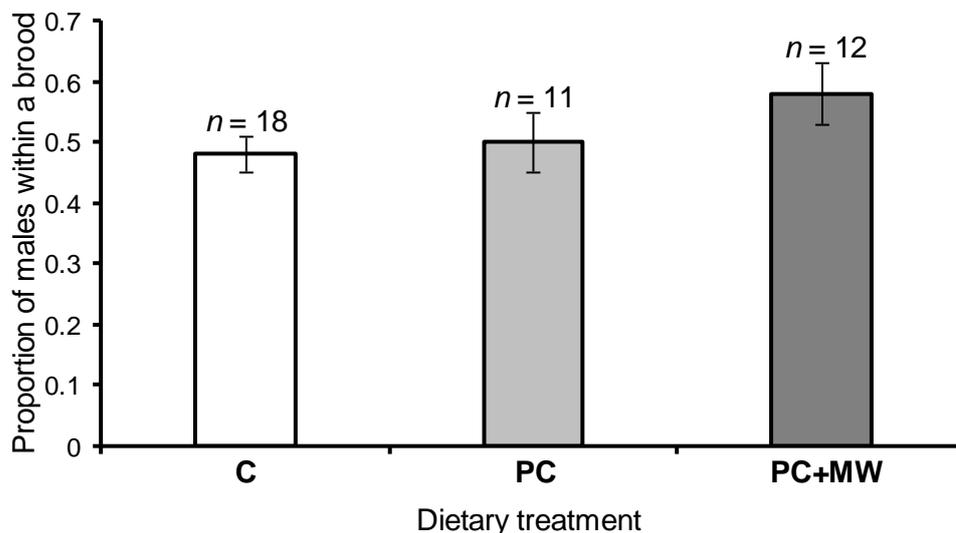
Figure 6.2. Proportion of males per brood (mean ± 1 SE from statistical estimates) in relation to food supplementation (C: control; PC: peanut cake; and PC+MW: peanut cake + mealworms) of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2009. The number of broods (*n*) is given above each bar.

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Table 6.2). However, there was no significant effect of supplementary feeding on either female or male body condition (ANOVA: Female – $F_{2,38} = 0.00$, $P = 1.00$; Male – $F_{2,38} = 0.00$, $P = 0.99$; Fig. 6.3). Moreover, there was no significant effect of either
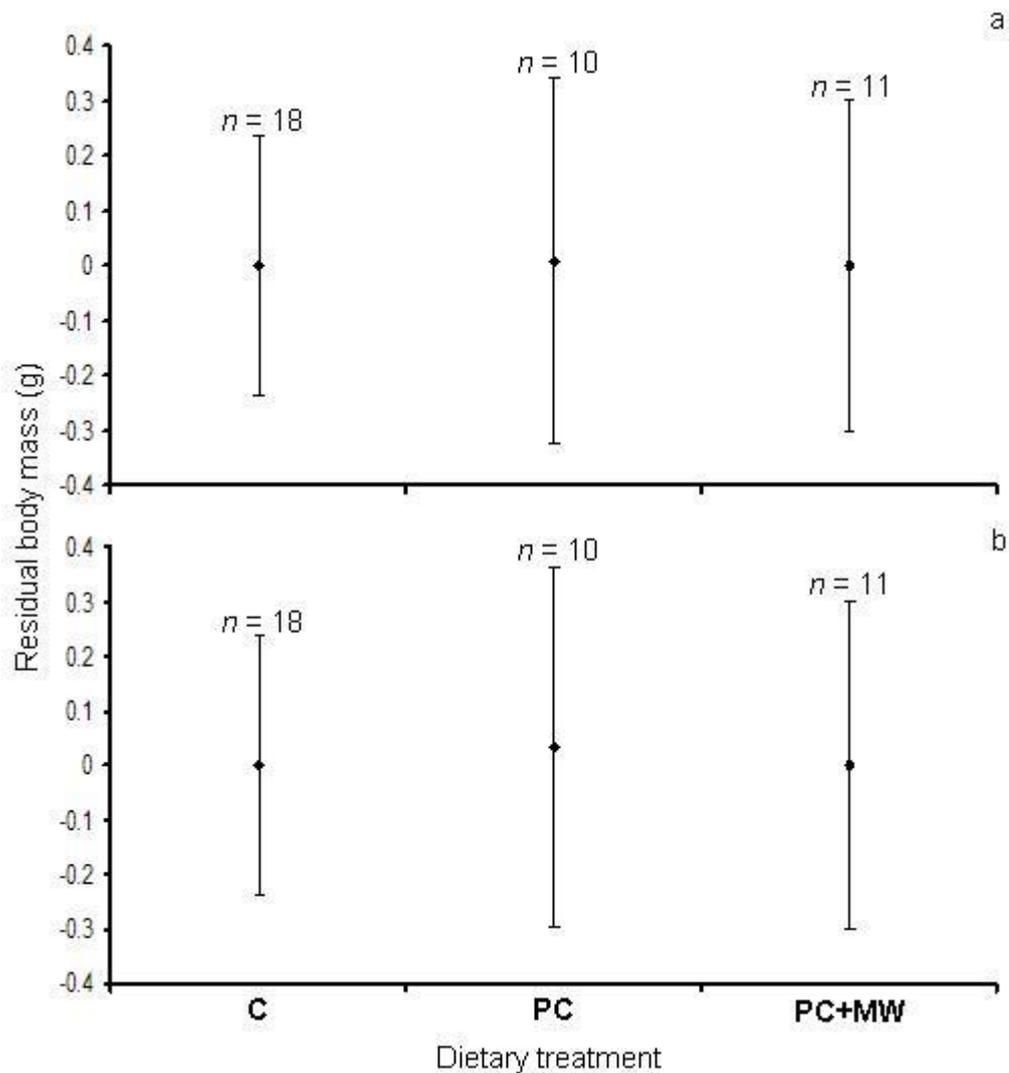
Figure 6.3. Adult body condition (residuals from the regression of body mass on tarsus length) of (**a**) female and (**b**) male Blue Tits (mean ± 1 SE from statistical estimates) in relation to food supplementation (C: control; PC: peanut cake; and PC+MW: peanut cake + mealworms) breeding in Chaddesley Woods National Nature Reserve in 2009. The number of broods (*n*) is given above each line.

Table 6.2. Results from the final statistical model (see text for details) explaining brood sex ratio of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2009. Significant main effects are given in bold. Dietary treatments: C – control; PC – peanut cake; and PC+MW – peanut cake + mealworms. Directions of significant effects are given: '+' and '-' denote significant positive and negative relationships, respectively, and 'NS' denotes nonsignificance ($P > 0.05$).

| Factor | F | df | P | Least squares means (SE) – Tukey-Kramer *post hoc P* values | | | Direction |
				C *vs.* PC	C *vs.* PC+MW	PC *vs.* PC+MW	
Dietary treatment	**4.23**	**2,30**	**0.02**	0.48(0.03) vs. 0.50(0.05) *P* = 0.93	0.48(0.03) vs. 0.58(0.05) *P* = 0.27	0.50(0.05) vs. 0.58(0.05) *P* = 0.57	PC+MW = highest; C = lowest
Hatch date	0.00	1,30	0.98				NS
Female body condition	0.44	1,30	0.51				NS
Male body condition	3.31	1,30	0.08				NS
Hatch date × dietary treatment	**4.35**	**2,30**	**0.02**				C = - ; PC = - ; PC+MW = +

female or male body condition on brood sex ratio both within and between dietary

treatment blocks ($P > 0.05$ for main effects of male and female condition and dietary

treatment $\times$ condition interactions; Fig. 6.4).
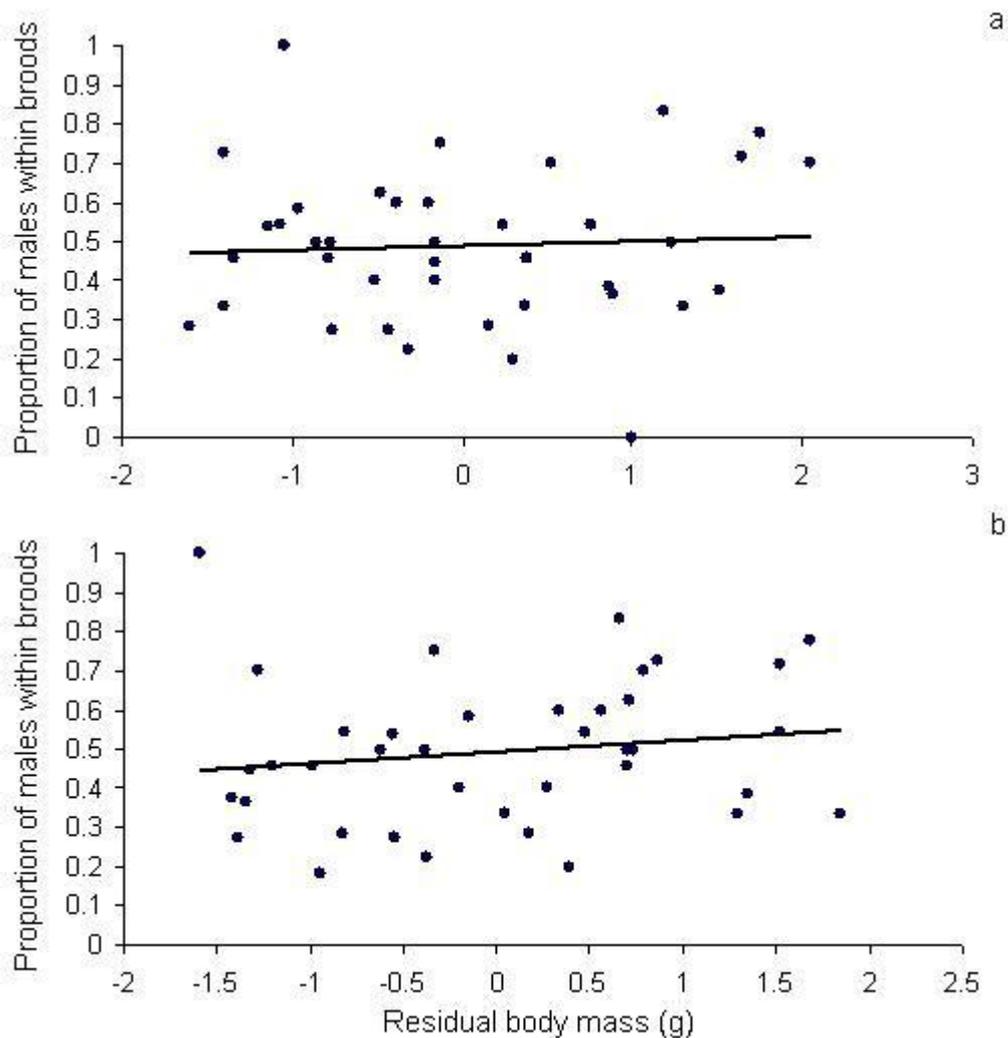
Figure 6.4. Proportion of males within a brood in relation to adult body condition
(residuals from the regression of body mass on tarsus length) in (**a**) female
(Spearman's $r = 0.05$, $d.f. = 39$, $P > 0.05$) and (**b**) male (Spearman's $r = 0.26$, $d.f. =$
39, $P > 0.05$) Blue Tits breeding in Chaddesley Woods National Nature Reserve in
2009. Dietary treatments are combined since there were no significant dietary
treatment $\times$ condition interactions in the statistical model.

6.5. DISCUSSION

I hypothesised that the supplementation of peanut cake during the fertile period would increase female condition enabling females supplemented with peanut cake to produce higher quality nestlings. Since higher quality male Blue Tits have higher reproductive success than high quality females (Dreiss et al. 2006), I predicted that broods in treatment blocks supplemented with peanut cake would be more-male biased than in the control treatment block. However, there was no significant effect of the supplementation of peanut cake on either female body condition or secondary brood sex ratio. Since males are approx. 6% larger than females (Råberg et al. 2005) and, therefore, are more likely to be susceptible to starvation and mortality than their smaller female conspecifics, I also hypothesised that the additional supplementation of mealworms that are probably consumed by nestlings would reduce male-biased mortality in broods. Therefore, I predicted that Blue Tits receiving both peanut cake and mealworms would have broods that were more male-biased at fledging than broods receiving no food or just peanut cake. In support of my prediction I found that the proportion of males in broods of Blue Tits receiving both food supplements was higher than in broods of Blue Tits receiving no food or just peanut cake.

6.5.1. Supplementary feeding during the fertile period, maternal body condition and sex allocation

Previous studies have demonstrated that supplementary feeding during the fertile period improves maternal body condition (e.g. Meijer et al. 1988, Elliot et al. 2001). In turn, this can influence the relative fitness benefits of male and female offspring and this can have consequences for brood sex ratio (e.g. Nager et al. 1999, Korpimäki et al. 2000). However, I found no significant effect of supplementary feeding during

the fertile period on female body condition or of female body condition on brood sex ratio. My findings are consistent with those of Leech et al. (2001) in this regard who examined female body condition through a number of indices including feather mite load, body mass, tarsus length and wing length and found no effect on brood sex ratio. My findings suggest that the absence of an effect of supplementary feeding during the fertile period on female body condition resulted in the relative reproductive values of male and female offspring during egg production in 2009 being equivalent between dietary treatments since there was no significant main effect of dietary treatment (i.e. peanut cake) during the fertile period on secondary brood sex ratio.

In contrast to my results, a number of laboratory-based studies have reported adaptive adjustments to brood sex ratio in response to food abundance and sex-specific differences in reproductive potential in Zebra Finches (e.g. Bradbury and Blakey 1998, Kilner 1998, Rutstein et al. 2004). In Zebra Finches female nestlings are more dependent on food than their male siblings and the effects of nutritional stress at the nestling stage have a more profound effect on female reproductive success than on male reproductive success (reviewed in Kilner 1998). In support of predictions made from sex allocation theory (Trivers and Willard 1973), Rutstein et al. (2004) demonstrated that diet quality affected primary brood sex ratio of the Zebra Finch. Females were provided with either a high quality or a low quality diet from 6 weeks pre-laying through to, and including, chick rearing and it was found that those on the high quality diet produced more female offspring compared with those on the low quality diet (Rutstein et al. 2004). Kilner (1998) also found that food-restricted Zebra Finches produced male-biased broods but she did not tissue sample unhatched eggs and deceased chicks so only determined secondary sex ratio. Likewise, I did not tissue sample unhatched eggs or deceased offspring and, therefore, it is still possible that

supplementary feeding during the fertile period may have had an effect on primary

sex ratio in my study.

In contrast to sex allocation theory (Trivers and Willard 1973), Arnold et al.

(2003) found that a low, medium or high quality diet had no overall effect on brood

sex ratio of Zebra Finches. Diets were provided from 4 weeks prior to pairing but

stopped once the breeding pairs had formed. Although it is likely that this feeding

regime manipulated pre-breeding condition of females, removing the experimental

diet at pairing would have affected the breeding female's perception of nestling

rearing conditions. This is likely to be problematic since females are likely to bias sex

ratio in relation to the expected rearing condition of the chicks (Rutstein et al. 2004).

6.5.2. Supplementary feeding during the nestling period and post-hatching

manipulation of brood sex ratio

The supplementation of mealworms during the nestling period appeared to result in

broods having significantly higher proportions of males than those produced in the

peanut cake-supplemented and control blocks. This suggests that the supplementation

of mealworms may have differentially affected sex-specific mortality of nestlings

post-hatching between treatment blocks.

In my study population mortality between hatching and fledging in Blue Tits

is significantly affected by dietary treatment with mortality being significantly higher

on supplemented treatments than in the control block (Harrison 2010). Since Blue Tits

exhibit slight size dimorphism (males are approx. 6% larger than females – Råberg et

al. 2005), it is expected that males are more likely to be susceptible to starvation and

mortality than smaller female conspecifics. While overall nestling mortality is not

significantly different between the peanut cake and peanut cake + mealworms dietary

treatment blocks in Blue Tits in my study population (Harrison 2010), it is possible that nestling mortality was more male-biased in the peanut cake compared to the peanut cake + mealworms as a result of lower food availability. Indeed, numerous other studies have demonstrated sex-biased mortality is affected by food availability (e.g. Teather and Weatherhead 1989, Hipkiss et al. 2002). However, the majority of studies that have demonstrated an effect of food abundance on secondary sex ratio have examined species that exhibit extreme sexual size dimorphism where one sex disproportionately suffers more when food-stressed (e.g. American Kestrel – Wiebe and Bortolotti 1992; Blue-footed Booby – Torres and Drummond 1997). Similar to my findings, a number of studies have provided empirical evidence that species that exhibit slight sexual size dimorphism can also show sex ratio bias through differential mortality (Cooch et al. 1997, González-Solis et al. 2005). For example, in Lesser Snow Geese in which males are 2-6% larger than females, male mortality between hatching and fledging was significantly larger than for females (Cooch et al. 1997). However, although I have suggested that differences in differential mortality between the dietary treatment blocks may account for my results, caution should be assigned when interpreting my results since I do not have data to confirm that differential mortality between male and female nestlings differed between dietary treatment blocks.

6.5.3. Further considerations and research directions

I have considered pre- and post-hatching manipulation of sex ratio separately. However, it is likely that my results are a consequence of a combination of both pre- and post-hatching manipulation. First, I suggest that the proportion of males in broods at hatching in both of the supplementary fed treatment blocks was equivalent, which,

in turn, was higher than in the nonsupplemented (control) block. Secondly, I suggest that a low nestling mortality rate in the control block coupled with more male-biased mortality in the peanut cake compared with the peanut cake + mealworms treatment resulted in broods from control and peanut cake treatments having equivalent secondary brood sex ratios. Broods from the peanut cake + mealworms treatment were more male-biased. To gain a better understanding of both pre- and post-hatching manipulation of brood sex ratio and to elucidate the effects of supplementary feeding, future research should consider obtaining tissues from unhatched eggs and dead nestlings to allow consideration of primary, as well as secondary, sex ratio. Since manipulations of sex ratio may be subtle and vary between years depending on local ecological circumstances (Korsten et al. 2006), future research should be repeated over many years to gain a better understanding of how supplementary feeding may affect sex ratio in Blue Tits.

Operational sex ratio, defined as the ratio of potentially receptive males to receptive females at any time (Emlen 1976), has been shown to be male-biased in approx. 60% of passerine species as a result of female-biased dispersal and mortality, for example (reviewed in Donald 2007). Operational sex ratio has also been shown to be related to brood sex ratio (reviewed in Donald 2007). However, since the relationship between operational and brood sex ratio is not consistent between species with either positive (Clarke et al. 2002), negative (Ewen et al. 2001) or no relationship (Bensch et al. 1999) at all, the association between the two remains unclear (reviewed in Donald 2007). Although I have no data that reflect differences in operational sex ratios between dietary treatment blocks, it is possible that they may exist due to differences in female-biased dispersal and mortality between treatments. Therefore,

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since operational sex ratio can partly drive brood sex ratio, future research should also consider operational sex ratio.

6.5.4 The next chapter

My findings from the last 5 chapters have provided evidence that supplementary feeding has marked effects on the reproductive behaviours of both Blue and Great Tits. Since food supplementation in my study was protracted and used commercially available food supplements, it mimicked feeding patterns of garden birds by the public (RSPB 2010); my findings may have implications for a wide range of species of birds that feed in our towns and cities (Jones and Reynolds 2008). However, my small-scale study was conducted in mixed broadleaved deciduous woodland and, therefore, did not mimic the mosaic of habitat types typical of urbanised landscapes in the UK. Moreover, the short-term nature of my study may mean that the results have limited applicability since breeding performance and, therefore, behaviour are likely to change in the future in relation to climate change (e.g. Dunn and Winkler 1999, Sanz 2003). To gain a better understanding of how the breeding performance of both Blue and Great Tits may vary across an urban gradient, how breeding performance may change temporally and to provide insights into the applicability of my research findings, I will now investigate differences in breeding performance of both Blue and Great Tits across an urban gradient over a 30-year period in Chapter Seven.

Chapter Seven

FOOD SUPPLEMENTATION ON BROAD SPATIAL, AND OVER LONG TEMPORAL, SCALES: BREEDING PATTERNS OF BLUE AND GREAT TITS IN URBAN AND NON-URBAN HABITATS

7.1. ABSTRACT

Small-scale (i.e. local) supplementary feeding studies conducted over short time periods (i.e. a few years) have shown that supplementary feeding has marked effects on breeding performance and phenology. However, the effects of supplementary feeding at a large spatial scale and over a long temporal period are unknown. Here, I investigate the breeding phenology and performance of Blue and Great Tits breeding in urban habitats (where supplementary food is regularly provided) and in deciduous woodland habitats across the UK between 1962 and 2008 using data from the British Trust for Ornithology's (BTO) Nest Record Scheme (NRS). Both Blue and Great Tits breeding in urban habitats bred earlier, produced smaller clutches and had higher failure rates (measured as brood size as a proportion of clutch size) than conspecifics breeding in deciduous woodland habitats. Over the 40+ years of this study the magnitude of the difference between clutch sizes, brood sizes and failure rates of Great Tits, but not of Blue Tits, breeding in urban and deciduous woodland habitats decreased. My results suggest that the probable widespread supplementary feeding of birds in urban habitats influenced their breeding parameters in both species but that the effects of supplementary feeding probably varied temporally. I conclude by considering the limitations of a food-focussed approach in a study such as this.

7.2. INTRODUCTION

Currently, the global human population stands at approx. seven billion people (United Nations 2009) of which approx. 50% live in urban areas (United Nations 2010). Urbanisation results in both functional and physical changes to the ecosystem (reviewed in Alberti 2005). For example, urbanisation often results in the degradation and fragmentation of natural habitats (reviewed in Marzluff 2001), significant changes in weather (reviewed in Haggard 1990), an increase in atmospheric pollution (reviewed in Fenger 1999), and an increase in food availability as a consequence of garden bird feeding (e.g. Davies et al. 2009, Fuller et al. In Press).

Feeding garden birds for a large proportion of humans living in urban areas, especially in the 'Western world', is a common phenomenon (e.g. Jones and Reynolds 2008, Davies et al. 2009) popularised by the publication of 'The Bird Table Book' by Soper (1965). Garden bird feeding has increased in popularity (CJ Wildlife Ltd. pers. comm.), and now approx. half of UK households feed garden birds at some point during the year (Davies et al. 2009) spending approx. £200-220 million annually (CJ Wildlife Ltd. pers. comm.).

Many bird species breed in urban habitats (Bland et al. 2004) and an increasing number of studies suggest that urbanisation has marked effects on avian reproduction (reviewed in Chamberlain et al. 2009). For example, birds breeding in urban areas often breed earlier than rural conspecifics (e.g. Dhondt et al. 1984, Cowie and Hinsley 1987, Harrison 2010) possibly as a result of supplementary feeding in gardens reducing the energetic cost of foraging, enabling birds to invest more time and energy in breeding attempts. Breeding earlier is beneficial since birds that fledge earlier are more likely to be recruited into the breeding population (e.g. Verboven and Visser 1998). Supplementary feeding investigations have shown that food promotes

breeding performance by, for example, increasing clutch size (e.g. Nilsson 1991, Schoech et al. 2008). However, although food availability is often higher in urban habitats as a consequence of garden bird feeding (e.g. Davies et al. 2009, Fuller et al. In Press), clutch sizes of birds breeding in urban habitats can be reduced (e.g. Perrins 1965, Solonen 2001, Harrison 2010). One possible explanation is that food quality is poorer in urban habitats during egg production (e.g. Solonen 2001, Chamberlain et al. 2009).

In addition to food availability, other aspects of urban ecology are likely to influence reproduction. For example, the warmer urban climate (Haggard 1990) is likely to reduce the energetic requirements for egg production (Stevenson and Bryant 2000) which may result in advanced laying (Dhondt and Eyckerman 1979) and increased investment into egg production (Nager and van Noordwijk 1992). The warmer climate of urban areas may also result in advanced breeding of birds with advances in the peak of availability of natural foods (e.g. caterpillars) that adult birds feed nestlings (e.g. Eden 1985, Both and Visser 2005). In addition, increased light levels associated with urban areas may both advance and prolong breeding seasons of some bird species (reviewed by Coppack and Pulido 2004) whilst increased urban pollution has been shown to decrease clutch size (Eeva and Lehikoinen 1995) and decrease hatching success (Janssens et al. 2003).

While there have been numerous studies that have investigated the effects of supplementary feeding on the reproductive performance of birds (reviewed by Robb et al. 2008a), the majority have done so at a small spatial scale and over a short temporal period (reviewed by Harrison et al. 2010). However, since garden bird feeding is a widespread activity here in the UK, is probably growing in popularity internationally as the world becomes increasingly urbanised (Fuller et al. In Press) and

may have consequences for a large number of bird species that breed in urban areas (Bland et al. 2004), it would clearly be valuable to know whether supplementary feeding affects avian reproductive performance at a landscape scale. Furthermore, since supplementary feeding can have differential effects on breeding performance between years (e.g. Schoech 1996, Dewey and Kennedy 2001, Hipkiss et al. 2002), it is important to consider the effects of supplementary feeding over extended periods if possible.

Here, I compare the breeding phenology and breeding performance (i.e. clutch size, brood size and failure rates [brood size as a proportion of clutch size]) of Blue and Great Tits breeding in urban and rural (deciduous woodland) habitats across the UK from 1962 to 2008. Although it is impossible to measure directly food availability across such wide spatial, and long temporal, scales, it is well documented that supplementary feeding occurred in urban habitats throughout the period of study (e.g. Soper 1965, Cowie and Hinsley 1988a, Davies et al. 2009).

7.3. METHODS

7.3.1. The Nest Record Scheme (NRS)

To investigate temporal trends in breeding performance of Blue and Great Tits breeding in deciduous woodland and urban habitats, I used breeding data from the NRS. The NRS was established by the BTO in 1939 to accumulate data on the breeding biology of birds using volunteer recorders (Mayer-Gross 1970). Since its establishment, 1,250,000 records for 232 species have been submitted (Crick et al. 2003) providing long-term datasets which have been used, for example, to produce breeding biology accounts (e.g. Newton 1964, Shaw 1978), to assess the effects of habitat modification on reproductive performance (e.g. Siriwardena et al. 2000, 2001)

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and to identify long-term temporal trends in breeding performance (e.g. Chamberlain and Crick 1999, Siriwardena and Crick 2002).

Using a separate nest record card for each focal nest, a nest recorder documents species, location (county, grid reference and altitude), date of visit and the habitat where the nest is located. Between 1962 and 1989 habitat was recorded using a hierarchical coding system whereby the nest recorder firstly selected the key habitat (rural, suburban or urban) and then provided a description of the most important features of the area surrounding the nest using a list of key terms provided (Mayer-Gross 1970). However, this system was modified and adapted from that of Yapp (1955), but it suffered drawbacks such as containing obsolete and irrelevant features (Crick 1992). Therefore, a new hierarchical habitat coding system was implemented in 1990 which was designed to provide more detailed information on habitat type with an emphasis on human-created sites (Crick 1992). For each nest, nest recorders also document specific details relating to the reproductive period at the time of the visit (i.e. the stage of nest building, number of eggs, developmental stage, number of nestlings, outcome of the breeding attempt [i.e. success or failure]) (Mayer-Gross 1970, Crick et al. 2003).

7.3.2. Selecting nest records

I selected nest records for Blue and Great Tits breeding between 1962 and 2008, inclusive, in either urban or deciduous woodland (broadleaved) habitats throughout the UK (Fig. 7.1.) by using either habitat descriptions (pre-1990) or habitat codes (post-1990) provided by nest recorders. Pre-1962 records were not selected due to small sample sizes per year. For each nest record the geographic location of the breeding attempt was determined using Ordnance Survey (OS) grid references
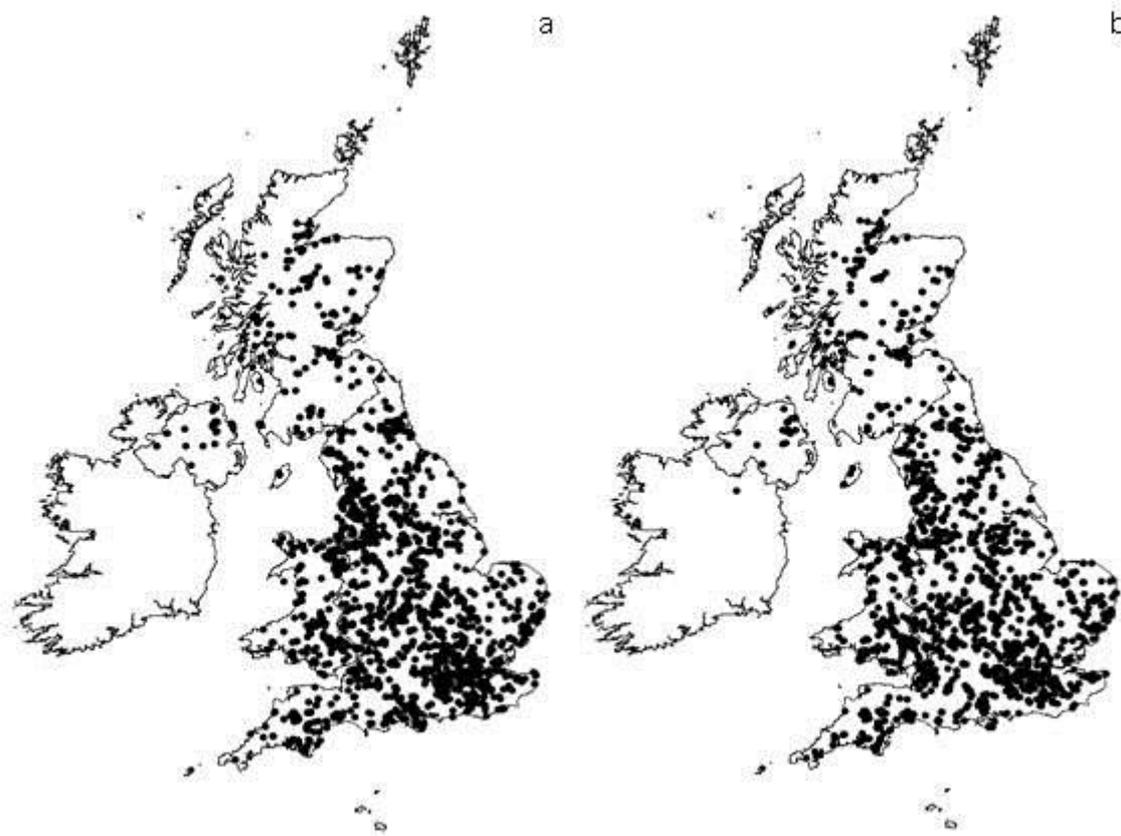
Figure 7.1. Spatial distribution of nest records selected from the British Trust for Ornithology's Nest Record Scheme for (**a**) Blue Tits and (**b**) Great Tits breeding between 1962 and 2008 in either urban or deciduous woodland (broadleaved) habitats throughout the UK.

provided by the nest recorder. Where an OS grid reference was not available, the OS grid reference at the mid-point of the county in which the breeding attempt occurred was used. Using the OS grid references, eastings and northings were calculated (Ordnance Survey 2010). Where multiple records for an individual site (i.e. a 1-km square) occurred within the same year, a single record was randomly selected and the remainder excluded in order to minimise non-independence of nest records.

7.3.3. Estimating breeding parameters from nest records

Nest records are often incomplete and, therefore, breeding parameters have to be estimated because of uncertainty in the data (Crick et al. 2003). I followed methods described in Crick et al. (2003) to estimate clutch initiation date, clutch size and brood size. In summary, clutch initiation date was estimated and defined as the mid-point between the earliest and latest first-egg dates (rounded to the nearest day). If the difference between the earliest and latest possible first-egg dates was > 10 days, the record was discarded (*sensu* Harrison 2010). Clutch and brood sizes were calculated as the maximum number of observed eggs and nestlings, respectively, but records were discarded from analysis if they were >16 (*sensu* Harrison 2010; such records may be erroneous since clutch sizes of both Blue and Great Tits do not usually exceed 16 – Cramp and Perrins 1993). Nest records were also excluded if brood size = 0 (i.e. indicating full brood mortality) and if maximum brood size exceeded maximum clutch size. NRS data do not allow failure rates at both egg and nestling stages to be estimated independently (Crick et al 2003). Instead, I used an alternative approach where failure rates (combined hatching success and/or partial brood mortality) were examined using the ratio of maximum brood size to maximum clutch size (i.e. brood size as a proportion of clutch size).

7.3.4. Statistical analyses

All statistical analyses were performed using PROC GENMOD in SAS version 9.2 (SAS Institute Inc. 2008). Clutch initiation date of both species was tested for normality using the Anderson-Darling Test and for homogeneity of variance using the *F*-test in Minitab 15 (Minitab 2007). Clutch initiation date of both species was square-root transformed to normalise its distributions and then fitted with normal error

distributions after examination of the model fit (Deviance/DF = approx. 1, Pearson

Chi-Square/DF = approx. 1). Clutch sizes and brood sizes were fitted with Poisson

error distributions while failure rates were fitted with a binomial error distribution.

In all analyses habitat was included as a categorical variable and year as a

continuous variable. Eastings, northings and altitude were also included as continuous

variables in all models to control for geographic variation in breeding phenology and

performance (e.g. Sanz 1998, Fargallo 2004). Since both clutch and brood sizes may

decline seasonally (e.g. Perrins and McCleery 1989, Smith 1993), clutch initiation

date was included as a continuous variable in clutch and brood size analyses. To

determine if the effects of covariates were consistent between habitats, covariate $\times$

habitat interactions were tested independently of one another within each model.

Where multiple covariate $\times$ habitat interactions were significant, they were both

included in the model as long as each stayed significant (Grafen and Hails 2002). I

performed backward model selections removing the least non-significant covariate

interactions ($P > 0.05$) one-by-one from models ensuring that there was no significant

change in deviance between models where applicable (Grafen and Hails 2002).

7.4. RESULTS

7.4.1. Breeding phenology

Clutch initiation date of both Blue and Great Tits was significantly affected by

urbanisation with birds in urban habitats breeding significantly earlier than those in

deciduous woodland habitats (Blue: $F_{1,5271} = 5.30$, $P = 0.02 - 0.3$ days; Great: $F_{1,4318}$

$= 29.97$, $P < 0.0001 - 3.9$ days; Table 7.1, Fig. 7.2a and b). In addition, clutch

initiation date significantly advanced over the years of the study for birds breeding in

Table 7.1. Results from the final statistical models (see text for details) explaining measurements of breeding phenology and performance of Blue (BT) and Great (GT) Tits breeding between 1962 and 2008 in urban (U) and deciduous woodland (D) habitats. Significant main and interaction term effects are given in bold. Directions of significant effects are given '+' and '-' denote significant positive and negative relationships, respectively, and 'NS' denotes nonsignficance ($P > 0.05$).

Breeding parameter	Species	Factor	F	df	P	Direction
Clutch initiation date	BT	Habitat	**5.30**	**1,5271**	**0.02**	U = earliest ; D = latest
		Year	**526.57**	**1,5271**	**< 0.0001**	-
		Northings	**11.00**	**1,5271**	**< 0.001**	-
		Eastings	**463.08**	**1,5271**	**< 0.0001**	+
		Altitude	**87.74**	**1,5271**	**< 0.0001**	+
		Northings × habitat	**6.11**	**1,5271**	**0.01**	D = negative; U = less negative
	GT	Habitat	**29.97**	**1,4318**	**< 0.0001**	U = earliest; D = latest
		Year	**444.05**	**1,4318**	**< 0.0001**	-
		Northings	**19.68**	**1,4318**	**< 0.0001**	-
		Eastings	**137.59**	**1,4318**	**< 0.0001**	+
		Altitude	**46.29**	**1,4318**	**< 0.0001**	+
		Altitude × habitat	**4.21**	**1,4318**	**0.04**	D = positive; U = less positive
		Eastings × habitat	**5.87**	**1,4318**	**0.02**	D = ; U = less positive
Clutch size	BT	Habitat	**173.57**	**1,3097**	**< 0.0001**	U = smaller; D = larger
		Year	**250.04**	**1,3097**	**< 0.0001**	-

Table 7.1. continued

Breeding parameter	Species	Factor	F	df	P	Direction
		Clutch initiation date	418.96	1,3097	< 0.0001	-
		Altitude	6.95	1,3097	0.01	+
		Eastings	11.13	1,3097	< 0.01	-
	GT	Habitat	12.39	1,2232	< 0.001	U = smaller; D = larger
		Year	23.17	1,2232	< 0.0001	-
		Clutch initiation date	124.66	1,2232	< 0.0001	-
		Altitude	4.55	1,2232	0.03	-
		Eastings	5.04	1,2232	0.02	-
		Northings	21.41	1,2232	< 0.0001	+
		Year × habitat	12.05	1,2232	< 0.01	D = negative; U = less negative
Brood size	BT	Habitat	172.32	1,3801	< 0.0001	U = smaller ; D = larger
		Year	273.72	1,3801	< 0.0001	-
		Clutch initiation date	295.93	1,3801	< 0.0001	-
		Eastings	8.79	1,3801	< 0.01	-
		Altitude	9.56	1,3801	< 0.01	+
	GT	Habitat	22.35	1,3184	< 0.0001	U = smaller; D = larger
		Year	24.56	1,3184	< 0.0001	-

Table 7.1. continued

Breeding parameter	Species	Factor	*F*	*df*	*P*	Direction
		Clutch initiation date	**106.49**	**1,3184**	**< 0.0001**	-
		Eastings	**8.02**	**1,3184**	**< 0.01**	-
		Northings	**9.28**	**1,3184**	**< 0.01**	+
		Altitude	**9.56**	**1,3184**	**< 0.01**	+
		Year × habitat	**21.80**	**1,3184**	**< 0.0001**	D = negative; U = less negative
Failure rates (brood size as a proportion of clutch size)	BT	Habitat	**4.49**	**1,2250**	**0.03**	U = highest ; D = lowest
		Year	**13.28**	**1,2250**	**< 0.001**	+
		Northings	**5.88**	**1,2250**	**0.02**	+
		Altitude	1.05	1,2250	0.31	N/S
		Clutch initiation date	**4.21**	**1,2250**	**0.04**	+
	GT	Habitat	**6.43**	**1,1652**	**0.01**	U = highest ; D = lowest
		Year	0.00	1,1652	0.96	N/S
		Northings	**7.87**	**1,1652**	**0.01**	+
		Altitude	0.20	1,1652	0.65	N/S
		Year × habitat	**6.32**	**1,1652**	**0.01**	U = positive ; D = more positive
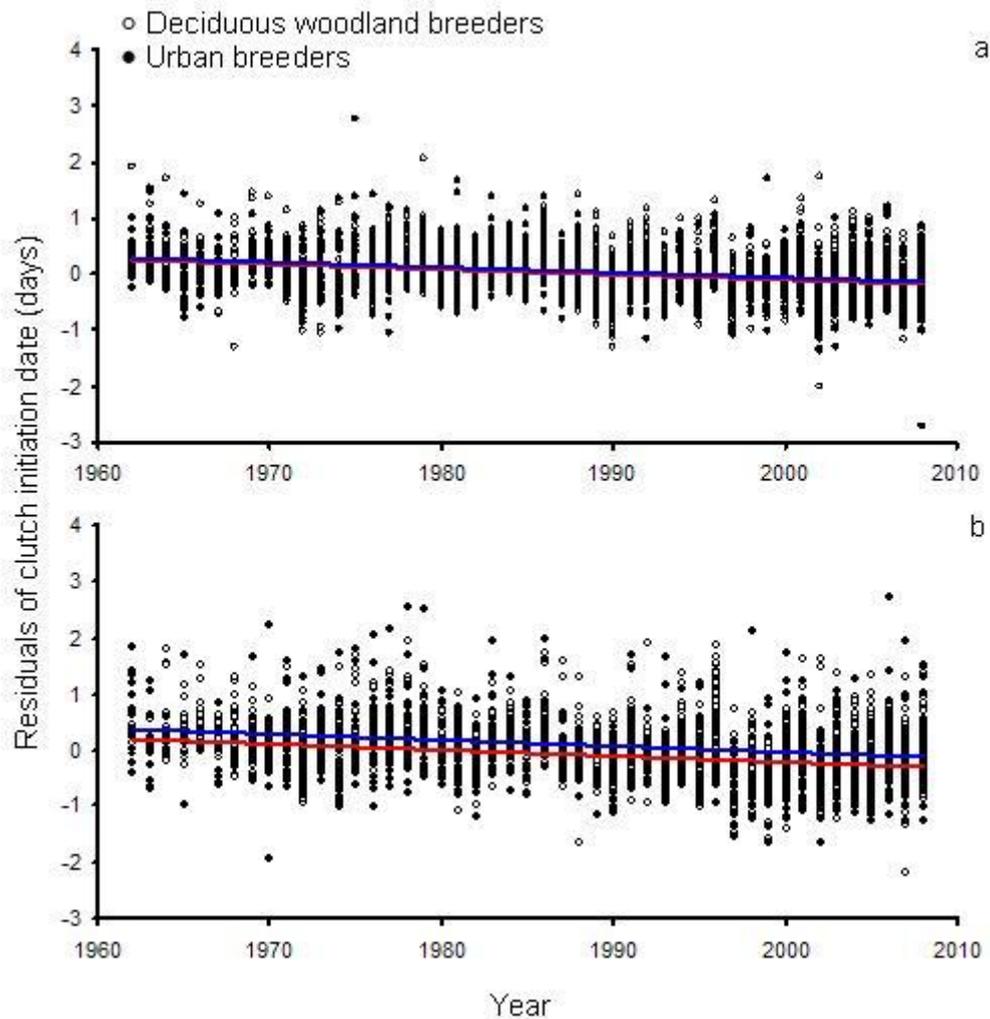
Figure 7.2. Temporal trend in clutch initiation date of (**a**) Blue Tits and (**b**) Great Tits breeding in urban (filled circles and red regression line) and deciduous woodland (open circles and blue regression line) habitats from 1962 to 2008 in the UK. Residuals of clutch initiation date were calculated after accounting for all predictors other than year and habitat in the final model (see Table 7.1 for details).

both habitats (Blue: $F_{1,5271} = 526.57$, $P < 0.0001 - 8.0$ days; Great: $F_{1,4318} = 444.05$, $P < 0.0001 - 10$ days; Table 7.1, Fig 7.2a and b). Furthermore, the advancement in clutch initiation date over time for both Blue and Great Tits breeding in urban habitats was equivalent to that of conspecifics breeding in deciduous woodland habitats (indicated by the absence of a significant year $\times$ habitat interaction in the statistical models [$P > 0.05$]).

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7.4.2. Breeding performance

Clutch sizes of Blue and Great Tits breeding in urban habitats were significantly

smaller than those of conspecifics breeding in deciduous woodland habitats (Blue:

$F_{1,3097} = 173.57$, $P < 0.0001 - 1.1$ eggs; Great: $F_{1,2232} = 12,39$, $P < 0.001 - 0.2$ eggs;

Table 7.1, Figs 7.3a and b). Clutch size significantly declined throughout the period of

study in Blue and
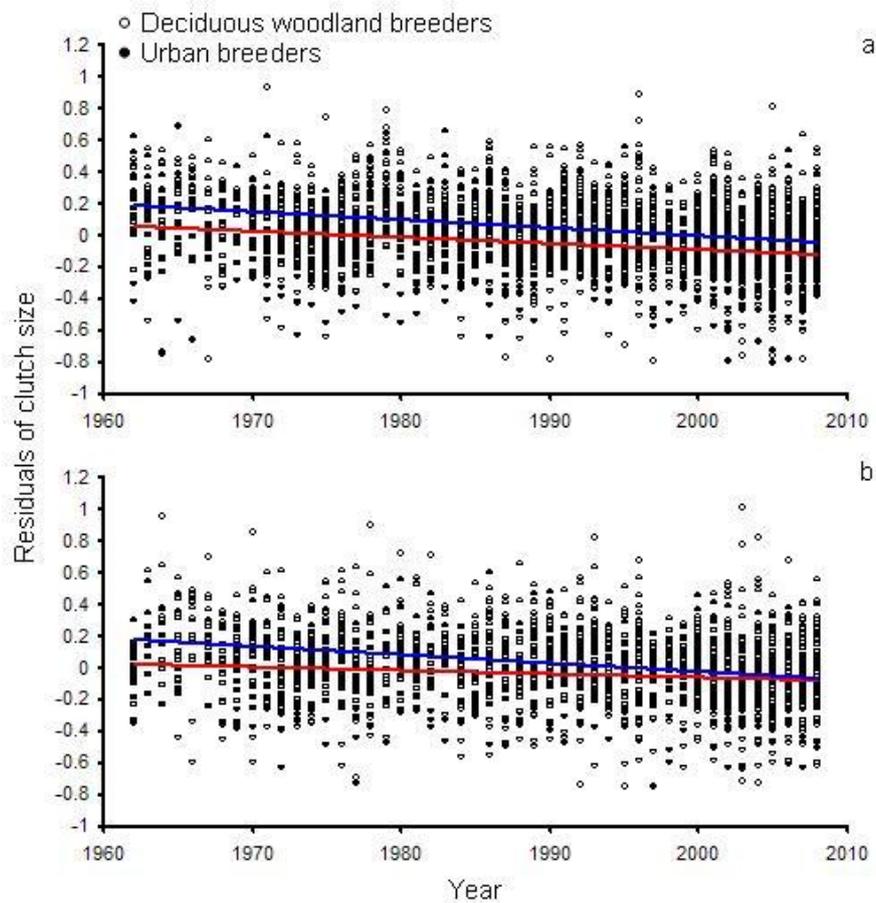
Figure 7.3. Temporal trend in clutch size of (**a**) Blue Tits and (**b**) Great Tits breeding
in urban (filled circles and red regression line) and deciduous woodland (open circles
and blue regression line) habitats between 1962 and 2008 in the UK. Residuals of
clutch size were calculated after accounting for all predictors other than year and
habitat in the final model (see Table 7.1 for details).

Great Tits breeding in urban and deciduous woodland habitats (Blue: $F_{1,3097} = 250.04$,

$P < 0.0001 - 1.0$ eggs; Great: $F_{1,2232} = 23.17$, $P < 0.0001 - 1.5$ eggs; Table 7.1, Fig.

7.3a and b). For Blue Tits this decline in clutch size was equivalent between urban

and deciduous woodland habitats (as indicated by the absence of a significant year $\times$

habitat interaction in the statistical models [$P > 0.05$]). However, in Great Tits the

decline in clutch size was more pronounced in deciduous woodland habitats compared

to urban habitats as indicated by a significant habitat $\times$ year interaction ($F_{1,2232} =$

12.05, $P < 0.001$ – deciduous: 1.8 eggs, urban: 0.9 eggs); Table 7.1 and Fig. 7.3b).

Both Blue and Great Tits breeding in urban habitats also had significantly

smaller brood sizes (Blue: $F_{1,3801} = 172,32$, $P < 0.0001$ – 0.9 chicks; Great: $F_{1,3184} =$

22.35, $P < 0.0001$ – 0.4 chicks; Table 7.1, Fig. 7.4a and b). Furthermore, there was a
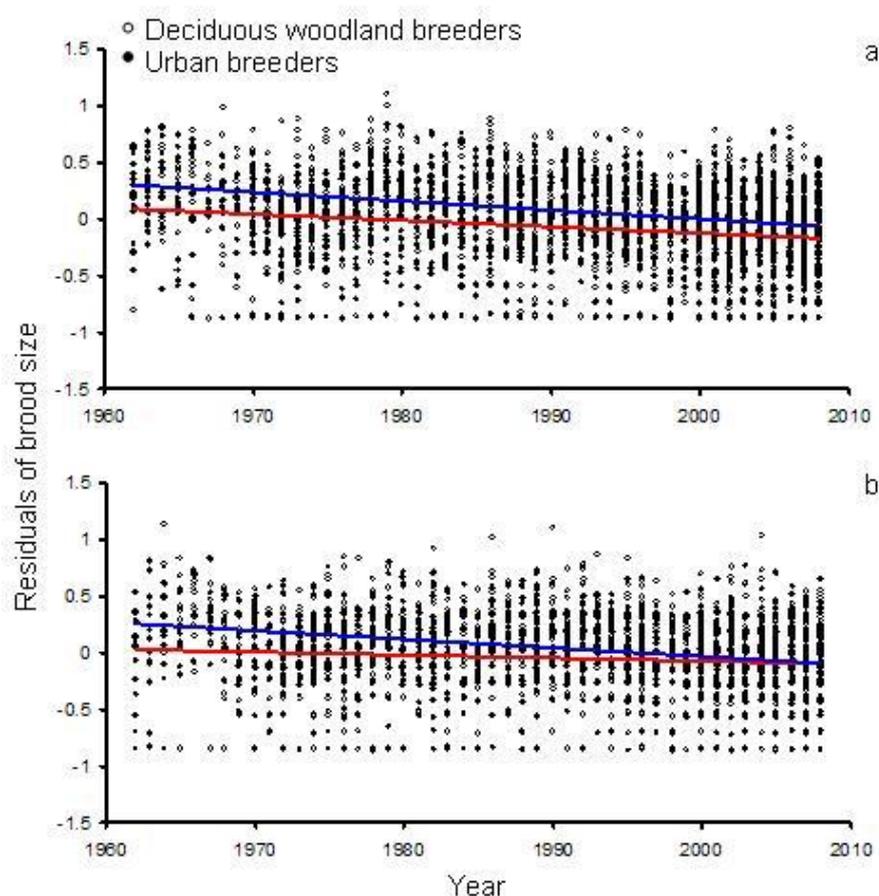
Figure 7.4. Temporal trend in brood size of (**a**) Blue Tits and (**b**) Great Tits breeding
in urban (filled circles and red regression line) and deciduous woodland (open circles
and blue regression line) habitats between 1962 and 2008 in the UK. Residuals of
brood size were calculated after accounting for all predictors other than year and
habitat in the final model (see Table 7.1 for details).

significant decline in brood size in both species throughout the period of study (Blue: $F_{1,3801} = 237.72$, $P < 0.0001 - 1.5$ chicks; Great: $F_{1,3184} = 24.56$, $P < 0.0001 - 0.5$ chicks; Table 7.1, Fig. 7.4a and b). Similar to the trend in clutch size, the declines in brood sizes of Blue Tits breeding in urban and deciduous woodland habitats were equivalent (as indicated by the absence of a significant year $\times$ habitat interaction in the statistical models [$P > 0.05$]). However, the decline in brood sizes of Great Tits breeding in deciduous woodland habitat was more pronounced than that of urban birds as indicated by a significant habitat $\times$ year interaction ($F_{1,3184} = 21.80$, $P < 0.0001 -$ urban habitats $= 0.5$ chicks; deciduous woodland habitats $= 1.1$ chicks; Table 7.1 and Fig. 7.4b).

7.4.3. Failure rates (brood size as a proportion of clutch size)

Failure rates were significantly higher for Blue and Great Tits breeding in urban habitats than for conspecifics breeding in deciduous woodland habitats (Blue: $F_{1,2250} = 4.49$, $P = 0.03 - 1.6$ %; Great: $F_{1,1652} = 6.43$, $P = 0.01 - 3.5$ %; Table 7.1, Fig. 7.5a and b). Failure rates of Blue Tits increased significantly during the study ($F_{1,2250} = 13.28$, $P < 0.001 - 4.5\%$; Table 7.1 and Fig. 7.5a) and the rate of increase was equivalent for birds breeding in urban versus deciduous woodland habitats (as indicated by the absence of a significant year $\times$ habitat interaction in the statistical models [$P > 0.05$]). In contrast, failure rates of Great Tits were consistent throughout the study ($F_{1,1652} = 0.00$, $P = 0.96$; Table 7.1 and Fig. 7.5b). However, a significant year $\times$ habitat interaction highlighted that, while failure rates appeared to remain consistent in urban habitats, failure rates increased for Great Tits breeding in deciduous woodland habitats throughout the study ($F_{1,1652} = 6.32$, $P = 0.01 - 8$ %; Table 7.1 and Fig. 7.5b).
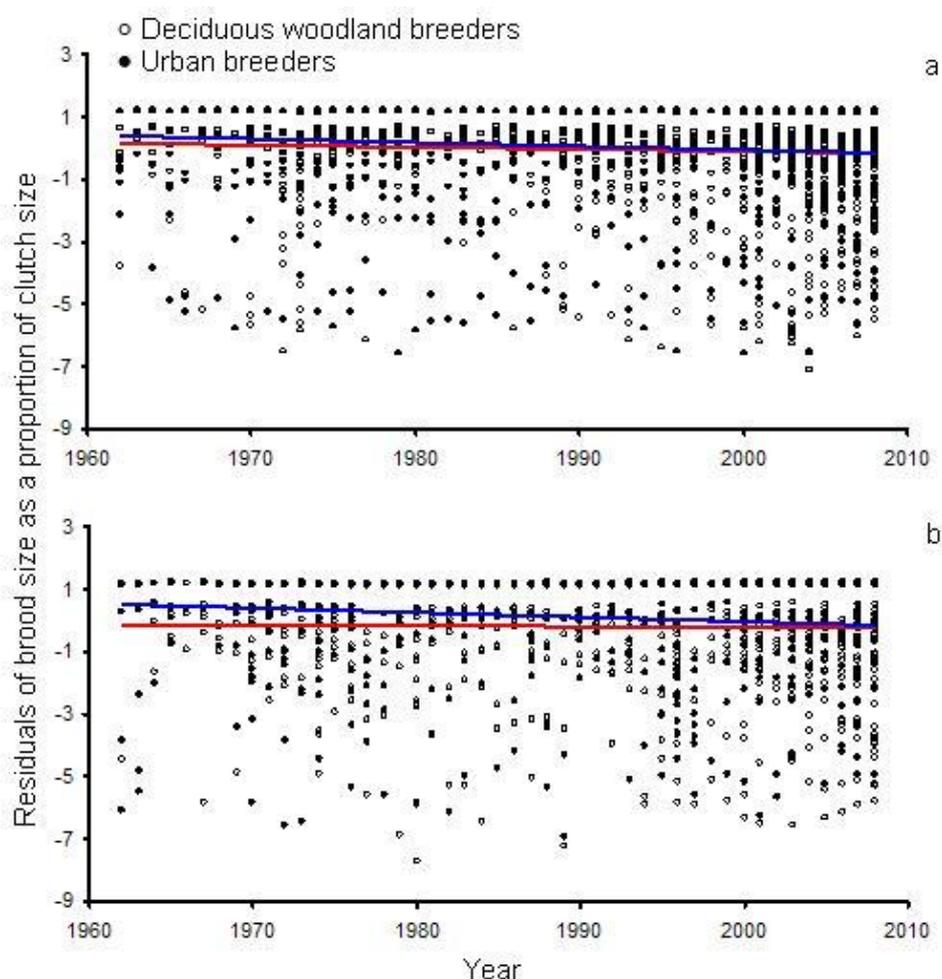
Figure 7.5. Temporal trend in failure rates (brood size as a proportion of clutch size) of (**a**) Blue Tits and (**b**) Great Tits breeding in urban (filled circles and red regression line) and deciduous woodland (open circles and blue regression line) habitats between 1962 and 2008 in the UK. Residuals of failure rates were calculated after accounting for all predictors other than year and habitat in the final model (see Table 7.1 for details).

7.5. DISCUSSION

Both Blue and Great Tits in urban habitats bred significantly earlier than conspecifics breeding in deciduous woodland habitats suggesting that breeding was advanced perhaps by heightened food availability. Furthermore, in both species the difference between breeding phenologies of birds breeding in the urban and deciduous woodland

habitats remained consistent throughout the study. This suggests that if the effect was driven by supplementary feeding in urban areas, it remained consistent across the study. Clutch and brood sizes of both Blue and Great Tits breeding in urban habitats were significantly smaller than those of conspecifics breeding in deciduous woodland habitats whilst failure rates were significantly higher for both Blue and Great Tits breeding in urban compared with deciduous woodland habitats. However, the magnitude of the difference changed over time for Great Tits. In contrast, the differences in breeding outputs of Blue Tits breeding in urban and deciduous woodland habitats were equivalent over the study.

7.5.1. Breeding phenology

The timing of breeding of birds is likely to have consequences for their fitness since birds that fledge earlier are more likely to be recruited into the breeding population (e.g. Verboven and Visser 1998). Similar to numerous other studies (e.g. Dhondt et al. 1984, Cowie and Hinsley 1987, Harrison 2010), my results indicate that two species of small passerine in urban habitats initiated clutches significantly earlier than conspecifics breeding in deciduous woodland habitats. My thesis in this chapter is that increased availability of food in urban areas might drive phenology and performance of breeding; indeed, egg formation and laying may be constrained by food availability (Perrins 1970) but it is also possible that elevated temperatures in urban habitats (Haggard 1990) resulting in reduced energetic investment in foraging (e.g. Norberg 1977) might enable birds to accumulate resources to form and lay eggs earlier than birds in deciduous woodland habitats. Alternatively, higher urban temperatures (Haggard 1990) may result in advanced phenology of prey availability and of avian breeding attempts in response (e.g. Eden 1985, Both and Visser 2005).

During this study both species in both urban and deciduous woodland habitats progressively advanced their timing of breeding. Advancement in breeding over the past few decades has also been demonstrated for a number of other species (e.g. European Pied Flycatcher [*Ficedula hypoleuca*] – Both and Visser 2005; Eurasian Reed Warbler [*Acrocephalus scirpaceus*] – Halupka et al. 2008) corresponding to increasing spring temperatures as a result of climate change (IPCC 2001, King 2005). Both and Visser (2005) suggested that the ultimate reason that a number of bird species have advanced the timing of breeding in relation to spring time temperatures is so that they are able to breed in synchrony with invertebrate food sources that have also advanced their emergence in response to higher spring temperatures. Although birds advanced the timing of breeding over the study, the difference in breeding phenology between birds breeding in the urban and deciduous woodland habitats remained of constant magnitude across years. If supplementary feeding does drive breeding phenology in urban habitats then this suggests that the effect of supplementary feeding on breeding phenology remained consistent in urban areas throughout the study. Alternatively, the effects of higher urban temperatures (Haggard 1990) which may result in advanced phenology may have remained consistent over the period of this study.

7.5.2. Breeding performance

Clutch size can be limited by food availability (e.g. Martin 1987, Williams 1996). Therefore, it is surprising that both species in urban habitats, where supplementary feeding is a widespread activity (e.g. Davies et al. 2009, Fuller et al. In Press), produced smaller clutches than birds breeding in deciduous woodland habitats. However, a recent study by Harrison et al. (2010) showed that Blue and Great Tits

supplemented with commercially available garden bird food produced smaller clutches than nonsupplemented (control) conspecifics. Therefore, it is plausible that supplementary food in urban habitats reduced clutch size of both species but the mechanism by which is mediated remains to be elucidated. Parids breeding in urban habitats may adaptively reduce clutch size when they breed out of synchrony with peaks in natural food availability that they need for egg formation, nestling provisioning or both (e.g. Sanz 2003, Laaksonen et al. 2006). Mistiming breeding relative to peaks in natural food availability may occur in urban habitats due to advanced breeding (see Section 7.5.1). Reducing clutch size when birds mistime breeding is likely to be adaptive since it results in a truncation of the time between laying and hatching, allowing birds to concentrate the limited food that is available on fewer offspring (Buse et al. 1999). It is also possible that the higher risk of predation in urban, compared to non-urban, habitats (Haskell et al. 2001, Lepczyk et al. 2004) may result in a responsive reduction in clutch size (and, therefore, brood size – see Fig. 7.4) in urban areas (reviewed in Lima 2009).

Failure rates were highest for both species when breeding in urban, compared with deciduous woodland, habitats suggesting that hatching success was reduced and/or nestling mortality was higher in the former. I suggest that the latter is more likely as previous studies (e.g. Mennechez and Clergeau 2006, Newhouse et al. 2008) have found that nestlings in urban habitats are lighter in weight and are more likely to starve than non-urban nestlings (e.g. Antonov and Atanasova 2003, Shawkey et al. 2004). Differences in starvation rates between habitats may occur as a result of natural food availability being lower in urban habitats compared with deciduous woodland areas (Marciniak et al. 2007), or as a result of the adults provisioning nutritionally unsuitable supplementary food to the nestlings (Chamberlain et al. 2009).

I found that the advancement in breeding of both species in this study was accompanied by declines in clutch size and brood size. Although Both and Visser (2005) suggested the advancement in breeding over time was a response to the advancement of peaks in prey availability, advancing breeding in many species has resulted in a mismatch between the peaks of natural food availability and of the energetic/nutritional demands of growing and developing nestlings (reviewed in Visser and Both 2005). Such mistimed breeding relative to food supply may result in an adaptive reduction in clutch size since producing smaller clutches shortens the time between laying and hatching and allows adults to concentrate limited food resources in feeding fewer offspring (Buse et al. 1999). Similar to my results, previous studies (e.g. Sanz 2003, Laaksonen et al. 2006) have shown that birds that have advanced breeding over the last approx. 50 yrs in relation to increasing spring temperatures have also shown declines in clutch size. I also found that failure rates increased throughout the period of this study for Blue Tits breeding in urban and deciduous woodland habitats and for Great Tits breeding in urban, but not deciduous woodland, habitats. This suggests that, although Blue and Great Tits appeared to reduce clutch size adaptively, birds may have still not have been able to match the peak energetic/nutritional demands of the growing and developing nestlings with the peaks of natural food availability. However, failure rates of Great Tits breeding in urban habitats remained constant over time suggesting that supplementary food buffered the effect of mistiming breeding by enabling more efficient feeding efforts by adults and greater survival of nestlings.

I found that the magnitude of the difference between clutch sizes, brood sizes and failure rates of Great Tits in urban and deciduous woodland habitats decreased through the course of the study period suggesting that the effects of supplementary

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feeding also decreased over time (if, indeed, supplementary feeding did drive breeding performance in urban habitats; see Section 7.2). Alternatively, deciduous woodland habitat quality may have improved over time with respect to natural food availability (but see Fuller et al. 2005), for example, decreasing the difference in food availability and, therefore, the constraints on breeding between the two habitats. In contrast, differences between clutch sizes, brood sizes and failure rates of Blue Tits breeding in urban and deciduous woodland habitats remained consistent throughout my study suggesting that the effects of supplementary feeding may have remained consistent over time. Alternatively, deciduous woodland habitat quality may have remained consistent over time with respect to natural food availability, for example, with the differences in the constraints on breeding between the two habitats being maintained over time. However, establishing historic and current patterns in food availability on national spatial scales is problematic in terms of logistics and, besides, food availability does not operate in isolation from other ecological factors such as climate change that influence breeding performance.

7.5.3. Future considerations and direction

The results of this study suggest that food availability in urban habitats in the form of food supplements may have complex effects on the breeding performance of two small passerines. Furthermore, they suggest that the effect of supplementary feeding in urban habitats may not be consistent over time, at least for Great Tits. However, although I have assumed that differences in breeding phenology and performance between birds breeding in urban and deciduous woodland habitats can be explained by supplementary food provided in gardens, estimating food availability over such large spatial, and long temporal, scales is problematic. It remains to be seen how such

data may be collected and used in such a study. Finally, it is clear that a wide range of

species that breed in rural and urban habitats, and use supplementary food sources,

should be studied if we are to understand how food availability influences avian life

history (e.g. Schoech 1996, Dewey and Kennedy 2001, Hipkiss et al. 2002) and

productivity in the 21st century.

Chapter Eight

GENERAL DISCUSSION

8.1. SUMMARY OF RESULTS

Supplementary feeding of two species of small passerine during the spring and summer had significant effects on their reproductive behaviours at all stages of their breeding cycles. During nest construction supplemented Blue and Great Tits advanced nest building while supplemented Blue Tits also constructed shallower nests over a shorter period of time than unfed (control) conspecifics (Chapter Two). Supplementary feeding also had a significant effect on both the incubation (Chapter Three) and brood provisioning (Chapter Four) behaviours of both species. However, the directions of effects were inconsistent between years suggesting that the influence of supplementary feeding may depend on other ecological factors such as the abundance of natural food (e.g. Schoech 1996, Dewey and Kennedy 2001, Hipkiss et al. 2002). In contrast, the effects of supplementary feeding on mating behaviour were consistent between years with broods of supplemented Blue Tits in 2008 and 2009 containing a higher proportion of EPY than those of control birds (Chapter Five). Supplementary feeding also appeared to influence sex allocation and/or sex-specific differential mortality between nestlings since broods of supplemented Blue Tits were significantly more male-biased than those of control birds (Chapter Six).

Over a broader (i.e. national), and a longer temporal (i.e. 45 yrs), scale than the Chaddesley study, my analysis of the BTO's NRS data revealed that both Blue and Great Tits breeding in urban habitats bred significantly earlier and experienced reduced productivity (i.e. smaller clutch and brood sizes, higher failure rates) than conspecifics breeding in deciduous woodland habitats (Chapter Seven) suggesting

that supplementary feeding in urban areas may have reduced breeding performance in both species. During the period of the study (i.e. from 1962 to 2008), however, the magnitude of the difference between clutch size, brood size and failure rates between Great Tits, but not Blue Tits, breeding in urban and deciduous woodland habitats decreased. This suggests that the strength of the effects of supplementary feeding on breeding performance (and reproductive behaviours) of Great Tits also declined during the period of the study (if, indeed, supplementary feeding did drive breeding performance in urban habitats – see Section 7.2). This suggests that the reduction in breeding performance (and reproductive behaviours) of Great Tits in urban habitats, possibly as a result of supplementary feeding, became less pronounced during the period of the study (if, indeed, supplementary feeding did drive breeding performance in urban habitats – see Section 7.2).

8.2 THE MECHANISMS UNDERLYING THE EFFECTS OF SUPPLEMENTARY FEEDING

8.2.1. Direct and indirect effects of supplementary feeding

Throughout my thesis I have considered separately the effects of supplementary feeding on individual behaviours that are elicited during reproduction. However, it is likely that supplementary feeding also acted indirectly between reproductive stages. For example, supplementary feeding in my field study significantly reduced incubation recess lengths of Great Tits in 2009 suggesting that increased food availability alleviated the energetic and temporal costs of foraging, thereby allowing Great Tits to reduce time away from the nest (Chapter Three). However, supplementary feeding may have indirectly influenced incubation behaviour through directly affecting nest construction behaviour (Chapter Two). Nest properties are likely to influence heat loss from a clutch of eggs (Hilton et al. 2004) and that, in turn,

influences incubation strategies of incubators that optimise egg temperature (Deeming 2002a). The effects of supplementary feeding on nest construction (Chapter Two) may also have had indirect effects on other downstream reproductive behaviours (e.g. brood provisioning). For example, nest construction behaviour can act as a sexually-selected signal influencing parental investment in other reproductive phases (e.g. de Neve et al. 2004). By increasing the proportion of EPY in broods (Chapter Five), supplementary feeding may have also indirectly influenced parental investment in other reproductive phases. This is because social pair males may adjust the extent of parental care in relation to their perceived level of paternity loss (Birkhead and Møller 1992). For example, Hoi-Leitner et al. (1999) provided evidence that male feeding rates were positively related to WPY. Therefore, it is possible that supplementary feeding could have indirectly affected brood provisioning behaviour (Chapter Four) by directly affecting EPP (Chapter Five). Future research to investigate how supplementary feeding directly affects each reproductive behaviour should target each breeding phase for exclusive food supplementation.

8.2.2. What does supplementary feeding provide?

A key hypothesis underlying the predictions throughout my thesis is that supplementary feeding reduces the energetic and temporal costs of foraging allowing supplemented birds to allocate more time and energy in reproductive behaviours. However, the mechanism(s) for such effects on reproductive behaviours remain unknown, partly because of limited knowledge on the food supplement consumption by birds. Recent stable isotope analysis, however, revealed that supplementary food does not make up a substantial proportion of the diet of Blue and Great Tits in my study population (Harrison 2010) suggesting that Blue and Great Tits do not

extensively consume the supplementary food. One possible mechanism by which food supplementation acts is as a cue to habitat quality (i.e. to high food availability for nestling/fledglings). This could result in modifications in behaviours of adult birds with their elevated perceptions about habitat quality (see Robertson and Hutto 2006 for discussion of ecological traps). However, since both species were observed on feeders throughout each year of my study (pers. obs.), and there were multiple effects of supplementary feeding on reproductive behaviours (Chapters Two-Six), it appears that supplementary food provided birds with a direct source of energy. It is also likely that supplementary feeding provided both species with temporal benefits as a supplemented bird has to spend less time foraging (e.g. Norberg 1977, Davies and Lundberg 1985).

8.3. THE APPLIED PERSPECTIVE

8.3.1. Implications for garden bird feeding

Supplementary feeding in my field study had marked effects on reproductive behaviours in all three major constituent phases of reproduction (i.e. nest construction – Chapter Two; incubation – Chapter Three; and brood rearing – Chapter Four) in a way which might predict increased fitness benefits. For example, supplementary feeding significantly advanced breeding in both Blue and Great Tits (Chapter Two) that could be beneficial given earlier fledged birds have a higher probability of being recruited into the breeding population (e.g. Verboven and Visser 1998). Supplementary feeding also decreased incubation recess lengths of Great Tits in 2009 (Chapter Three) which may have resulted in a truncation of incubation period and, consequently, a reduction in predation risk (Martin and Ghalambor 1999, Conway and Martin 2000a, Martin et al. 2000b). In addition, supplementary feeding significantly

affected brood provisioning rates (Chapter Four) and, although the effects were not consistent between species or years, it is likely that behavioural changes in response to feeding may be adaptive and, therefore, provide fitness benefits (e.g. Naef-Daenzer and Keller 1999, Ringsby et al. 2009). Furthermore, supplementary feeding significantly increased the proportion of EPY in Blue Tit broods (Chapter Five) which, through genetic benefits (reviewed in Petrie and Kempenaers 1998, Griffith et al. 2002), may have enhanced post-fledging survival. Indeed, supplementary feeding has previously been shown to increase post-fledging 'apparent' survival of both Blue and Great Tits in my study population (Harrison 2010). These results suggest that supplementary feeding may have substantial fitness benefits for garden birds when considered over the entire reproductive period.

However, supplemented Blue and Great Tits in my study population advanced breeding but also experienced declines in breeding outputs (Harrison et al. 2010). Blue and Great Tits breeding in urban habitats also experience advances in breeding phenology but reduced breeding output (Chapter Seven) suggesting that breeding phenology and performance of birds in urban areas might be influenced, at least in part, by supplementary feeding. However, many ecological factors, other than food availability, may influence avian breeding performance and, therefore, care should be taken when drawing conclusions from the NRS analyses, especially since estimation of broad-scale availability of food supplements remains so intractable (as discussed in Chapter Seven).

In my field study I also found that supplementary feeding of Blue Tits with both peanut cake + mealworms resulted in broods being male-biased (Chapter Six). In comparison, brood sex ratio of Blue Tits breeding in either the control or peanut cake dietary treatment block were not biased towards either sex (i.e. the sex ratio was

approx. 50:50) (Chapter Six). While the relationship between operational sex ratio

(Emlen 1976) and brood sex ratio is not consistent between species (reviewed in

Donald 2007), it is possible that supplementary feeding might indirectly elevate the

proportion of breeding males in a population profoundly influencing population

dynamics (reviewed in Donald 2007). For example, as the proportion of breeding

males in a population increases, intra-sexual competition for access to breeding

females intensifies (e.g. Emlen and Oring 1977, Grant and Foam 2002), resulting in

some species exhibiting a greater degree of polygyny (Emlen and Oring 1977) with

accompanying reductions in breeding success (e.g. Réale et al. 1996, Marchesan

2002). Furthermore, a highly male-biased breeding population (e.g. where males

outnumber females approx. 2:1 – Steifetten and Dale 2006) may contain a high

proportion of unpaired males (e.g. approx. 50% – Steifetten and Dale 2006), thereby

reducing the potential for population growth and, indeed, potentially resulting in

population decline (e.g. Steifetten and Dale 2006, Grüebler et al. 2008).

8.3.2. Future considerations in garden bird feeding

Combining my results from the Chaddesely Woods population of birds with those of

Harrison (2010), it appears that advocating garden bird feeding during the spring and

summer may not be appropriate, especially when considering bird populations that are

endangered. For example, the effects of reduced breeding performance are likely to be

more severe for species such as Seychelles Black Paradise-flycatchers (*Terpsiphone*

corvine) that exist in low numbers (approx. 150-200 individuals – Currie et al. 2003).

Furthermore, the potential indirect effects of supplementary feeding on operational

sex ratio may have more profound consequences for populations which are small

and/or isolated such as the last remaining population of Ortolan Buntings (*Emberiza*

hortulana) in Norway that consists of less than 160 breeding pairs (Steifetten and

Dale 2006), partly because the potential for female immigration to small isolated

populations is so limited (Steifetten and Dale 2006). Therefore, extreme caution

should be applied when planning to employ supplementary feeding for the

conservation of garden bird species (e.g. House Sparrows – Robinson et al. 2005) and

of critically endangered species (e.g. Kakapo [*Strigops habroptilus*] – Clout et al.

2002; Spanish Imperial Eagle [*Aquila adalberti*] – Gonzalez et al. 2006).

I urge caution in applying my results to supplementary feeding of birds

generally. My two focal species did not always respond to supplementary feeding in a

consistent manner. For example, supplementary feeding resulted in a truncation of

nest construction period of Blue, but not Great, Tits (Chapter Two) whilst

supplementary feeding decreased brood provisioning rates more markedly in Great

Tits compared with Blue Tits in 2008 (Chapter Four). To gain further insight, it is

essential that future research considers a wider range of species which utilise garden

bird feeders, particularly those which have different feeding ecologies from tits (e.g.

European Greenfinch, Common Chaffinch). In addition, as exemplified by an

apparent diminution of the effects of food supplementation on breeding outputs of

Great Tits to the present day (Chapter Seven), my findings suggest that protracted

studies are imperative.

Perhaps more fundamentally, it is unclear whether the results from my field

study have direct applicability within an urban garden bird feeding context since the

effects of supplementary feeding may partly depend on the underlying ecology of the

habitat in which supplementary food is provided. For example, birds breeding in

urban habitats may experience increased predation risk (e.g. Jokimäki and Huhta

2000, Thorington and Bowman 2003), warmer air temperatures (e.g. Haggard 1990)

and lower natural food availability (e.g. Marciniak et al. 2007). Such fundamental ecological differences are likely to result in birds of different habitat types differentially adjusting time and energy budgets in response to supplementary feeding since both air temperature and natural food availability influence the energetic costs of foraging (e.g. Tinbergen and Dietz 1994, Stevenson and Bryant 2000, Thomas et al. 2001), and predation risk influences time budgets as a result of vigilance behaviour (e.g. de Laet 1985, Hegner 1985). Therefore, it seems plausible that the effects of supplementary feeding on both reproductive behaviours and performance may vary considerably between habitats.

It is fundamental that future supplementary feeding studies consider conducting supplementary feeding experiments in urban habitats (e.g. town and city gardens – Jones and Reynolds 2008). However, this presents many logistical problems and, therefore, elucidating the effects of supplementary feeding in urban habitats may remain challenging (as discussed in Chapter One).

8.4. FUTURE RESEARCH AT CHADDESLEY WOODS NATIONAL NATURE RESERVE

In Section 8.3.2. I stressed the need for both long running and urban-based supplementary feeding studies. However, conducting small-scale scientific supplementary feeding studies are vital too since they enable the researcher to manipulate supplementary food directly and, therefore, to answer specific questions under tightly-controlled conditions. Therefore, here I will make recommendations for future research to be conducted at my field site, Chaddesley Woods National Nature Reserve, where the effects of supplementary feeding during spring and summer on reproduction have been investigated extensively for the last 5 years.

8.4.1. Mechanism(s) underlying the effects of supplementary feeding on reproductive behaviour

Future research should focus on the potential energetic advantages to the consumer of supplementary feeding during breeding attempts (e.g. Tinbergen and Dietz 1994). If supplementary feeding reduces the energetic cost of foraging, I would expect the energetic expenditure on breeding attempts to be lower in supplemented compared with control Blue and Great Tits. The doubly-labelled water (DLW) technique (reviewed in Speakman 1997) could be used in such an investigation but simpler, less invasive techniques such as individually marking tits with colour rings before the breeding season could be used to monitor feeder visitation rates by focal birds.

Further research into more subtle behavioural effects of supplementary feeding may prove fruitful: supplemented female Blue Tits may have spent more time collecting nesting material resulting in a truncation of nest construction period (Chapter Two); supplemented male Blue Tits may have spent more time defending territories, and less time mate-guarding, resulting in increased EPP rates (Chapter Five); and supplemented males and females may have differentially invested into brood provisioning influencing overall combined brood provisioning rates (Chapter Four). A range of remote sensing techniques could be employed such as PIT tag technology that allows the automatic identification of individual birds as they enter a nestbox (e.g. Freitag et al. 2001), and radio-tracking (e.g. Naef-Daenzer et al. 2001) that allows the movements of birds away from the nest to be monitored.

8.4.2. Downstream effects of supplementary feeding

Supplementary feeding is likely to have important consequences for fledgling survival and fitness (e.g. Naef-Daenzer and Keller 1999, Ringsby et al. 2009), and for

dispersal (e.g. Greenwood and Harvey 1982, Lambin et al. 2001). For example, one key factor that influences the latter is intra-sexual competition for resources (reviewed in Lambin et al. 2001). As it intensifies, the propensity to disperse increases (Arcese 1989). Since supplementary feeding increased the proportion of male fledglings (Chapter Six), intra-sexual post-fledging competition in supplemented treatment blocks may intensify compared with that in the control block, resulting in increased male dispersal. However, supplementary feeding may partially alleviate competition (both inter- and intra-sexual) by increasing the availability of food (e.g. Arcese 1989, Kennedy and Ward 2003). Therefore, the effects of supplementary feeding on dispersal remain unclear; dispersal plays a major role in population dynamics (reviewed in Hanski 2001) and, therefore, understanding the effects of supplementary feeding on dispersal is a research priority. Many approaches might be available. A mark-recapture technique could be employed where nestlings and adults are ringed between nestling days 10 and 14 and re-sighted or recaptured by using mist-nets at multiple sites at set distance intervals (e.g. at 500 m distance bands) away from the study site. However, mark-recapture methods are restrictive because they are time-consuming and labour-intensive. Radio-tracking could be employed by attaching radio tags to nestlings as they near fledging (but no later than day 15 to avoid force-fledging) (e.g. Naef-Daenzer et al. 2001). However, this method is problematic due to short battery life (e.g. <5 weeks – Biotrack Ltd. 2010) and limited detection distance (e.g. 500 m from the ground – Bulyuk et al. 2010). An alternative method to quantify dispersal would be to examine the genetic structure of a population because dispersal results in gene flow, the movement and integration of genes from one population to another (Rousset 2001). It would be straightforward to employ the tissue sampling

methods outlined in Chapter Five and to develop a large set of genetic markers to enable detailed characterisation of our focal populations.

8.4.3. Effects of supplementary feeding at a population level

Another prime area for research is how effects at the individual level translate into those at the level of the population. For example, breeding performance of individuals may be reduced by supplementary feeding (Chapter Seven, Harrison et al. 2010) but breeding 'performance' and dynamics of the population remain relatively poorly understood. Studying population size in this regard may be crucial, especially in an urban garden bird feeding context, since the size of bird assemblages at feeders may influence the rate of disease transmission and the risk of predation (Brittingham and Temple 1986, Robb et al. 2008a). To estimate population size at our study site, a survey of breeding attempts in both nestboxes (e.g. clutch size, fledgling success, double brooding) and natural cavities would be essential but problematic.

8.5. CONCLUSIONS

I have provided empirical evidence that supplementary feeding during the spring and summer has marked effects on reproductive behaviours throughout the breeding season of both Blue and Great Tits. Furthermore, my results suggest that supplementary feeding may reduce breeding performance and may influence the genetic structure of avian populations which, for small isolated populations, may lead to population decline. Therefore, I suggest that the recommendation by organisations such as the RSPB (RSPB 2010) and BTO (BTO 2010) of providing food to birds during the spring and summer may be premature.

However, caution should be taken when directly applying the results of my field study to an urban garden bird feeding context since the effects of supplementary feeding may partly depend on the underlying ecology of the habitat in which the supplementary food is provided. Moreover, the results of my NRS study revealed that the effects of supplementary feeding, at least in urban habitats, may be diminishing in recent decades (Chapter Seven). Therefore, a definitive conclusion about whether garden bird feeding should be advocated during the spring and summer cannot currently be drawn.

Perhaps most importantly in this regard, future research should concentrate on investigating the effects of supplementary feeding on reproduction in urban habitats using an avian community-based approach since effects may be species-specific (Chapter Two, Four and Seven). With the global human population predicted to increase from just less than seven billion today to over nine billion by 2050 and, consequently, with an increase in the world's population living in urban areas from approx. 50% today to 70% over the same time period (United Nations 2008), the influence of supplementary feeding on avian breeding performance is sure to intensify (Fuller et al. In Press). Therefore, studies such as mine will become ever important in advancing our knowledge of many aspects of avian breeding biology.

Appendix One

MEASURING CATERPILLAR AVAILABILITY

Both Blue and Great Tits time their breeding to coincide with the peak availability of caterpillars, specifically those of Winter Moths (*Operophtera brumata*) in addition to other moths (*Geometridae* spp.) (Gosler 1993, van Noordwijk et al. 1995). Winter Moths tend to concentrate on oak (*Quercus* spp.) to lay their eggs (Wint 1983). As such, Blue and Great Tits preferentially forage on oaks (Naef-Daenzer and Keller 1999). Once the caterpillar eggs have hatched the caterpillars feed on shoots and leaves until they are fully grown and then descend from the canopy to pupate in the forest floor, usually in late May (Carter and Hargreaves 1986).

In 2007, to investigate the abundance and timing of caterpillar emergence 10 evenly spaced oak trees were chosen in each dietary treatment block. At each focal tree two pairs of plastic cat litter trays (combined surface area of approx.. 4800 cm^2) were positioned on the ground under the canopy halfway between the tree trunk and the edge of the tree's canopy (to control for differences in canopy size between focal trees) and filled with water (*sensu* Perrins 1991) approx. 2 weeks before the first clutch of eggs was expected to hatch. Caterpillars descend from the forest canopy, fall into the traps, drown and are then be collected for further analyses. The trays were covered with wire mesh (approx. 25 mm diameter holes) to prevent either the caterpillars or water being disturbed/consumed and secured to the ground using metal tent pegs. To control for potential effects of aspect on caterpillar hatching and abundance (Buse et al. 1999), where possible, one pair of trays was positioned to the north, and one to the south of the tree trunk. On hatching, trays were visited every 2-3 days. The contents of the trays were sieved and the caterpillar biomass was transferred

into freezer bags and frozen in a domestic freezer pending laboratory analysis. In

2008 and 2009 the protocol was repeated using the same focal trees as in 2008.

However, in 2008 and 2009 I used half the number of trays that were used in 2007 to

reduce the labour intensity of the method.

In the laboratory Winter Moth and other geometrid caterpillars from defrosted

samples were separated from other debris (e.g. leaf litter) in each sample. Samples

were then dried to constant weight in an oven (Electrolux, Sweden) at 60°C for

approx. 3 days. Biomass $^{-day}$ was calculated for the period between caterpillar

collections.

Appendix Two

DEVELOPMENT OF PRIMER SETS

A brief introduction to microsatellite loci and primers

Microsatellites are nucleotide sequences in DNA that consist of tandem repeats of short nucleotide units (i.e. repeat motifs) which are flanked at each end with sequences of nucleotides known as flanking regions (Alberts et al. 1983). Microsatellite loci (referring to the specific location of the microsatellite on the chromosome) are common in DNA across most taxa (Ellegren 2004 – but see Primmer et al. 1997) and are often polymorphic (i.e. have multiple alleles – e.g. Ellegren 1992, Lagercrantz et al. 1993). Therefore, microsatellites are commonly used for paternity analysis (reviewed in Queller et al. 1993, Selkoe and Toonen 2006).

Knowledge of the sequence of microsatellite flanking regions that are generally conserved (i.e. identical between individuals – Selkoe and Toonen 2006), allows us to design and synthesise fluorescently labelled genetic markers (hereafter referred to as primers) (Ziegle et al. 1992). Primers are designed to bind to the flanking region and guide the amplification (i.e. the copying) of the microsatellite region during the polymerase chain reaction (PCR), a technique used to amplify target sections of DNA (i.e. microsatellites) sufficiently to enable genetic analysis (Alberts et al. 1983). At the end of the PCR the amplified microsatellite regions (i.e. the PCR products) remain labelled with the primer and, therefore, can be visualised by capillary electrophoresis using a DNA sequencer (e.g. Toh et al. 1996, Oda et al. 1997).

Testing microsatellite loci suitability

I tested the suitability of 36 primer sets that had been designed for 36 unique microsatellite loci isolated from a microsatellite-enriched Blue Tit genomic library (G. Horsburgh pers. comm.) and four designed for cross-species utility (POCC1 – Bensch et al. 1997; Pca3, Pca7 and Pca9 – Dawson et al. 2000). They were initially tested for suitability using DNA from blood taken from four adult Blue Tits, which I assumed were unrelated, captured in Chaddesley Woods National Nature Reserve in 2009 (see Section 5.3.2).

Genomic DNA was extracted from blood samples using the Ammonium acetate method (Nicholls et al. 2000). Extracted DNA was quantified by measuring the optimal density of samples at 250 nm using a Fluostar Optima fluorimeter (BMG Labtech, Offenburg, Germany). The microsatellite loci were then amplified using PCR. Each 2-µl PCR contained approximately 15 ng of genomic DNA, 0.5 µl of each primer (0.2 µM) and 1.0 µl of QIAGEN Multiplex PCR Master Mix (Kenta et al. 2008). PCR amplification was performed using a DNA Engine Tetrad PTC-225 Peltier thermal cycler (MJ Research, Bio-Rad, Hemel Hempstead, UK). A touchdown PCR was used (TD65-55) with the following profile: 95ºC for 15 min, then 10 cycles of 94ºC for 30 sec, 65ºC for 90 sec (dropping 1.0ºC per cycle), 72ºC for 1 min, followed by 25 cycles of 94ºC for 30 sec, 55ºC for 90 sec, and 72ºC for 1 min. PCR products were diluted using high grade water (1 in 400 dilution). One µl of each diluted sample was then mixed with 9.5 µl of formamide/size standard mix (made up of 1 ml of formamide and 3 µl of ABI ROX 500 internal size standard [Applied Biosystems, California, USA]). Samples were then denatured for 5 min at 95ºC and quenched on ice prior to being separated on an ABI 3730 DNA Analyser (Applied Biosystems, California, USA).

Genotypes were scored relative to the 500 ROX internal size standard (Applied

Biosystems, California, USA).

Of the 40 Blue Tit loci tested, 28 were polymorphic (i.e. they had many alleles),

four were monomorphic (i.e. only had one allele), four failed to amplify during PCR

and four were difficult to score either due to stuttery or weak profiles. The 28

polymorphic microsatellite loci were then tested further in 28 adult Blue Tits that I

assumed were unrelated from the same population in Chaddesley Woods National

Nature Reserve using the same laboratory protocol as before. At the same time the 28

unrelated Blue Tits were also sexed using the sexing primers P2/P8 (see Section 6.6.3 –

Griffith et al. 1998) and Z-002 (Dawson 2007) to identify whether any of the

microsatellite loci were sex-linked (i.e. located on the Z sex chromosome).

A brief introduction to sex-linked loci

In birds males are homogametic and possess two copies of the Z sex chromosome.

Females are heterogametic and possess one copy of the Z and one copy of the W sex

chromosome. A sex-linked locus is one linked to the Z sex chromosome and can be

identified when heterozygous profiles for that locus (i.e. when two alleles are present)

only exist in males. The male is, therefore, able to have two copies of a Z-linked locus

(i.e. one from the mother and one from the father). Conversely, a female cannot be

heterozygous for a sex-linked locus since she only possesses one copy of the Z sex

chromosome. Sex-linked loci can be problematic in avian parentage studies since

female offspring (ZW) will not have copies of Z-linked loci from the mother (i.e. the

origin of the W chromosome if maternal; the Z chromosome paternal) and, therefore, in

female offspring the mother cannot be determined from Z-linked loci. However, sex-

linked loci are useful in sex determination since males can be identified from

heterozygous genotypes (see Ellegren 2000 for review of avian sex determination).

Characterisation of microsatellite loci and genotyping errors

Genotyping errors (reviewed in Hoffman and Amos 2005) can seriously disrupt

estimates of parentage through false assignment of paternity (reviewed in Marshall et al.

1998). For example, genotyping errors may arise when one allele fails to amplify

resulting in heterozygotes appearing as homozygotes through a process known as allelic

dropout (Gagneux et al. 1997) or when genotypes are scored incorrectly by the analyst

(reviewed in Hoffmann and Amos 2005). Another way in which errors can arise is when

microsatellite null alleles occur. A microsatellite null allele is any allele at a

microsatellite locus that consistently fails to amplify during PCR (Dakin and Avise

2004). It arises when the nucleotide sequence of the microsatellite flanking region does

not complement the primer sequence because, for example, mutations occur within the

flanking region that result in poor primer annealing and failure of allele amplification

during PCR (reviewed in Dakin and Avise 2004). False paternity assignment occurs

when the paternal allele does not amplify during PCR and an 'allelic mismatch' occurs

between the genotypes of the offspring and father (Fig. 2.1.A).

Genotyping errors can be detected by testing for significant deviations from

Hardy-Weinberg equilibrium where allelic frequencies in a population are in the

proportion:

$$P^2 + 2pq + q^2 = 1$$

where P^2 is the frequency of the genotype 'AA', $2pq$ is the frequency of the genotype 'Aa' and q^2 is the frequency of the genotype 'aa'. It describes the expected genotype frequencies in a population when gametes fuse randomly, there is random mating and mutation, and migration and natural selection do not occur.
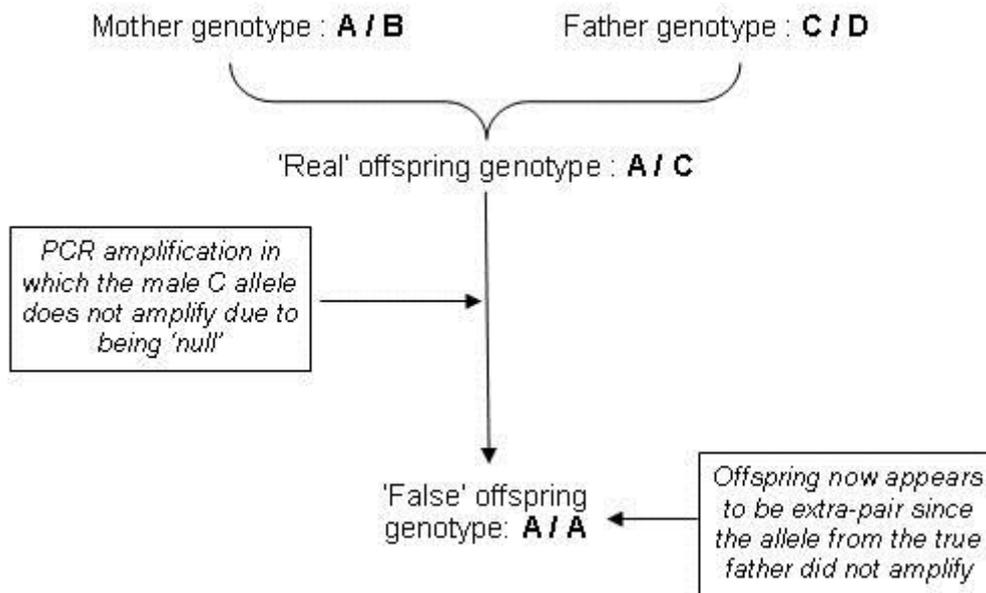
Figure 2.1.A. False paternity assignment resulting from the presence of a null allele. The 'real' offspring genotype (A/C) consists of one allele from the mother (A) and one from the father (C). However, the offspring genotype determined through genetic analysis is 'false' (A/A) as a result of 'C' being null.

Significant deviations from Hardy-Weinberg equilibrium (when $P < 0.05$) indicate either an excess of homozygote or heterozygote genotypes in a population which may be indicative of high genotyping error rates (see Russell 1996 for overview of the Hardy-Weinberg equilibrium).

Linkage disequilibrium (the association between alleles at different loci) (Amos et al. 1992) can strongly increase the likelihood of close relatives sharing particular combinations of alleles. Linkage disequilibrium, therefore, reduces the number of

informative loci during paternity analysis and may lead to spurious paternity assignment (Amos et al. 1992).

To determine which loci were likely to be the most powerful for paternity assignment, analytical tests for departures from Hardy-Weinberg equilibrium and linkage equilibrium were conducted using a Markov-chain method implemented in GENEPOP v.4.0 (Rousset 2008). In addition, the observed and expected heterozygosities (i.e. measurements of genetic diversity – Weir 1990) and estimated null allele frequencies were calculated using CERVUS v3.0 (Marshall et al. 1998) using the genotypes from all unrelated individuals.

The repeat testing of the 28 polymorphic loci highlighted that seven loci were significantly out of Hardy-Weinberg equilibrium ($P < 0.05$; Table 2.1.A) and six loci possessed high estimated null allele frequencies (> 0.1 [10%] estimated as being null; Table 2.1.A). Following a Bonferroni correction for multiple comparisons (Rice 1989), tests for linkage disequilibrium between pairs of loci revealed that no pairs of loci were in linkage disequilibrium. However, linkage disequilibrium between pairs of loci may remain undetected since few individuals were tested.

Testing sex-linked microsatellite loci suitability

Five loci previously known to be sexed-linked (BT22H03, BT23C07 [unpubl.], Z040, Z037 and Z037B – Dawson 2007) (Table 2.1.A) were also tested for their suitability by using 10 unrelated male Blue Tits from Chaddesley Woods National Nature Reserve using the same molecular protocol as used to test the aforementioned primer sets. Of the five loci tested, BT22H03 did not amplify and BT23C07 was hard to score. For the other three loci, observed and expected heterozygosities and estimated null allele frequencies were calculated using CERVUS v3.0 (Marshall et al. 1998) using the

genotypes from the 10 males. Tests for departures from Hardy-Weinberg and linkage

equilibrium were conducted as above. All three loci possessed high estimated null allele

frequencies (>0.1 [10%] estimated as being null; Table 2.1.A) while one was

significantly out of Hardy-Weinberg equilibrium ($P < 0.05$; Table 2.1.A). While no

pairs of loci were found to be in linkage disequilibrium after Bonferroni correction (see

above), linkage disequilibrium may have remained undetected since few individuals

were tested.

Table 2.1.A. Details of polymorphic microsatellite loci tested during the development of primer sets used in the paternity analysis of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009.

Note: N is the number of birds tested ([*] indicates were a locus either did not amplify, was monomorphic or was hard to score); H_O is the observed heterozygosity; H_E is the expected heterozygosity; HWE is the Hardy-Weinberg Equilibrium from which the P value is generated (values in bold indicate a departure from Hardy-Weinberg equilibrium where $P < 0.05$); NE-1P is the non-exclusion probability (first parent); F(null) is the expected null allele frequency as a proportion (values in bold indicate where proportion of null allele frequencies for the loci are > 0.1; ND = not determined); EMBL accession number is the unique identifier given to each primer once submitted to the EMBL nucleotide sequence database - http://www.ebi.ac.uk/embl/; and Reference is the place of publication.

Locus	Repeat motif	Primer sequence (5' - 3')	N	Number of alleles	Product size range (base pairs)	H_O	H_E	HWE P-value	NE-1P	F(Null)	EMBL accession number	Reference
BT19A04	GTGT	GGGAGGCATTGCAGAAGTC AAAGCTTATTAGGGACTGGGAGAG	27	10	232-259	0.889	0.860	0.097	0.470	0.027	FM878043	*Unpubl*
BT19B12	ACAC	TTTATGGCGTTGCTGCTTTC AACAGCAAATTGTCAGCGTAATAAG	26	10	207-226	0.808	0.857	0.069	0.478	0.023	FM878045	*Unpubl*
BT19F01	GATA	GCAGGAGGCCTCAATTCTCTTC GCCTCAGTCTTATTCACACACTCTCC	27	14	283-335	0.889	0.849	0.356	0.492	-0.043	FM878046	*Unpubl*
BT19G06	ACAC	CCTTTAATATACAACCCATGTCCAAC GCAGAGTTACTCCATGGGAATG	27	13	176-199	0.815	0.911	0.765	0.354	0.045	FM878047	*Unpubl*
BT19G08	GGTT	CGTGAACAAATTCAGAGGGTGAGAC CAGCTGCATAGAGGTTGTGTCTGG	26	5	280-296	0.615	0.735	0.039	0.694	0.069	FM878048	*Unpubl*

Table 2.1.A continued

BT19H02	CTT	TTCATCTAAATCGTCTTCTTCTTT AGGAATTACAGTGAGCATAACAA	4*								FM878050	*Unpubl*
BT19H11	GATA	TCCCATTGTCAGAATTAGGTTT TTTGTGTGTGGGTCAAACAG	27	9	210-250	0.889	0.894	0.473	0.401	-0.009	FM878051	*Unpubl*
BT20C03	GTGT	TCACTGGAGTCCTTAATGCTGGTG GAATAAGGGACCCAGAAACATAATGAATAAC	25	8	216-236	0.84	0.818	0.454	0.564	-0.021	FM878052	*Unpubl*
BT20C06	TAGA	CCTAATGTTGTGGTGCTTTCTCTC GATTTGCTTGCTTGTTATCCTGAG	26	23	229-355	0.808	0.947	**0.043**	0.248	0.071	FM878053	*Unpubl*
BT20E11	ACAC	AACAGCATGGAAATAGCTGGGTTTAAG CGCAATGTTATTTGAACATGCTTCTG	27	10	223-239	0.741	0.718	0.797	0.683	-0.037	FM878054	*Unpubl*
BT20F11	GT	TGGTCCCTTCCAACCTGACC TGGCTGGAAATGTGCTCTGG	27	7	242-269	0.741	0.804	0.429	0.585	0.033	FM878055	*Unpubl*
BT21A10	AAGA	TGGTGGAACAACAAGTGACCTACC CTGCTGGTAAACACAAGGCACTG	28	6	190-205	0.571	0.612	**0.030**	0.807	0.016	FM878056	*Unpubl*
BT21C03	ATAG	GAGGCTTAGAGGCAGGGCTTT AAACCAGTAATTCCCTTGATTGAAAGA	4*								FM878057	*Unpubl*
BT21C07	GTGT	TGGTGCTATCTTCCCTTACCC GCACCACTCACCAGCAAAG	22	5	194-202	0.773	0.735	0.412	0.696	-0.044	FM878058	*Unpubl*

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Table 2.1.A continued

BT21G02	TCTA	TAAGGTCCAGCTCACCAACC TGGTACTAGCCCAGCATCAAC	4*								FM878060	*Unpubl*
BT21G04	CA	TCAACCTTTTGGAACTTGACCT CCTCATTGGGGCATGTGTA	27	7	179-191	0.741	0.697	0.270	0.715	-0.058	FM878061	*Unpubl*
BT21H05	GAGT	CCAGGGACTCTGGTCCTATG TGTGTCTCAACACCCTCACTG	4*								FM878062	*Unpubl*
BT21H09	TC	TTCCTTTAGCAACCTTGATGC AGATGGGTTTCATGGTCCTG	4*								FM878063	*Unpubl*
BT21H10	TT	TGGTGAGTAGGTTCCAGAAGAGCA TCTGCTCTATATCCTTTTCCAGTATGAATTTT	23	8	210-219	0.391	0.700	**0.000**	0.720	**0.307**	FM878064	*Unpubl*
BT22B10	AC	GTTGCAGAATTCTTGTCATGG TGTCTCACACAGGCACAAAG	27	3	174-178	0.296	0.520	**0.004**	0.870	**0.255**	FM878067	*Unpubl*
BT22C08	CA	TGGGTATAACAAACTCAACCCAGTACC AAGCATCCTTGCACCTTCAGC	4*								FM878069	*Unpubl*
BT22D06	TATC	AAGTATGCAGTTGCCAAGTGTGC GCATCCCTGTAGCCATGCTG	24	16	152-237	0.833	0.918	0.289	0.332	0.041	FM878073	*Unpubl*
BT22F05	AG	TGCATACATTCTATGTGTTATATGTTC TGACTTGCACAACTTAACTGC	4*								FM878079	*Unpubl*

Table 2.1.A continued

BT22G07	CACA	AGCCCTCAGGGAGTATCTGG GCAGAAGAGGACTCGCAGAC	4*									FM878080	*Unpubl*
BT22G10	GGTT	ACACTTCGGTGTTCTGTGACCTG CCCTTGCATCTGCCAAGAAAC	27	7	209-238	0.889	0.844	0.338	0.516	-0.033	FM878081	*Unpubl*
BT22H03	TC	CGTCTCGTCCCTTGGTAGC TTAGTGCCAACAAGGGTTATGC	4*								FM878082	*Unpubl*
BT22H09	GT	TGAGGCATAGGAACTATATTCCAGTTGTAG ACCAGGGAACCATTGGCATC	25	4	148-155	0.480	0.520	0.695	0.866	0.021	FM878083	*Unpubl*
BT23A03	AC	TTTCCCCAGTTATGGTTCCATTTT GCAGCACATTAGGACTATGAATCCAC	25	4	164-171	0.360	0.559	**0.004**	0.841	**0.212**	FM878084	*Unpubl*
BT23A11	TCCTC	TGGAAATATATGCATACAGATACATGG CACCTCCAGCTTACAATGCTC	4*								FM878085	*Unpubl*
BT23C07	AGAAG	TCTTTATGAGGTTGCAATGACAG AGAGGTCAGAGAGTTGGACTAGATG	4*								FM878086	*Unpubl*
BT23D06	GTGT	TGTCTGGTGTAGTGAAGAGACAATCAG TGGAGAAGGTCTGGCATTGTG	4*								FM878087	*Unpubl*
BT23D08	TG	TCTTCTTCCAATAGTCAAAGTCATCCTG GAATTCCTCGACTGGAACAACTGAC	4*								FM878088	*Unpubl*

Table 2.1.A continued

BT23D11	GTGT	CTTCAGCACAGGAATAGCTTCAG CATGAGCATGCCTTTATGTCC	28	11	154-172	0.750	0.741	0.467	0.641	-0.021	FM878089	*Unpubl*
BT23E01	CA	TGCTTAGCTAATATGGGCCAACTG TCAAGGGACATTTAGTGGTGTTCTG	4*								FM878090	*Unpubl*
BT23E05	GT	CTCTGTCTGGGCACTGAGG GACCCAATGTCTCTTTCTACAGC	26	13	236-272	0.731	0.758	0.736	0.634	0.009	FM878091	*Unpubl*
BT23F02	GTGT	AAGTGTTGGAGGAACATCGAAGG GAAGCAGGGCACTGGACTTTC	26	13	229-258	0.962	0.916	0.251	0.344	-0.036	FM878092	*Unpubl*
BT23F09	ATGG	GGCTCTGGTTACCTGCAGAGG TCTCAAGTTCACACTGCTTTCCTG	27	20	287-347	0.778	0.943	**0.011**	0.262	0.087	FM878093	*Unpubl*
BT23H11	GA	TCCAGAGCAACACTACTGACAAACATTAG TTGCTAAGTTGATTGTTTGCACAGC	25	3	185-196	0.520	0.523	1.000	0.869	-0.011	FM878096	*Unpubl*
Pca3	(GT)6CT(GT) 3CT(GT)5CT (GT)3CT(GT)13	GGTGTTTGTGAGCCGGGG TGTTACAACCAAAGCGGTCATTTG	9	12	162-206	0.889	0.935	0.644	0.378	ND	AJ279805	Dawson et al. 2000
Pca7	(TG)24	TGAGCATCGTAGCCCAGCAG GGTTCAGGACACCTGCACAATG	9	9	124-144	0.778	0.882	0.636	0.493	ND	AJ279809	Dawson et al. 2000
Pca9	(GT)21	ACCCACTGTCCAGAGCAGGG AGGACTGCAGCAGTTTGTGGG	10	7	120-142	0.900	0.842	0.934	0.558	-0.058	AJ279811	Dawson et al. 2000

Table 2.1.A continued

POCC1	GTGT	TTCTGTGCTGCAATCACACA GCTTCCAGCACCACTTCAAT	10	10	221-250	1.000	0.874	1.000	0.496	**-0.102**	U59113	Bensch et al. 1997
Z037	ACAC		7	2	164-166	0.286	0.440	0.443	0.917	ND		Dawson 2007
Z037B	GATA		13	3	97-103	0.462	0.385	1.000	0.932	**-0.124**		Dawson 2007
Z040	ACAC		10	5	131-139	0.400	0.695	**0.034**	0.756	**0.264**		Dawson 2007

Assembling multiplex primer sets

After examination of the loci characteristics (Table 2.1.A), I selected the loci which were most likely to be powerful in paternity analysis (i.e. those which were polymorphic, in Hardy-Weinberg and in linkage equilibria and did not possess high frequencies of null allele frequencies). To minimise expense whilst maximising the number of loci employed, I designed four multiplex primer sets using the selected loci. A multiplex primer set is a set of primers which can be used together in one PCR. Since some pairs of primers react if put together in a PCR, Autodimer (Vallone and Butler 2004) was used to screen sets of primer pairs initially for cross-reactivity. Multiplex Manager Version 1.0 (Holleley and Geerts 2009) was then used to design efficient combinations of primers for four multiplex primer sets. Since PCR products from different loci can overlap in size making it challenging to analyse genoptypes visually (Fig. 2.2.A.a.), Multiplex Manager Version 1.0 was programmed to design primer sets where the expected PCR product sizes from each loci in the set were at least 30 base pairs apart. However, this heavily restricted the number of loci I could use and, therefore, to allow me to maximise the number of primers in each multiplex, I included an untested locus, Pca4 (Dawson et al. 2000), and I used a set of three different fluorescent labels (HEX [green], 6-FAM [blue] and NED [yellow]) so that PCR products from different loci could easily be separated visually even if the products were similar in size (compare Fig. 2.2.A.a with Fig 2.2.A.b). The final multiplex primer sets contained a combined total of 16 loci (14 autosomal [i.e. not sex-linked] and two sex-linked ones; Table 2.2.A).
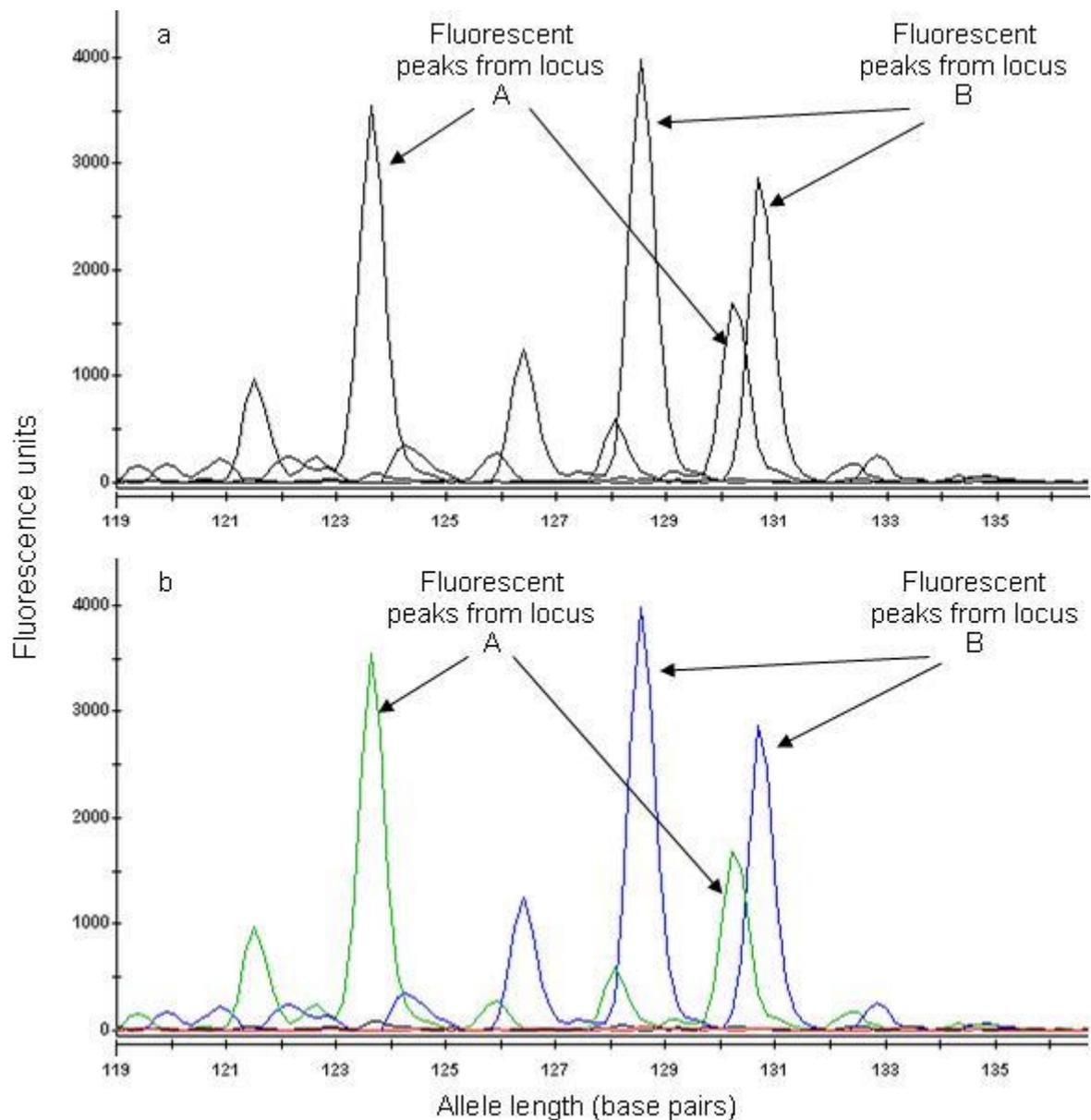
Figure 2.2.A. Two electropherograms with fluorescent peaks corresponding to PCR products from two microsatellite loci which overlap in size where (**a**) the primers for both loci are labelled using the same dye and (**b**) the primers for each loci are labelled with different dyes. The use of multiple dyes enables PCR products from different loci to be visually separated with ease.

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Table 2.2.A. Details of the four multiplex primer sets used in the paternity analysis and sexing of Blue Tits breeding in Chaddesley Woods National Nature Reserve in 2008 and 2009. The locus name refers both to the locus and to the name of the primers which are used to guide its amplification during PCR. PCR product sizes refer to the size of the DNA fragments from each locus after PCR. Primers within each multiplex are labelled with a fluorescent dye: HEX – green; FAM – blue; or NED – yellow.

Multiplex	Locus	PCR product size range (base pairs)	Fluorescent label trade name	Fluorescent label colour
1	Pca4	161-211	HEX	Green
	Pca3	162-206	FAM	Blue
	BT19F01	283-335	FAM	Blue
2	Pca9	120-142	FAM	Blue
	Pca7	124-144	HEX	Green
	BT21C07	194-202	FAM	Blue
	BT22D06	152-237	NED	Yellow
	POCC1	221-250	HEX	Green
3	BT19G06	176-199	FAM	Blue
	BT20C03	216-236	HEX	Green
	BT22G10	209-238	NED	Yellow
	BT23E05	236-272	FAM	Blue
4	Z037B	97-103	HEX	Green
	Z040	131-139	FAM	Blue
	BT19H11	210-250	FAM	Blue
	BT23F02	229-258	NED	Yellow

PUBLICATIONS

Cooper, C. B., and **J. A. Smith**. 2010. Gender patterns in bird-related recreation in the USA and UK. Ecology and Society 15:4-16.

Halsey, L.G., S. J. Portugal, **J. A. Smith**, C. P. Murn, and R. P. Wilson. 2009. Recording raptor behavior on the wing via accelerometry. Journal of Field Ornithology 80: 171-177.

Harrison, T. J. E., **J. A. Smith**, G. R. Martin, D. E. Chamberlain, S. Bearhop, G. Robb, and S. J. Reynolds. 2010. Does food supplementation really enhance productivity in breeding birds? Oecologia 164:311-320.

Smith, J.A., T. J. E. Harrison, G. R. Martin, and S. J. Reynolds. In revision. Feathering the nest: food supplementation influences nest construction by woodland birds. Auk.

REFERENCES

Alabrudzińska, J., A. Kaliński, R. Słomczynski, J. Wawrzyniak, P. Zieliński, and J. Bańbura. 2003. Effects of nest characteristics on breeding success of Great Tits *Parus major*. Acta Ornithologica 38:151-154.

Alberti, M. 2005. The effects of urban patterns on ecosystem function. International Regional Science Review 28:168-192.

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. 1983. Molecular Biology of the Cell. Garland Publishing, New York, USA.

Alonso-Alvarez, C. 2006. Manipulation of primary sex-ratio: an updated review. Avian and Poultry Biology Reviews 17:1-20.

Álvarez, E., and E. Barba. 2008. Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits *Parus major*. Acta Ornithologica 43:3-9.

Amos, W., J. A. Barrett, and J. M. Pemberton. 1992. DNA Fingerprinting: parentage studies in natural populations and the importance of linkage analysis. Proceedings of the Royal Society of London, Series B 249:157-162.

Ankney, C. D. 1984. Nutrient reserve dynamics of breeding and moulting Brant. Auk 101:361-370.

Ankney, C. D., and C. D. MacInnes. 1978. Nutrient reserves and reproductive performance of female Lesser Snow Geese. Auk 95:459-471.

Antonov, A., and D. Atanasova. 2003. Small-scale differences in the breeding ecology of urban and rural Magpies *Pica pica*. Ornis Fennica 80:21-30.

Ar, A., and Y. Sidis. 2002. Nest microclimate during incubation. Pages 143-160 *in* Avian Incubation: Behaviour, Environment, and Evolution (D. C. Deeming, Ed.). Oxford University Press, Oxford, UK.

Arcese, P. 1989. Intrasexual competition, mating system and natal dispersal in Song Sparrows. Animal Behaviour 38:958-979.

Arcese, P., and J. N. M. Smith. 1988. Effects of population density and supplemental food on reproduction in Song Sparrows. Journal of Animal Ecology 57:119-136.

Ardia, D. R. 2005. Super size me: an experimental test of the factors affecting lipid content and the ability of residual body mass to predict lipid stores in nesting European Starlings. Functional Ecology 19:414-420.

Ardia, D. R., and E. D. Clotfelter. 2006. Individual quality and age affect responses to an energetic constraint in a cavity-nesting bird. Behavioral Ecology 18:259-266.

Arnold, K. E., R. Griffiths, D. J. Stevens, K. J. Orr, A. Adams, and D. C. Houston. 2003. Subtle manipulation of egg sex ratio in birds. Proceedings of the Royal Society of London, Series B 270:S216-S219.

Babiker, E. M., and G. K. Baggott. 1995. The role of ion transport in the formation of sub-embryonic fluid by the embryo of the Japanese Quail. British Poultry Science 36:371-383.

Barba, E., F. Atiénzar, M. Marín, J. S. Monrós and J. A. Gil-Delgado. 2009. Patterns of nestling provisioning by a single-prey loader bird, Great Tit *Parus major*. Bird Study 56:187-197.

Barba, E., J. A. Gil-Delgado, and J. S. Monros. 1995. The costs of being late: consequences of delaying Great Tit *Parus major* first clutches. Journal of Animal Ecology 64:642-651.

Barnett, C. A., and J. V. Briskie. 2007. Energetic state and the performance of dawn chorus in Silvereyes (*Zosterops lateralis*). Behavioral Ecology and Sociobiology 61:579-587.

Belant, J. L., T. W. Seamans, S. W. Gabrey, and S. K. Ickes. 1993. Importance of landfills to nesting Herring Gulls. Condor 95:817-830.

Bennett, P. M., and I. P. F. Owens. 2002. Evolutionary Ecology of Birds: Life Histories, Mating Systems and Extinction. Oxford University Press, Oxford, UK.

Bensch, S., T. Price, and K. Khon. 1997. Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. Molecular Ecology 6:91-92.

Bensch, S., H. Westerdahl, B. Hansson, and D. Hasselquist. 1999. Do females adjust the sex of their offspring in relation to the breeding sex ratio? Journal of Evolutionary Biology 12:1104-1109.

Berg, M. L., N. H. Beintema, J. A. Welbergen, and J. Komdeur. 2005. Singing as a handicap: the effects of food availability and weather on song output in the Australian Reed Warbler *Acrocephalus australis*. Journal of Avian Biology 36:102-109.

Berryman, A. A. 1996. What causes population cycles of forest Lepidoptera? Trends in Ecology and Evolution 11:28-32.

Betts, M. M. 1955. The food of titmice in Oak woodland. Journal of Animal Ecology 24:282-323.

Biotrack Ltd. 2010.

http://www.biotrack.co.uk/

Birkhead, T. R. 1988. Behavioral aspects of sperm competition in birds. Advances in the Study of Behaviour 18:35-72.

Birkhead, T. R. 1998. Sperm competition in birds. Reviews of Reproduction 3:123-129.

Birkhead, T. R., and A. P. Møller. 1992. Sperm Competition in Birds: Evolutionary Causes and Consequences. Academic Press, London, UK.

Bland, R. L., J. Tully, and J. J. D. Greenwood. 2004. Birds breeding in British gardens: an underestimated population? Bird Study 51:97-106.

Bollmann, K., H. –U. Reyer, and P. A. Brodmann. 1997. Territory quality and reproductive success: can Water Pipits *Anthus spinoletta* assess the relationship reliably? Ardea 85:83-98.

Bolton, M. 1991. Determinants of chick survival in the Lesser Black-backed Gull: relative contributions of egg size and parental quality. Journal of Animal Ecology 60:949-960.

Bolton, M., D. Houston, and P. Monaghan. 1992. Nutritional constraints on egg formation in the Lesser Black-backed Gull: an experimental study. Journal of Animal Ecology 61:521-532.

Boone, R. B., and R. S. Mesecar, III. 1989. Telemetric egg for use in egg-turning studies. Journal of Field Ornithology 60:315-322.

Booth, D. T. 1987. Effect of temperature on development of Mallee Fowl *Leipoa ocellata* eggs. Physiological Zoology 60:437-445.

Both, C., and M. E. Visser. 2005. The effect of climate change on the correlation between avian life-history traits. Global Change Biology 11:1606-1613.

Bradbury, R. B., and J. K. Blakey. 1998. Diet, maternal condition, and offspring sex ratio in the Zebra Finch, *Poephila guttata*. Proceedings of the Royal Society of London, Series B 265:895-899.

British Trust for Ornithology. 2010.
http://www.bto.org/gbw/birds_feeding_home.htm

Brittingham, M. C., and S. A. Temple. 1986. A survey of avian mortality at winter feeders. Wildlife Society Bulletin 14:445-450

Broggi, J., and J. C. Senar. 2009. Brighter Great Tit parents build bigger nests. Ibis 151:588-591.

Brommer, J. E., P. Korsten, K. M. Bouwman, M. L. Berg, and J. Komdeur. 2007. Is extrapair mating random? On the probability distribution of extrapair young in avian broods. Behavioral Ecology 18:895-904.

Bryant, D. M., and P. Tatner. 1991. Intraspecies variation in avian energy expenditure: correlates and constraints. Ibis 133:236-245.

Bulyuk, V. N., and A. Mukhin. 2010. Do weather and lunar cycle influence the decision of juvenile Reed Warblers *Acrocephalus scirpaceus* to perform post-fledging nocturnal flights over the natal areas? Acta Ornithologica 45:27-32

Burley, N. 1986. Sex ratio manipulation in color-banded populations of Zebra Finches. Evolution 40:1191-1206.

Burley, N. 1988. The differential-allocation hypothesis: an experimental test. American Naturalist 132:611-628.

Buse, A., S. J. Dury, R. J. W. Woodburn, C. M. Perrins, and J. E. G. Good. 1999. Effects of elevated temperature on multi-species interactions: the case of Pedunculate Oak, Winter Moth and Tits. Functional Ecology 13: S74-S82.

Byers, B. E. 2007. Extrapair paternity in Chestnut-sided Warblers is correlated with consistent vocal performance. Behavioral Ecology 18:130-136.

Carpenter, F. L. 1978. A spectrum of nectar-eater communities. American Zoology 18:809-819.

Carpenter, F. L. 1987. Food abundance and territoriality: to defend or not to defend? American Zoologist 27:387-399.

Carpenter, F. L., and R. E. MacMillen. 1976. Threshold model of feeding territoriality and test with a Hawaiian Honeycreeper. Oecologia 26:213-224.

Carter, D., and B. Hargreaves. 1986. A Field Guide to Caterpillars of Butterflies and Moths in Britain and Europe. William Collins & Sons Ltd., London, UK.

Chalfoun, A. D., and T. E. Martin. 2007. Latitudinal variation in avian incubation attentiveness and a test of the food limitation hypothesis. Animal Behaviour 73:579-585.

Chamberlain, D. E., A. R. Cannon, M. P. Toms, D. I. Leech, B. J. Hatchwell, and K. J. Gaston. 2009. Avian productivity in urban landscapes: a review and meta-analysis. Ibis 151:1-18.

Chamberlain, D. E., and H. Q. P. Crick. 1999. Population declines and reproductive performance of Skylarks *Alauda arvensis* in different regions and habitats in the United Kingdom. Ibis 141:38-51.

Chamberlain, D. E., J. A. Vickery, D. E. Glue, R. A. Robinson, G. J. Conway, R. J. W. Woodburn, and A. R. Cannon. 2005. Annual and seasonal trends in the use of garden feeders by birds in winter. Ibis 147:563-575.

Charmantier, A., and J. Blondel. 2003. A contrast in extra-pair paternity levels on mainland and island populations of Mediterranean Blue Tits. Ethology 109:351-363.

Charmantier, A., J. Blondel, P. Perret, and M. M. Lambrechts. 2004. Do extra-pair paternities provide genetic benefits for female Blue Tits *Parus caeruleus*? Journal of Avian Biology 35:524-532.

Christe, P., A. P. Møller, and F. de Lope. 1998. Immunocompetence and nestling survival in the House Martin: the tasty chick hypothesis. Oikos 83:175-179.

Christians, J. K. 2002. Avian egg size: variation within species and inflexibility within individuals. Biological Reviews of the Cambridge Philosophical Society 77:1-26.

Chuang-Dobbs, H. C., M. S. Webster, and R. T. Holmes. 2001. The effectiveness of mate guarding by male Black-throated Blue Warblers. Behavioral Ecology 12:541-546.

Cichoń, M., J. Sendecka, and L. Gusrafsson. 2005. Male-biased sex ratio among unhatched eggs in Great Tit *Parus major*, Blue Tit *P. caeruleus* and Collard Flycatcher *Ficedula albicollis*. Journal of Avian Biology 36:386-390.

CJ Wildlife Ltd. 2010.
http://www.birdfood.co.uk

Clarke, M. F., D. A. Jones, J. G. Ewen, R. J. Robertson, R. Griffiths, J. Painter, P. T. Boag, and R. Crozier. 2002. Male-biased sex ratios in broods of the cooperatively breeding Bell Miner. Journal of Avian Biology 33:71-76.

Clotfelter, E. D. 1996. Mechanisms of facultative sex-ratio variation in Zebra Finches (*Taeniopygia guttata*). Auk 113:441-449.

Clout, M. N., G. P. Elliott, and B. C. Robertson. 2002. Effects of supplementary feeding on the offspring sex ratio of Kakapo: a dilemma for the conservation of a polygynous parrot. Biological Conservation 107:13-18.

Collias, N. E., and E. C. Collias. 1984. Nest Building and Bird Behavior. Princeton University Press, Princeton, New Jersey, USA.

Conway, C. J., and T. E. Martin. 2000a. Evolution of passerine incubation behavior: influence of food, temperature, and nest predation. Evolution 54:670-685.

Conway, C. J., and T. E. Martin. 2000b. Effects of ambient temperatures on avian incubation behavior. Behavioral Ecology 11:178-188.

Cooch, E., D. Lank, R. Robertson, and F. Cooke. 1997. Effects of parental age and environmental change on offspring sex ratio in a precocial bird. Journal of Animal Ecology 66:189-202.

Coppack, T., and F. Pulido. 2004. Photoperiodic response and the adaptability of avian life cycles to environmental change. Advances in Ecological Research 35:131-150.

Cordero, P. J., J. Vinuela, J. M. Aparicio, and J. P. Veiga. 2001. Seasonal variation in sex ratio and sexual egg dimorphism favouring daughters in first clutches of the Spotless Starling. Journal of Evolutionary Biology 14:829-834.

Cowie, R. J., and S. A. Hinsley. 1987. Breeding success of Blue Tits and Great Tits in suburban gardens. Ardea 75:81-90.

Cowie, R. J., and S. A. Hinsley. 1988a. The provision of food and the use of bird feeders in suburban gardens. Bird Study 35:163-168.

Cowie, R. J., and S. A. Hinsley. 1988b. Feeding ecology of Great Tits (*Parus major*) and Blue Tits (*Parus caeruleus*), breeding in suburban gardens. Journal of Animal Ecology 57:611-626.

Cramp, S. 1985. Handbook of the Birds of the Western Palearctic Vol. 4. Oxford University Press, Oxford, UK.

Cramp, S., and C. M. Perrins. 1993. Handbook of the Birds of the Western Palearctic Vol. 7. Flycatchers to shrikes. Oxford University Press, Oxford, UK.

Crick, H. Q. P. 1992. A bird-habitat coding system for use in Britain and Ireland incorporating aspects of land-management and human activity. Bird Study 39:1-12.

Crick, H. Q. P., S. R. Baillie, and D. I. Leech. 2003. The UK Nest Record Scheme: its value for science and conservation. Bird Study 50:254-270.

Cucco, M., and G. Malacarne. 1997. The effect of supplemental food on time budget and body condition in the Black Redstart *Phoenicurus ochruros*. Ardea 5:211-221.

Currie, D., M. Hill, J. Millett, R. Bristol, J. Nevill, and N. J. Shah. 2003. Conservation options for the critically endangered Seychelles Black Paradise-Flycatcher *Terisphone corvina*. Bird Conservation International 13:97-114.

Dakin, E. E., and J. C. Avise. 2004. Microsatellite null alleles in parentage analysis. Heredity 93:504-509.

Davies, Z. G., R. A. Fuller, A. Loram, K. N. Irvine, V. Sims, and K. J. Gaston. 2009. A national scale inventory of resource provision for biodiversity within domestic gardens. Biological Conservation 142:761-771.

Dawson, D. A. 2007. Genomic analysis of passerine birds using conserved microsatellite loci. PhD Thesis, University of Sheffield, Sheffield, UK.

Dawson, D. A., O. Hanotte, C. Greig, I. R. K. Stewart, and T. Burke. 2000. Polymorphic microsatellites in the Blue Tit *Parus caeruleus* and their cross-species utility in 20 songbird families. Molecular Ecology 9:1919-1952.

de Laet, J. F. 1985. Dominance and anti-predator behaviour of Great Tits *Parus major*: a field study. Ibis 127:372-377.

de Neve, L., J. J. Soler, M. Soler, T. Pérez-Contreras, M. Martín-Vivaldi, and J. G. Martínez. 2004. Effects of a food supplementation experiment on reproductive investment and a post-mating sexually selected trait in Magpies *Pica pica.* Journal of Avian Biology 35:246-251.

Deeming, D. C. 1989. Characteristics of unturned eggs: a critical period, retarded embryonic growth and poor albumen utilisation. British Poultry Science 30:239-249.

Deeming, D. C. 2002a. Importance and evolution of incubation in avian reproduction. Pages 1-7 *in* Avian Incubation: Behaviour, Environment, and Evolution (D. C. Deeming, Ed.). Oxford University Press, Oxford, UK.

Deeming, D. C. 2002b. Patterns and significance of egg turning. Pages 161-178 *in* Avian Incubation: Behaviour, Environment, and Evolution (D. C. Deeming, Ed.). Oxford University Press, Oxford, UK.

Deeming, D. C., and M. W. J. Ferguson. 1991. Physiological effects on incubation temperature on embryonic development in reptiles and birds. Pages 147-172 *in* Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles (D. C. Deeming, and M. W. J. Ferguson, Eds.). Cambridge University Press, Cambridge, UK.

Deeming, D. C., K. Rowlett, and K. Simkiss. 1987. Physical influences on embryo development. Journal of Experimental Zoology, Supplement 1:341-345.

Dewey, S. R., and P. L. Kennedy. 2001. Effects of supplemental food on parental-care strategies and juvenile survival of Northern Goshawks. Auk 118:352-365.

Dhondt, A. A., and R. Eyckerman. 1979. Temperature and date of laying by tits *Parus* spp. Ibis 121:329-331.

Dhondt, A. A., R, Eyckerman, R. Moermans, and J. Huble. 1984. Habitat and laying date of Great Tit *Parus major* and Blue Tit *Parus caeruleus*. Ibis 126:388-397.

Dijkstra, C., L. Vuursteen, S. Daan, and D. Masman. 1982. Clutch size and laying date in the Kestrel *Falco tinnunculus*: effect of supplementary food. Ibis 124:210-213.

Dixon, A., D. Ross, S. L. C. O'Malley, and T. Burke. 1994. Paternal investment inversely related to degree of extra-pair paternity. Nature 371:698-700.

Donald, P. F. 2007. Adult sex ratios in wild bird populations. Ibis 149:671-692.

Doutrelant, C., A. Grégoire, N. Grnac, D. Gomez, M. M. Lambrechts, and P. Perret. 2008. Female coloration indicates female reproductive capacity in Blue Tits. Journal of Evolutionary Biology 21:226-233.

Dreiss, A., M. Richard, F. Moyen, J. White, A. P. Møller, and E. Danchin. 2006. Sex ratio and male sexual characteristics in a population of Blue Tits, *Parus caeruleus*. Behavioral Ecology 17:13-19.

Drent, R. H. 1975. Incubation. Pages 333-420 *in* Avian Biology Vol. 5 (D. S. Farner, and J. R. King, Eds.). Academic Press, New York, USA.

Dunn, P. O., and S. J. Hannon. 1992. Effects of food abundance and male parental care on reproductive success and monogamy in Tree Swallows. Auk 109:488-499.

Dunn, P. O., and D. W. Winkler. 1999. Climate change has affected the breeding date of Tree Swallows throughout North America. Proceedings of the Royal Society of London, Series B 266:2487-2490.

Eden, S. F. 1985. The comparative breeding biology of Magpies *Pica pica* in an urban and a rural habitat (Aves: Corvidae). Journal of Zoology 205:325-334.

Eeva, T., and E. Lehikoinen. 1995. Egg shell quality, clutch size and hatching success of the Great Tit (*Parus major*) and the Pied Flycatcher (*Ficedula hypoleuca*) in an air pollution gradient. Oecologia 102:312-323.

Eguchi, K. 1980. The feeding ecology of the nestling Great Tit, *Parus major minor*, in the temperate ever-green broadleaved forest II. With reference to breeding ecology. Researches on Population Ecology 22:284-300.

Eikenaar, C., M. L. Berg, and J. Komdeur. 2003. Experimental evidence for the influence of food availability on incubation attendance and hatching asynchrony in the Australian Reed Warbler *Acrocephalus australis*. Journal of Avian Biology 34:419-427.

Ellegren, H. 1992. Polymerase-chain-reaction (PCR) analysis of microsatellites: a new approach to studies of genetic relationships in birds. Auk 109:886-895.

Ellegren, H. 2000. Evolution of the avian sex chromosomes and their role in sex determination. Trends in Ecology and Evolution 15:188-192.

Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics 5:435-445.

Elliott, G. P., D. V. Merton, and P. W. Jansen. 2001. Intensive management of a critically endangered species: the Kakapo. Biological Conservation 99:121-133.

Emlen, S. T. 1976. Lek organization and mating strategies in the Bullfrog. Behavioral Ecology and Sociobiology 1:283-313.

Emlen, S. T. 1997. When mothers prefer daughters over sons. Trends in Ecology and Evolution 12:291-292.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197:215-223.

Enoksson, B. 1990. Time budgets of Nuthatches *Sitta europaea* with supplementary food. Ibis 132:575-583.

Erikstad, K. E. 1986. Relationship between weather, body condition and incubation rhythm in Willow Grouse. Fauna Norvegica Series C, Cinclus 9:7-12.

Ewald, P. W., and S. Rohwer. 1982. Effects of supplemental feeding on timing of breeding, clutch-size and polygyny in Red-winged Blackbirds *Agelaius phoeniceus*. Journal of Animal Ecology 51:429-450.

Ewen, J. G., R. H. Clarke, E. Moysey, R. L. Boulton, R. H. Crozier, and M. F. Clarke. 2001. Primary sex ratio bias in an endangered cooperatively breeding bird, the Black-eared Miner, and its implications for conservation. Biological Conservation 101:137-145.

Fargallo, J. A. 2004. Latitudinal trends of reproductive traits in the Blue Tit *Parus caeruleus*. Ardeola 51:177-190.

Feast, M., R. C. Noble, B. K. Speake., and M. W. J. Ferguson. 1998. The effect of temporary reductions in incubation temperature on growth characteristics and lipid utilisation in the chick embryo. Journal of Anatomy 193:383-390.

Fenger, J. 1999. Urban air quality. Atmospheric Environment 33:4877-4900.

Fleischer, A. L., R. Bowman, and G. E. Woolfenden. 2003. Variation in foraging behavior, diet, and time of breeding of Florida Scrub-Jays in suburban and wildland habitats. Condor 105:515-527.

Ford, H. A. 1981. Territorial behaviour in an Australian nectar-feeding bird. Australian Journal of Ecology 6:131-134.

Forstmeier, W., B. Kempenaers, A. Meyer, and B. Leisler. 2002. A novel song parameter correlates with extra-pair paternity and reflects male longevity. Proceedings of the Royal Society of London, Series B 269:1479-1485.

Freitag, A., A. Martinoli, and J. Urzelai. 2001. Monitoring the feeding activity of nesting birds with an autonomous system: case study of the endangered Wryneck *Jynx torquilla*. Bird Study 48:102-109.

Fuller, R. A., K. N. Irvine, Z. G. Davies, and K. J. Gaston. In Press. Interactions between people and birds in urban landscapes. Studies in Avian Biology.

Fuller, R. A., P. H. Warren, P. R. Armsworth, O. Barbosa, and K. J. Gaston. 2008. Garden bird feeding predicts the structure of urban avian assemblages. Diversity and Distributions 14:131-137.

Fuller, R. J., D. G. Noble, K. W. Smith, and D. Vanhinsbergh. 2005. Recent declines in populations of woodland birds in Britain: a review of possible causes. British Birds 98:116-143.

Gagneux, P., C. Boesch, and D. S. Woodruff. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology 6:861-868.

Gibb, J. 1950. The breeding biology of the Great and Blue Titmice. Ibis 92:507-539.

Gibb, J. A. 1955. Feeding rates of Great Tits. British Birds 48:49-58.

Gil, D., and M. Gahr. 2002. The honesty of bird song: multiple constraints for multiple traits. Trends in Ecology and Evolution 17:133-141.

Gill, F. B., and L. L. Wolf. 1975. Economics of feeding territoriality in the Golden-winged Sunbird. Ecology 56:333-345.

Gill, F., and M. Wright. 2006. Birds of the World: Recommended English Names. Princeton University Press, Princeton, New Jersey, USA.

Glue, D. 2006. Variety at winter bird tables. Bird Populations 7:212-215.

González, L. M., A. Margalida, R. Sánchez, and J. Oria. 2006. Supplementary feeding as an effective tool for improving breeding success in the Spanish Imperial Eagle (*Aquila adalberti*). Biological Conservation 129:477-486.

Goodbred, C. O., and R. T. Holmes. 1996. Factors affecting food provisioning of nestling Black-throated Blue Warblers. Wilson Bulletin 108:467-479.

Gorman, H. E., and R. G. Nager. 2003. State-dependent incubation behaviour in the Zebra Finch. Animal Behaviour 65:745-754.

Gosler, A. G. 1993. The Great Tit. Hamlyn, London, UK.

Gosler, A.G., and P. Clement. 2007. Family Paridae (Tits and Chickadees). Pages 662-750 *in* Handbook of the Birds of the World Vol. 12 Picathartes to Tits and Chickadees (J. del Hoyo, A. Elliot, and D. A. Christie, Eds.). Lynx Editions, Barcelona, Spain.

Gosler, A. G., J. J. D. Greenwood, J. K. Baker, and N. C. Davidson. 1998. The field determination of body size and condition in passerines: A report to the British Ringing Committee. Bird Study 45:92-103.

Gowaty, P. A. 1993. Differential dispersal, local resource competition, and sex ratio variation in birds. American Naturalist 141:263-280.

Gowaty, P. A. 1996. Battles of the sexes and origins of monogamy. Pages 21-52 *in* Partnerships in Birds (J. M. Black, Ed.) Oxford University Press, Oxford, UK.

Grafen, A., and R. Hails. 2002. Modern Statistics for the Life Sciences. Oxford University Press, Oxford, UK.

Grant, J. W. A., and P. E. Foam. 2002 Effect of operational sex ratio on female-female versus male-male competitive aggression. Canadian Journal of Zoology 80:2242-2246.

Gray, E. M. 1997. Female Red-winged Blackbirds accrue material benefits from copulating with extra-pair males. Animal Behaviour 53:625-639.

Greenwood, P. J., and P. H. Harvey. 1982. The natal and breeding dispersal of birds. Annual Review of Ecology and Systematics 13:1-21.

Greig, S. A., J. C. Coulson, and P. Monaghan. 1986. A comparison of foraging at refuse tips by three species of gull (Laridae). Journal of Zoology 210:459-472.

Grieco, F. 2001. Short-term regulation of food-provisioning rate and effect on prey size in Blue Tits, *Parus caeruleus*. Animal Behaviour 62:107-116.

Grieco, F. 2002a. Time constraint on food choice in provisioning Blue Tits, *Parus caeruleus*: the relationship between feeding rate and prey size. Animal Behaviour 64:517-526.

Grieco, F. 2002b. How different provisioning strategies result in equal rates of food delivery: an experimental study of Blue Tits *Parus caeruleus*. Journal of Avian Biology 33:331-341.

Griffith, S. C., J. Oernborg, A. F. Russell, S. Andersson, and B. C. Sheldon. 2003. Correlations between ultraviolet coloration, overwinter survival and offspring sex ratio in the Blue Tit. Journal of Evolutionary Biology 16:1045-1054.

Griffith, S. C., I. P. F. Owens, and K. A. Thuman. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11:2195-2212.

Griffths, R. 1992. Sex-biased mortality in the Lesser Black-backed Gull *Larus fuscus* during the nestling stage. Ibis 134:237-244.

Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071-1075.

Griffiths, R., and B. Tiwari. 1995. Sex of the last wild Spix's Macaw. Nature 375:454.

Grüebler, M. U., H. Schuler, M. Muller, R. Spaar, P. Horch, and B. Naef-Daenzer. 2008. Female biased mortality caused by anthropogenic nest loss contributes to population decline and adult sex ratio of a meadow bird. Biological Conservation 141:3040-3049.

Haftorn, S. 1981. Incubation during the egg-laying period in relation to clutch-size and other aspects of reproduction in the Great Tit *Parus major*. Ornis Scandinavica 12:169-185.

Haftorn, S. 1994. The act of tremble-thrusting in tit nests, performance and possible function. Fauna Norvegica Series C, Cinclus 17:55-74.

Haftorn, S., and R. E. Reinertsen. 1985. The effect of temperature and clutch size on the energetic cost of incubation in a free-living Blue Tit (*Parus caeruleus*). Auk 102:470-478.

Haftorn, S., and T. Slagsvold. 1995. Egg covering in birds: description of the behaviour in tits (*Parus* spp.) and a test of hypothesis of its function. Fauna Norvegica Series C, Cinclus 18:85-106.

Haggard, W. H. 1990. Urban weather. International Journal of Environmental Studies 36:73-82.

Halupka, L., A. Dyrcz, and M. Borowiec. 2008. Climate change affects breeding of Reed Warblers *Acrocephalus scirpaceus*. Journal of Avian Biology 39:95-100.

Hamer, K. C., P. Quillfeldt, J. F. Masello, and K. L. Fletcher. 2006. Sex differences in provisioning rules: responses of Manx Shearwaters to supplementary chick feeding. Behavioral Ecology 17:132-137.

Hansell, M., and G. D. Ruxton. 2002. An experimental study of the availability of feathers for avian nest building. Journal of Animal Biology 33:319-321.

Hanski, I. 2001. Population dynamic consequences of dispersal in local populations and in metapopulations. Pages 283-298 *in* Dispersal (J. Clobert, E. Danchin, A. A. Dhondt, and J. D. Nichols, Eds.). Oxford University Press, Oxford, UK.

Harrison, T. J. E. 2010. A curate's egg: Feeding birds during reproduction is 'good in parts'. A study of Blue Tits *Cyanistes caeruleus* and Great Tits *Parus major*. PhD Thesis, University of Birmingham, Birmingham, UK.

Harrison, T. J. E., J. A. Smith, G. R. Martin, D. E. Chamberlain, S. Bearhop, G. N. Robb, and S. J. Reynolds. 2010. Does food supplementation really enhance productivity in breeding birds? Oecologia 164:311-320.

Hartshorne, J. M. 1962. Behavior of the Eastern Bluebird at the nest. The Living Bird 1:131-150.

Harvey, M. G., D. N. Bonter, L. M. Stenzler, and I. J. Lovette. 2006. A comparison of plucked feathers versus blood samples as DNA sources for molecular sexing. Journal of Field Ornithology 77:136-140.

Haskell, D. G., A. M. Knupp, and M. C. Schneider. 2001. Nest predator abundance and urbanization. Pages 243-258 *in* Avian Ecology and Conservation in an Urbanizing World (J. M. Marzluff, R. Bowman, and R. Donnelly, Eds.). Kluwer Academic Publishers, Boston, Massachusetts, USA.

Hasselquist, D., S. Bensch, and T. Von Schantz. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the Great Reed Warbler. Nature 381:229-232.

Haywood, S., and C. M. Perrins. 1992. Is clutch size in birds affected by environmental conditions during growth? Proceedings of the Royal Society of London, Series B 249:195-197.

Heeb, P., I. Werner, H. Richner, and M. Kölliker. 1996. Horizontal transmission and reproductive rates of Hen Fleas in Great Tit nests. Journal of Animal Ecology 65:474-484.

Hegner, R. E. 1985. Dominance and anti-predator behaviour in Blue Tits (*Parus caeruleus*). Animal Behaviour 33:762-768.

Hegyi, Z. 1996. Laying date, egg volumes and chick survival in Lapwing (*Vanellus vanellus* L.), Redshank (*Tringa totanus* L.), and Black-tailed Godwit (*Limosa limosa* L.). Ornis Hungarica 6:1-7.

Hiesemann, M. 1908. How to Attract and Protect Wild Birds. Witherby, London, UK.

Hilton, G. M., M. H. Hansell, G. D. Ruxton, J. M. Reid, and P. Monaghan. 2004. Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. Auk 121:777-787.

Hinde, R. A. 1952. The behaviour of the Great Tit (*Parus major*) and some other related species. Behaviour supplement 2:1-201.

Hipkiss, T., B. Hornfeldt, U. Eklund, and S. Berlin. 2002. Year-dependent sex-biased mortality in supplementary-fed Tengmalm's Owl nestlings. Journal of Animal Ecology 71:693-699.

Hoffman, J. I., and W. Amos. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology 14:599-612.

Hoi-Leitner, M., H. Hoi, M. Romero-Pujante, and F. Valera. 1999. Female extra-pair behaviour and environmental quality in the Serin (*Serinus serinus*): a test of the 'constrained female hypothesis'. Proceedings of the Royal Society of London, Series B 266:1021-1026.

Holleley, C. E., and P. G. Geerts. 2009. Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. BioTechniques 46:511-517.

Horvath, T., and A. M. Roelans. 1991. Backyard feeders: not entirely for the birds. Anthrozoos: A Multidisciplinary Journal of the Interactions of People and Animals 4:232-236.

Hunter, M. D. 1998. Interactions between *Operophtera brumata* and *Tortrix viridana* on oak: new evidence from time-series analysis. Ecological Entomology 23:168-173.

IPCC 2001. Climate change 2001: The Scientific Basis. Contribution of Working Group 1 to the Third Assessment Report of the Intergovernmental Panel on Climate Change (J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell, and C. A. Johnson, Eds.). Cambridge University Press, Cambridge, UK.

Janssens, E., T. Dauwe, R. Pinxten, and M. Eens. 2003. Breeding performance of Great Tits (*Parus major*) along a gradient of heavy metal pollution. Environmental Toxicology and Chemistry 22:1140-1145.

Johnson, L. S., C. F. Thompson, S. K. Sakaluk, M. Neuhauser, B. G. P. Johnson, S. S. Soukup, S. J. Forsythe, and B. S. Masters. 2009. Extra-pair young in House Wren broods are more likely to be male than female. Proceedings of the Royal Society of London, Series B 276:2285-2289.

Jokimäki, J., and E. Huhta. 2000. Artificial nest predation and abundance of birds along an urban gradient. Condor 102:838-847.

Jones, A. G., and W. R. Ardren. 2003. Methods of parentage analysis in natural populations. Molecular Ecology 12: 2511-2523.

Jones, D. N., and S. J. Reynolds. 2008. Feeding birds in our towns and cities: a global research opportunity. Journal of Avian Biology 39:265-271.

Jönsson, K. I. 1997. Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78:57-66.

Kacelnik, A., and I. Cuthill. 1990. Central place foraging in Starlings (*Sturnus vulagris*). II. Food allocation to chicks. Journal of Animal Ecology 59:655-674.

Källander, H., and J. Karlsson. 1993. Supplemental food and laying date in the European Starling. Condor 95:1031-1034.

Kalmbach, E., R. G. Nager, R. Griffiths, and R. W. Furness. 2001. Increased reproductive effort results in male-biased offspring sex ratio: an experimental study in a species with reversed sexual size dimorphism. Proceedings of the Royal Society of London, Series B 268:2175-2179.

Kelly, J. F., and B. van Horne. 1997. Effects of food supplementation on the timing of nest initiation in Belted Kingfishers. Ecology 78:2504-2511.

Kempenaers, B., G. R. Verheyen, and A. A. Dhondt. 1995. Mate guarding and copulation behaviour in monogamous and polygynous Blue Tits: do males follow a best-of-a-bad-job strategy? Behavioral Ecology and Sociobiology 36:33-42.

Kempenaers, B., G. R. Verheyen, and A. A. Dhondt. 1997. Extrapair paternity in the Blue Tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behavioral Ecology 8:481-492.

Kempenaers, B., G. R. Verheyen, M. van den Broeck, T. Burke, C. van Broeckhoven, and A. A. Dhondt. 1992. Extra-pair paternity results from female preference for high-quality males in the Blue Tit. Nature 357:494-496.

Kennedy, P. L., and J. M. Ward. 2003. Effects of experimental food supplementation on movements of juvenile Northern Goshawks (*Accipter gentilis atricapillus*). Oecologia 134:284-291.

Kenta, T., J. Gratten, G. Hinten, J. Slate, R. K. Butlin, and T. Burke. 2008. Multiplex SNP-SCALE: a cost-effective medium-throughput SNP genotyping method. Molecular Ecology Resources 8:1230-1238.

Kern, M. D., M. K. Sogge, R. B. Kern, and C. van Riper. 1993. Nests and nest sites of the San-Miguel Island Song Sparrow. Journal of Field Ornithology 64:367-381.

Kilner, R. 1998. Primary and secondary sex ratio manipulation by Zebra Finches. Animal Behaviour 56:155-164.

King, D. 2005. Climate change: the science and the policy. Journal of Applied Ecology 42:779-783.

Kirkpatrick, C., C. J. Conway, and M. H. Ali. 2009. Sanitation of entire broods of dead nestlings may bias cause-specific nest failure rates. Ibis 151:207-211.

Kluijver, H. N. 1950. Daily routines of the Great Tit, *Parus m. major* L. Ardea 38:99-135.

Kölliker, M., P. Heeb, I. Werner, A. C. Mateman, C. M. Lessells, and H. Richner. 1999. Offspring sex ratio is related to male body size in the Great Tit (*Parus major*). Behavioral Ecology 10:68-72.

Komdeur, J. 1996. Facultative sex ratio bias in the offspring of Seychelles Warblers. Proceedings of the Royal Society of London, Series B 263:661-666.

Komdeur, J., F. Kraaijeveld-Smit, K. Kraaijeveld, and P. Edelaar. 1999. Explicit experimental evidence for the role of mate guarding in minimizing loss of paternity in the Seychelles Warbler. Proceedings of the Royal Society of London, Series B 266:2075-2081.

Komdeur, J., M. J. L. Magrath, and S. Krackow. 2002. Pre-ovulation control of hatchling sex ratio in the Seychelles Warbler. Proceedings of the Royal Society of London, Series B 269:1067-1072.

Komdeur, J., and I. Pen. 2002. Adaptive sex allocation in birds: the complexities of linking theory and practice. Philosophical Transactions of the Royal Society of London, Series B 357:373-380.

Korpimäki, E., C. A. May, D. T. Parkin, J. H. Wetton, and J. Wiehn. 2000. Environmental and parental condition related variation in sex ratio of Kestrel broods. Journal of Avian Biology 31:218-234.

Korsten, P., C. M. Lessells, A. C. Mateman, M. van der Velde, and J. Komdeur. 2006. Primary sex ratio adjustment to experimentally reduced male UV attractiveness in Blue Tits. Behavioral Ecology 27:539-546.

Krakow, S. 1995. Potential mechanisms for sex ratio adjustment in mammals and birds. Biological Reviews of the Cambridge Philosophical Society 70:225-241.

Krebs, E. I., D. I, Green, M. I. Double, and R. Griffiths. 2002. Laying date and laying sequence influence the sex ratio of Crimson Rosella broods. Behavioral Ecology and Sociobiology 51:447-454.

Krokene, C, K. Rigstad, M. Dale, and J. T. Lifjeld. 1998. The function of extrapair paternity in Blue Tits and Great Tits: good genes or fertility insurance? Behavioral Ecology 9:649-656.

Laaksonen, T., M. Ahola, T. Eeva, R. A. Väisänen, and E. Lehikoinen. 2006. Climate change, migratory connectivity and changes in laying date and clutch size of the Pied Flycatcher. Oikos 114:277-290.

Lack, D. 1954. The Natural Regulation of Animal Numbers. Clarendon Press, Oxford, UK.

Lack, D. 1968. Ecological Adaptations for Breeding in Birds. Methuen & Co., London, UK.

Lagercrantz, U., H. Ellegren, and L. Andersson. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Research 21:1111-1115.

Lambin, X., J. Aars, and S. B. Piertney. 2001. Dispersal, intraspecific competition and kin facilitation: a review of the empirical evidence. Pages 110-122 *in* Dispersal (J. Clobert, J, E. Danchin, A. A. Dhondt, and J. D. Nichols, Eds.). Oxford University Press, Oxford, UK.

Lambrechts, M. M., P. Perret, M. Maistre, and J. Blondel. 1999. Do experiments with captive non-domesticated animals make sense without population field studies? A case study with Blue Tits' breeding time. Proceedings of the Royal Society of London, Series B 266:1311-1315.

Lea, R. W., and H. Klandorf. 2002. The brood patch. Pages 100-118 *in* Avian Incubation: Behaviour, Environment, and Evolution (D. C. Deeming, Ed.). Oxford University Press, Oxford, UK.

Leech, D. I., I. R. Hartley, I. R. K. Stewart, S. C. Griffith, and T. Burke. 2001. No effect of parental quality or extrapair paternity on brood sex ratio in the Blue Tit (*Parus caeruleus*). Behavioral Ecology 12:674-680.

Lens, L., L. A. Wauters, and A. A. Dhondt. 1994. Nest-building by Crested Tit *Parus cristatus* males: an analysis of costs and benefits. Behavioral Ecology and Sociobiology 35:431-436.

Lepczyk, C. A., A. G. Mertig, and J. Liu. 2004. Landowners and cat predation across rural-to-urban landscapes. Biological Conservation 115:191-201.

Lessells, C. M., A. C. Mateman, and J. Visser. 1996. Great Tit hatchling sex ratios. Journal of Avian Biology 27:135-142.

Lima, S. L. 2009. Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. Biological Reviews of the Cambridge Philosophical Society 84:485-513.

Lombardo, M. P., R. M. Bosman, C. A. Faro, S. G. Houtteman, and T. S. Kluisza. 1995. Effect of feathers as nest insulation on incubation behavior and reproductive performance of Tree Swallows (*Tachycineta bicolor*). Auk 112:973-981.

Londoño, G. A., D. J. Levey, and S. K. Robinson. 2008. Effects of temperature and food on incubation behaviour of the Northern Mockingbird, *Mimus polyglottos*. Animal Behaviour 76:669-677.

Lubjuhn, T., T. Gerken, T. Brun, and T. Schmoll. 2007. Yearling male Great Tits, *Parus major*, suffer more strongly from cuckoldry than older males. Zoology 110:387-397.

McFarland, D. J. 1977. Decision making in animals. Nature 269:15-21.

McGinley, M. A., D. H. Temme, and M. A. Geber. 1987. Parental investment in offspring in variable environments: Theoretical and empirical considerations. American Naturalist 130:370-398.

McGowan, A., S. P. Sharp, and B. J. Hatchwell. 2004. The structure and function of nests of Long-tailed Tits *Aegithalos caudatus*. Functional Ecology 18:578-583.

Magrath, M. J. L., O. Vedder, M. van der Velde, and J. Komdeur. 2009. Maternal effects contribute to the superior performance of extra-pair offspring. Current Biology 19:792-797.

Mainwaring, M. C., C. McW. H. Benskin, and I. R. Hartley. 2008. The weight of female-built nests correlates with female but not male quality in the Blue Tit *Cyanistes caeruleus*. Acta Ornithologica 43:43-48.

Mainwaring, M. C., and I. R. Hartley. 2008. Seasonal adjustments in nest cup lining in Blue Tit *Cyanistes caeruleus*. Ardea 96:278-282.

Mainwaring, M. C., and I. R. Hartley. 2009. Experimental evidence for state-dependent nest weight in the Blue Tit, *Cyanistes caeruleus*. Behavioural Processes 81:144-146.

Malacarne, G., M. Cucco, and E. Bertolo. 1994. Sibling competition in asynchronously hatched broods of the Pallid Swift (*Apus pallidus*). Ethology, Ecology and Evolution 6:293-300.

Marchesan, M. 2002. Operational sex ratio and breeding strategies in the Feral Pigeon *Columba livia*. Ardea 90:249-257.

Marciniak, B., J. Nadolski, M. Nowakowska, B. Loga, and J. Bańbura. 2006. Habitats and annual variation in arthropod abundance affects Blue Tit *Cyanistes caeruleus* reproduction. Acta Ornithologica 42:53-62.

Markman, S., B. Pinshow, and J. Wright. 2002. The manipulation of food resources reveals sex-specific trade-offs between parental self-feeding and offspring care. Proceedings of the Royal Society of London, Series B 269:1931-1938.

Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655.

Martin, T. E. 1987. Food as a limit on breeding birds: a life-history perspective. Annual Review of Ecology, Evolution, and Systematics 18:453-487.

Martin, T. E., S. K. Auer, R. D. Bassar, A. M. Niklison, and P. Lloyd. 2007. Geographic variation in avian incubation periods and parental influences on embryonic temperature. Evolution 61:2558-2569.

Martin, T. E., and C. K. Ghalambor. 1999. Males feeding females during incubation. I. Required by microclimate or constrained by nest predation. American Naturalist 153:131-139.

Martin, T. E., and P. Li. 1992. Life history traits of open- *v.s.* cavity-nesting birds. Ecology 73:579-592.

Martin, T. E., P. R. Martin, C. R. Olson, B. J. Heidinger., and J. J. Fontaine. 2000a. Parental care and clutch sizes in North and South American Birds. Science 287:1482-1485.

Martin, T. E., J. Scott, and C. Menge. 2000b. Nest predation increases with parental activity: separating nest site and parental activity effects. Proceedings of the Royal Society of London, Series B 267: 2287-2293.

Marzluff, J. M. 2001. Worldwide urbanization and its effects on birds. Pages 19-47 *in* Avian Ecology in an Urbanizing World (Marzluff, J. M., R. Bowman, and R. Donnelly, Eds.). Kluwer, Boston, Massachusetts, USA.

Maxim Integrated Products. 2010. http://www.maxim-ic.com/products/ibutton/ibuttons/

Mayer-Gross, H. 1970. Nest Record Scheme. British Trust for Ornithology, Tring, UK.

Mazgajski, T. D., and Z. Rykowska. 2008. Dependence of nest mass on nest hole depth in the Great Tit *Parus major*. Acta Ornithologica 43:49-55.

Meek, S. B., R. J. Robertson, and P. T. Boag. 1994. Extrapair paternity and intraspecific brood parasitism in Eastern Bluebirds revealed by DNA fingerprinting. Auk 111:739-744.

Meijer, T., S. Daan, and C. Dijkstra. 1988. Female condition and reproduction: effects of food manipulation in free-living and captive Kestrels. Ardea 76:141-154.

Meijer, T., and R. Drent. 1999. Re-examination of the capital and income dichotomy in breeding birds. Ibis 141:399-414.

Mennechez, G., and P. Clergeau. 2006. Effect of urbanisation on habitat generalists: Starlings not so flexible? Acta Oecologica 30:182-191.

Met Office. 2010. http://www.metoffice.gov.uk/climate/uk/

Minitab. 2007. Meet Minitab 15 for Windows. Minitab Inc., State College, Pennsylvania, USA.

Minot, E. 1981. Effects of interspecific competition for food in breeding Blue and Great Tits. Journal of Animal Ecology 50:375-385.

Møller, A. P. 1982. Clutch size in relation to nest size in the Swallow *Hirundo rustica*. Ibis 124:339-343.

Møller, A. P. 1987. Mate guarding in the Swallow *Hirundo rustica*: an experimental study. Behavioral Ecology and Sociobiology 21:119-123.

Møller, A. P., and T. R. Birkhead. 1991. Frequent copulations and mate guarding as alternative paternity guards in birds: a comparative study. Behaviour 118:170-186.

Monrós, J. S., and E. Barba. 2002. Post-fledging survival of individual Great Tits: the effect of hatching date and fledging mass. Oikos 99:481-488.

Moreno, J. 1989. Energetic constraints on uniparental incubation in the Wheatear *Oenanthe oenanthe* (L.). Ardea 77:107-115.

Moreno, J., and A. Carlson. 1989. Clutch size and the costs of incubation in the Pied Flycatcher *Ficedula hypoleuca*. Ornis Scandinavica 20:123-128.

Moreno, J., J. Martínez, C. Corral, E. Lobato, S. Merino, J. Morales, J. Martínez-de la Puente, and G. Tomás. 2008. Nest construction rate and stress in female Pied Flycatchers *Ficedula hypoleuca*. Acta Ornithologica 43:57-64.

Moreno, J., J. –G. Martínez, J. Morales, E. Lobato, S. Merino, G. Tomás, R. A. Vásquez, E. Möstl, and J. L. Osorno. 2010. Paternity loss in relation to male age, territorial behaviour and stress in the Pied Flycatcher. Ethology 116:76-84.

Morris, D. W. 1987. Optimal allocation of parental investment. Oikos 49:332-339.

Morrison, S. A., and D. T. Bolger. 2002. Variation in a sparrow's reproductive success with rainfall: food and predator-mediated processes. Oecologia 133:315-324.

Murphy, M. T. 1986. Body size and condition, timing of breeding, and aspects of egg production in Eastern Kingbirds. Auk 103:465-476.

Murton, R. K., and N. J. Westwood. 1977. Avian Breeding Cycles. Clarendon Press, Oxford, UK.

Naef-Daenzer, B. 2000. Patch time allocation and patch sampling by foraging Great and Blue Tits. Animal Behaviour 59:989-999.

Naef-Daenzer, B., and L. F. Keller. 1999. The foraging performance of Great and Blue Tits (*Parus major* and *P. caeruleus*) in relation to caterpillar development, and its consequence for nestling growth and fledging weight. Journal of Animal Ecology 68:708-718.

Naef-Daenzer, L., B. Naef-Daenzer, and R. G. Nager. 2000. Prey selection and foraging performance of breeding Great Tits *Parus major* in relation to food availability. Journal of Avian Biology 31:206-214.

Naef-Daenzer, B., F. Widmer, and M. Nuber. 2001. Differential post-fledging survival of Great and Coal Tits in relation to their condition and fledging date. Journal of Animal Ecology 70:730-738.

Nager, R. G., P. Monaghan, R. Griffiths, D. C. Houston, and R. Dawson. 1999. Experimental demonstration that offspring sex ratio varies with maternal condition. Proceedings of the National Academy of Sciences of the United States of America 96:570-573.

Nager, R. G., C. Ruegger, and A. J. van Noordwijk. 1997. Nutrient or energy limitation on egg formation: a feeding experiment in Great Tits. Journal of Animal Ecology 66:495-507.

Nager, R. G., and A. J. van Noordwijk. 1992. Energetic limitation in the egg-laying period of Great Tits. Proceedings of the Royal Society of London, Series B 249:259-263.

New, D. A. T. 1957. A critical period for the turning of hens' eggs. Journal of Embryology and Experimental Morphology 5:293-299.

Newhouse, M. J., P. P. Marra, and J. L. Scott. 2008. Reproductive success of House Wrens in suburban and rural landscapes. Wilson Journal of Ornithology 120:99-104.

Newton, I. 1964. The breeding biology of the Chaffinch. Bird Study 11:47-68.

Nicholls, J. A., M. C. Double, D. M. Rouell, and R. D. Magrath. 2000. The evolution of cooperative and pair breeding in Thornbills *Acanthiza* (Pordalotidae). Journal of Avian Biology 31:165-176.

Nilsson, J. -Å. 1991. Clutch size determination in the Marsh Tit (*Parus palustris*). Ecology 72:1757-1762.

Nilsson, J. -Å. 1994. Energetic bottle-necks during breeding and the reproductive cost of being too early. Journal of Animal Ecology 63:200-208.

Nilsson, J. -Å., and H. G. Smith. 1988. Incubation feeding as a male tactic for early hatching. Animal Behaviour 36:641-647.

Norberg, R. A. 1977. An ecological theory on foraging time and energetics and choice of optimal food-searching method. Journal of Animal Ecology 46:511-529.

Norris, K. 1993. Seasonal variation in the reproductive success of Blue Tits: an experimental study. Journal of Animal Ecology 62:287-294.

Nuechterlein, G. L. 1981. Asynchronous hatching and sibling competition in Western Grebes. Canadian Journal of Zoology 59:994-998.

Nur, N. 1984. The consequences of brood size for breeding Blue Tits II. Nestling weight, offspring survival and optimal brood size. Journal of Animal Ecology 53:497-517.

O'Brien, E. L., and R. D. Dawson. 2007. Context-dependent genetic benefits of extra-pair mate choice in a socially monogamous passerine. Behavioral Ecology and Sociobiology 61:775-782

O'Connor, R. J. 1984. The Growth and Development of Birds. John Wiley and Sons, Chichester, UK.

Oda, S., E. Oki, Y. Maehara, and K. Sugimachi. 1997. Precise assessment of microsatellite instability using high resolution fluorescent microsatellite analysis. Nucleic Acids Research 25:3415-3420.

Olson, C. R., C. M. Vleck., and D. C. Adams. 2008. Decoupling morphological development from growth in periodically cooled Zebra Finch embryos. Journal of Morphology 269:875-883.

Ordnance Survey. 2010. http://www.ordnancesurvey.co.uk/oswebsite/aboutus/reports/misc/calculate.html

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Reviews of the Cambridge Philosophical Society 45:525-567.

Parsons, J. 1970. Relationship between egg size and post-hatching chick mortality in the Herring Gull (*Larus argentatus*). Nature 226:1221-1222.

Pearse, A. T., J. F. Cavitt, and J. F. Cully, Jr. 2004. Effects of food supplementation on female nest attentiveness and incubation mate feeding in two sympatric wren species. Wilson Bulletin 116:23-30.

Perrins, C. M. 1965. Population fluctuations and clutch-size in the Great Tit, *Parus major* L. Journal of Animal Ecology 34:601-647.

Perrins, C. M. 1970. The timing of birds' breeding seasons. Ibis 112:242-255.

Perrins, C. M. 1979. British Tits. Collins, London, UK.

Perrins, C. M. 1991. Tits and their caterpillar food supply. Ibis 133:49-54.

Perrins, C. M., and R. H. McCleery. 1985. The effect of age and pair bond on the breeding success of Great Tits *Parus major*. Ibis 127:306-315.

Perrins, C. M., and R. H. McCleery. 1989. Laying dates and clutch size in the Great Tit. Wilson Bulletin 101:236-253.

Petrie, M., C. Doums, and A. P. Møller. 1998. The degree of extra-pair paternity increases with genetic variability. Proceedings of the National Academy of Sciences of the United States of America 95:9390-9395.

Petrie, M., and B. Kempenaers. 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends in Ecology and Evolution 13:52-58.

Pike, T. W., and M. Petrie. 2005. Maternal body condition and plasma hormones affect offspring sex ratio in Peafowl. Animal Behaviour 70:745-751.

Pike, T. W., and M. Petrie. 2006. Experimental evidence that corticosterone affects offspring sex ratio in Quail. Proceedings of the Royal Society London, Series B 273:1093-1098.

Powell, L. A., and K. L. Rangen. 2000. Variation in Wood Thrush nest dimensions and construction. North American Bird Bander 25:89-96.

Primmer, C. R., T. Raudsepp, B. P. Chowdharry, A. P. Møller, and H. Ellegren. 1997. Low frequency of microsatellites in the avian genome. Genome Research 7:471-482.

Queller, D. C., J. E. Strassmann, and C. R. Hughes. 1993. Microsatellites and kinship. Trends in Ecology and Evolution 8:285-288.

Quillfeldt, P., and J. F. Masello. 2004. Context-dependent honest begging in Cory's Shearwaters (*Calonectris diomedea*): influence of food availability. Acta Ethologica 7:73-80.

Råberg, L., A. Stjernman and J. -Å. Nilsson. 2005. Sex and environmental sensitivity in Blue Tit nestlings. Oecologia 145:496-503.

Radford, A. N., R. H. McCleery, R. J. W. Woodburn, and M. D. Morecroft. 2001. Activity patterns of parent Great Tits *Parus major* feeding their young during rainfall. Bird Study 48:214-220.

Ramsay, S. L., and D. C. Houston. 1997. Nutritional constraints on egg production in the Blue Tit: a supplementary feeding study. Journal of Animal Ecology 66:649-657.

Rastogi, A. D., L. Zanette, and M. Clinchy. 2006. Food availability affects diurnal nest predation and adult antipredator behaviour in Song Sparrows, *Melospiza melodia*. Animal Behaviour 72:933-940.

Rauter, C. M., P. A. Brodmann, and H. -U. Reyer. 2000. Provisioning behaviour in relation to food availability and nestling food demand in the Water Pipit *Anthus spinoletta*. Ardea 88:81-90.

Réale, D., P. Boussès, and J. -L. Chapuis. 1996. Female-biased mortality induced by male sexual harassment in a feral sheep population. Canadian Journal of Zoology 74:1812-1818.

Redfern, C. P. F., and J. A. Clark. 2001. Ringers' Manual. British Trust for Ornithology, Thetford, UK.

Reid, J. M., P. Monaghan, and R. G. Nager. 2002. Incubation and the costs of reproduction. Pages 314-325 *in* Avian Incubation: Behaviour, Environment, and Evolution (D. C. Deeming, Ed.). Oxford University Press, Oxford, UK.

Reyer, H. -U., K. Bollmann, A. R. Schlapfer, A. Schymainda, and G. Klecack. 1997. Ecological determinants of extrapair fertilizations and egg dumping in Alpine Water Pipits (*Anthus spinoletta*). Behavioral Ecology 8:534-543.

Reynolds, S. J., S. J. Schoech, and R. Bowman. 2003a. Nutritional quality of prebreeding diet influences breeding performance of the Florida Scrub-Jay. Oecologia 134:308-316.

Reynolds, S. J., S. J. Schoech, and R. Bowman. 2003b. Diet quality during pre-laying and nestling periods influences growth and survival of Florida Scrub-Jay (*Aphelocoma coerulescens*) chicks. Journal of Zoology 261:217-226.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Richner, H., A. Oppliger, and P. Christie.1993. Effect of an ectoparasite on reproduction in Great Tits. Journal of Animal Ecology 62:703-710.

Ringsby, T. H., T. Berge, B. -E. Saether, and H. Jensen. 2009. Reproductive success and individual variation in feeding frequencies of House Sparrows (*Passer domesticus*). Journal of Ornithology 150:469-481.

Robb, G. N., R. A. McDonald, D. E. Chamberlain, and S. Bearhop. 2008a. Food for thought: supplementary feeding as a driver of ecological change in avian populations. Frontiers in Ecology and the Environment 6:476-484.

Robb, G. N., R. A. McDonald, D. E. Chamberlain, S. J. Reynolds, T. J. E. Harrison, and S. Bearhop. 2008b. Winter feeding of birds increases productivity in the subsequent breeding season. Biology Letters 4:220-223.

Robertson, B. A., and R. L. Hutto. 2006. A framework for understanding ecological traps and an evaluation of existing evidence. Ecology 87:1075-1085.

Robinson, R. A., G. M. Siriwardena, and H. Q. P. Crick. 2005. Size and trends of the House Sparrow *Passer domesticus* populations in Great Britain. Ibis 147:552-562.

Rollinson, D. J., R. A. O'Leary, and D. N. Jones. 2003. The practice of wildlife feeding in suburban Brisbane. Corella 27:52-58.

Rousset, F. 2001. Genetic approaches to the estimation of dispersal rates. Pages 29-42 *in* Dispersal (J. Clobert, E. Danchin, A. A. Dhondt, and J. D. Nichols, Eds.). Oxford University Press, Oxford, UK.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103-106.

Royal Society for the Protection of Birds. 2010. http://www.rspb.org.uk/advice/helpingbirds/feeding/index.aspx

Rubenstein, D. R. 2007. Territory quality drives intraspecific patterns of extrapair paternity. Behavioral Ecology 18:1058-1064.

Russell, P. J. 1996. Genetics. Harper Collins, New York, USA.

Rutstein, A. N., P. J. B. Slater, and J. A. Graves. 2004. Diet quality and resource allocation in the Zebra Finch. Proceedings of the Royal Society of London, Series B 271:S286-S289.

Sanz, J. J. 1998. Effects of geographic location and habitat on breeding parameters of Great Tits. Auk 115:1034-1051.

Sanz, J. J. 2003. Large-scale effect of climate change on breeding performance of Pied Flycatchers in Western Europe. Ecography 26:45-50.

SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. SAS Institute Inc., Cary, North Carolina, USA.

Schoech, S. J. 1996. The effect of supplemental food on the body condition and the timing of reproduction in a cooperative breeder, the Florida Scrub-Jay. Condor 98:234-244.

Schoech, S. J., E. S. Bridge, R. K. Boughton, S. J. Reynolds, J. W. Atwell, and R. Bowman. 2008. Food supplementation: a tool to increase reproductive output? A case study in the threatened Florida Scrub-Jay. Biological Conservation 141:162-173.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9:615-629.

Shaw, G. 1978. The breeding biology of the Dipper. Bird Study 25:149-160.

Shawkey, M. D., R. Bowman, and G. E. Woolfenden. 2004. Why is brood reduction in Florida Scrub-Jays higher in suburban than in wildland habitats? Canadian Journal of Zoology 82:1427-1435.

Sheldon, B. C., S. Andersson, S. C. Griffith, J. Örnborg, and J. Sendecka. 1999. Ultraviolet colour variation influences Blue Tit sex ratios. Nature 402:874-877.

Sheldon, B. C., and H. Ellegren. 1996. Offspring sex and paternity in the Collard Flycatcher. Proceedings of the Royal Society of London, Series B 23:1017-1021.

Sibley, R., and D. McFarland. 1976. On the fitness of behavior sequences. American Naturalist 110:601-617.

Simons, L. S., and T. E. Martin. 1990. Food limitation of avian reproduction: an experiment with the Cactus Wren. Ecology 71:869-876.

Siriwardena, G. M., S. R. Baillie, H. Q. P. Crick, and J. D. Wilson. 2001. Changes in agricultural land-use and breeding performance of some granivorous farmland passerines in Britain. Agriculture, Ecosystems and Environment 84:191-206.

Siriwardena, G. M., and H. Q. P. Crick. 2002. National trends in the breeding performance of Starling *Sturnus vulgaris*. Pages 91-120 *in* Investigations into the Causes of the Declines of Starlings and House Sparrows in Great Britain (H. Q. P. Crick, R. A. Robinson, G. F. Appleton, N. A. Clark, and A. D. Rickard, Eds.) BTO Research Report No. 290. DEFRA, Bristol, UK.

Siriwardena, G. M., H. Q. P. Crick, S. R. Baillie, and J. D. Wilson. 2000. Agricultural habitat-type and the breeding performance of granivorous farmland birds in Britain. Bird Study 47:66-81.

Skutch, A. F. 1949. Do tropical birds rear as many young as they can nourish? Ibis 91:430-455.

Skutch, A. F. 1962. The constancy of incubation. Wilson Bulletin 74:115-152.

Smith, H. G. 1993. Seasonal decline in clutch size of the Marsh Tit (*Parus palustris*) in relation to date specific survival of offspring. Auk 110:889-899.

Smith, H. G., H. Källander, and J. -Å. Nilsson. 1989. The trade-off between offspring number and quality in the Great Tit *Parus major*. Journal of Animal Ecology 58:383-401.

Soler, J. J., A. P. Møller, and M. Soler. 1998. Nest building, sexual selection and parental investment. Evolutionary Ecology 12:427-441.

Solonen, T. 2001. Breeding of the Great Tit and Blue Tit in urban and rural habitats in southern Finland. Ornis Fennica 78:49-60.

Soper, T. 1965. The Bird Table Book. David & Charles, London, UK.

Speakman, J. R. 1997. Doubly Labelled Water: Theory and Practice. Chapman & Hall, London, UK.

Stanley, T. R. 2002. How many kilojoules does a Black-billed Magpie nest cost? Journal of Field Ornithology 73:292-297.

Starck, J. M., and R. E. Ricklefs. 1998. Avian Growth and Development. Oxford University Press, Oxford, UK.

Stauss, M. J., J. F. Burkhardt, and J. Tomiuk. 2005a. Foraging flight distances as a measure of parental effort in Blue Tits *Parus caeruleus* differ with environmental conditions. Journal of Avian Biology 36:47-56.

Stauss, M. J., G. Segelbacher, J. Tomiuk, and L. Bachmass. 2005b. Sex ratio of *Parus major* and *P. caeruleus* broods depends on parental condition and habitat quality. Oikos 109:367-373.

Stearns, S. C. 1989. Trade-offs in life-history evolution. Functional Ecology 3:259-268.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford, UK.

Steifetten, O., and S. Dale. 2006. Viability of an endangered population of Ortolan Buntings: the effect of a skewed operational sex ratio. Biological Conservation 132:88-97.

Stevenson, I. R., and D. M. Bryant. 2000. Avian phenology: climate change and constraints on breeding. Nature 406:366-367.

Stewart, S. L. M., D. F. Westneat, and G. Ritchison. 2010. Extra-pair paternity in Eastern Bluebirds: effects of manipulated density and natural patterns of breeding synchrony. Behavioral Ecology and Sociobiology 64:463-472.

Svensson, E., and J. -Å. Nilsson. 1995. Food supply, territory quality and reproductive timing in the Blue Tit (*Parus caeruleus*). Ecology 76:1804-1812.

Svensson, E., and J. -Å. Nilsson. 1996. Mate quality affects offspring sex ratio in Blue Tits. Proceedings of the Royal Society of London, Series B 263:357-361.

Szentirmai, I., J. Komdeur, and T. Szekely. 2005. What makes a nest-building male successful? Male behavior and female care in Penduline Tits. Behavioral Ecology 16:994-1000.

Taberlet, P., and J. Bouvet. 1991. A single plucked feather as a source of DNA for bird genetic studies. Auk 108:959-960.

Tasker, M. L., C. J. Camphuysen, J. Cooper, S. Garthe, W. A. Montevecchi, and S. J. M. Blaber. 2000. The impacts of fishing on marine birds. ICES Journal of Marine Science 57:531-547.

Teather, K. L., and P. J. Weatherhead. 1989. Sex-specific mortality in nestling Great-tailed Grackles. Ecology 70:1485-1493.

Thomas, T. W., J. Blondel, P. Perret, M. M. Lambrechts, and J. R. Speakman. 2001. Energetic and fitness costs of mismatching resource supply and demand in seasonally breeding birds. Science 291:2598-2600.

Thorington, K. K., and R. Bowman. 2003. Predation rate on artificial nests increases with human housing density in suburban habitats. Ecography 26:188-196.

Thusius, K. J., P. O. Dunn, K. A. Peterson, and L. A. Whittingham. 2001. Extrapair paternity is influenced by breeding synchrony and density in the Common Yellowthroat. Behavioral Ecology 12:633-639.

Tinbergen, J. M., and M. W. Dietz. 1994. Parental energy expenditure during brood rearing in the Great Tit (*Parus major*) in relation to body mass, temperature, food availability and clutch size. Functional Ecology 8:563-572.

Tinbergen, J. M., and J. B. Williams. 2002. Energetics of incubation. Pages 299-313 *in* Avian Incubation: Behaviour, Environment, and Evolution (D. C. Deeming, Ed.). Oxford University Press, Oxford, UK.

Toh, Y., E. Oki, S. Oda, M. Tomoda, S. Tomisaki, Y. Ichiyoshi, S. Ohno, and K. Sugimachi. 1996. An integrated microsatellite length analysis using an automated fluorescent DNA sequencer. Cancer Research 56:2688-2691.

Tomás, G., S. Merino, J. Moreno, J. J. Sanz, J. Morales, and S. Garcia-Fraile. 2006. Nest weight and female health in the Blue Tit (*Cyanistes caeruleus*). Auk 123:1013-1021.

Toms, M., and P. Sterry. 2008. Garden Birds and Wildlife. AA Publishing, Basingstoke, UK.

Török, T. 1986. Food segregation in three hole-nesting bird species during the breeding season. Ardea 74:129-136.

Torres, R., and H. Drummond. 1997. Female-biased mortality in nestlings of a bird with size dimorphism. Journal of Animal Ecology 66:859-865.

Tremblay, I., D. Thomas, J. Blondel, P. Perret, and M. M. Lambrechts. 2005. The effect of habitat quality on foraging patterns, provisioning rate and nestling growth in Corsican Blue Tits *Parus caeruleus*. Ibis 147:17-24.

Trivers, R. L., and D. E. Willard. 1973. Natural selection of parental ability to vary sex ratio of offspring. Science 179:90-92.

Tullett, S. G., and D. C. Deeming. 1987. Failure to turn eggs during incubation: effects on embryo weight, development of the chorioallantois and absorption of albumen. British Poultry Science 28:239-249.

Turner, J. S. 2002. Maintenance of egg temperature. Pages 119-142 *in* Avian Incubation: Behaviour, Environment, and Evolution (D. C. Deeming, Ed.). Oxford University Press, Oxford, UK.

United Nations. 2008. World Urbanization Prospects: The 2007 Revision (Executive Summary). United Nations Department of Economic and Social Affairs/Population Division, New York, USA.

United Nations. 2009. World Population Prospects: The 2008 Revision http://esa.un.org/unpd/wpp2008/all-wpp-indicators_components.htm

United Nations. 2010. http://esa.un.org/unpd/wup/index.htm

United States Department of the Interior, Fish and Wildlife Service. 2008. 2006 National survey of fishing, hunting and wildlife-associated recreation. http://www.census.gov/prod/2008pubs/fhw06-nat.pdf

Václav, R., H. Hoi, and D. Blomqvist. 2003. Food supplementation affects extrapair paternity in House Sparrows (*Passer domesticus*). Behavioral Ecology 14:730-735.

Vallone, P. M., and J. M. Butler. 2004. AutoDimer: a screening tool for primer-dimer and hairpin structures. BioTechniques 37:226-231.

van Noordwijk, A. J., R. H. McCleery, and C. M. Perrins. 1995. Selection for the timing of Great Tit breeding in relation to caterpillar growth and temperature. Journal of Animal Ecology 64:451-458.

Varley. G. C., and G. R. Gradwell. 1960. Key factors in population studies. Journal of Animal Ecology 29:399-401.

Verboven, N., and M. E. Visser. 1998. Seasonal variation in local recruitment of Great Tits: the importance of being early. Oikos 81:511-524.

Verhulst, S. 1994. Supplementary food in the nestling phase affects reproductive success in Pied Flycatchers (*Ficedula hypoleuca*). Auk 111:714-716.

Verhulst, S., and J. M. Tinbergen. 1997. Clutch size and parental effort in the Great Tit *Parus major*. Ardea 85:111-126.

Visser, M. E., and L. J. M. Holleman. 2001. Warmer springs disrupt the synchrony of oak and winter moth phenology. Proceedings of the Royal Society of London, Series B 268:289-294.

Visser, M. E., A. J. van Noordwijk, J. M. Tinbergen, and C. M. Lessells. 1998. Warmer springs lead to mistimed reproduction in Great Tits (*Parus major*). Proceedings of the Royal Society of London, Series B 265:1867-1870.

Vleck, C. M. 1981. Energetic costs of incubation in the Zebra Finch. Condor 83:229-237.

Votier, S. C., R. W. Furness, S. Bearhop, J. E. Crane, R. W. G. Caldow, P. Catry, K. Ensor, K. C. Hamer, A. V. Hudson, E. Kalmbach, N. I. Klomp, S. Pfeiffer, R. A. Phillips, I. Prieto, and D. R. Thompson. 2004. Changes in fisheries discard rates and seabird communities. Nature 427:727-730.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. Genetics 166:1963-1979.

Ward, J. M., and P. L. Kennedy. 1996. Effects of supplemental food on size and survival of juvenile Northern Goshawks. Auk 113:200-208.

Weatherhead, P. J., R. Montgomerie, H. L. Gibbs, and P. T. Boag. 1994. The cost of extra-pair fertilizations to female Red-winged Blackbirds. Proceedings of the Royal Society London, Series B 258:315-320.

Weathers, W. W.1985. Energy cost of incubation in the Canary. Comparative Biochemistry and Physiology Part A 81:411-413.

Weir, B. S. 1990 Genetic Data Analysis. Sinauer Associatiates, Sunderland, Massachusetts, USA.

Westneat, D. F. 1994. To guard mates or go forage: conflicting demands affect the paternity of male Red-winged Blackbirds. American Naturalist 144:343-354.

Westneat, D. F., P. W. Sherman, and Morton, M. L. 1990. The ecology and evolution of extra-pair copulations in birds. Current Ornithology 7:331-369.

White, F. N., and Kinney, J. L. 1974. Avian incubation. Science 186:107-115.

Whittingham, L. A., and P. O. Dunn. 2000. Offpsring sex ratios in Tree Swallows: females in better condition produce more sons. Molecular Ecology 9:1123-1129.

Wiebe, K. L., and G. R. Bortolotti. 1992. Facultative sex ratio manipulation in American Kestrels. Behavioral Ecology and Sociobiology 36:49-57.

Williams, G. 1975. Sex and Evolution. Princeton University Press, Princeton, New Jersey, USA.

Williams, J. B. 1996. Energetics of avian incubation. Pages 375-416 *in* Avian Energetics and Nutritional Ecology (C. Carey, Ed.). Chapman and Hall, New York, USA.

Williams, T. D. 2005. Mechanisms underlying the costs of egg production. Bioscience 55:39-48.

Wilkin, T. A., A. G. Gosler, D. Garant, S. J. Reynolds, and B. C. Sheldon. 2009a. Calcium effects on life-history traits in a wild population of the Great Tit (*Parus major*): analysis of long-term data at several spatial scales. Oecologia 159:463-472.

Wilkin, T. A., L. E. King, and B. C. Sheldon. 2009b. Habitat quality, nestling diet, and provisioning behaviour in Great Tits *Parus major*. Journal of Avian Biology 40:135-145.

Wilson, J. D. 1992. Correlates of agonistic display by Great Tits *Parus major*. Behaviour 121:168-214.

Winkler, D. W. 1993. Use and importance of feathers as nest lining in Tree Swallows (*Tachycineta bicolor*). Auk 110:29-36.

Wint, W. 1983. The role of alternative host-plants species in the life of a polyphagous moth, *Operophtera brumata* (Lepidoptera: Geometridae). Journal of Animal Ecology 52:439-450.

Withers, P. C. 1977. Energetic aspects of reproduction by the Cliff Swallow. Auk 94:718-725.

Yapp, W. B. 1955. A classification of the habitats of British birds. Bird Study 2:111-121.

Ydenberg, R. C. 1984. The conflict between feeding and territorial defence in the Great Tit. Behavioral Ecology 15:103-108.

Ydenberg, R. C. 1998. Behavioral decisions about foraging and predator avoidance. Pages 343-378 *in* Cognitive ecology: the evolutionary ecology of information processing and decision making (R. Dukas, Ed.). The University of Chicago Press, Chicago, Illinois, USA.

Ydenberg, R. C., C. V. J. Welham, R. Schmid-Hempel, P. Schmid-Hempel, and G. Beauchamp. 1994. Time and energy constraints and the relationships between currencies in foraging theory. Behavioral Ecology 5:28-34.

Young, B. E. 1994. Geographic and seasonal patterns of clutch-size variation in House Wrens. Auk 111:545-555.

Zanette, L., J. N. M. Smith, H. van Oort, and M. Clinchy. 2003. Synergistic effects of food and predators on annual reproductive success in Song Sparrows. Proceedings of the Royal Society of London, Series B 270:799-803.

Zerba, E., and M. L. Morton. 1983. The rhythm of incubation from egg laying to hatching in Mountain White-crowned Sparrows. Ornis Scandinavica 14:188-197.

Ziegle, J. A., Y. Su, K. P. Corcoran, L. Nie, P. E. Mayrand, L. B. Hoff, L. J. McBride, M. N. Kronick, and S. R. Diehl. 1992. Application of automated DNA sizing technology for genotyping microsatellite loci. Genomics 14:1026-1031.

Zimmerling, J. R., and C. D. Ankney. 2005. Variation in incubation patterns of Red-winged Blackbirds nesting at lagoons and ponds in Eastern Ontario. Wilson Bulletin 117:280-290.

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