THE ROLE OF FOOD AVAILABILITY IN DETERMINING THE ENERGETIC AND LIFE HISTORY COSTS OF REPRODUCTION IN SHORT-LIVED BIRDS

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by

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A thesis submitted to

The University of Birmingham

for the degree of

DOCTOR OF PHILOSOPHY

Centre for Ornithology

School of Biosciences

College of Life & Environmental Sciences

The University of Birmingham

September 2012

Abstract

Food availability strongly affects avian breeding success. Conflicting results from food supplementation studies have obscured the role of food availability in shaping the life history trajectories of birds. With the popularity of providing food for wild birds increasing, the effects of this resource for breeding birds need to be clarified. In this study Blue Tits (*Cyanistes caeruleus*) and Great Tits (*Parus major*) were provided with supplementary food to investigate whether food availability reduced the costs of breeding for adults, and affected life history traits. Food supplementation with peanut cake disrupted the timing of Great Tit breeding and reduced fecundity. There was no effect of food supplementation on Great Tit adult or juvenile survival, except in 2010 when females traded off fecundity against future survival. Blue Tit fecundity was largely unaffected by food supplementation, but the provision of mealworms improved adult female survival. Food supplementation reduced female Daily Energy Expenditure (DEE) for both species during egg laying and for Great Tits feeding nestlings, revealing unexpectedly complex life history strategies. Through the integration of physiological techniques and life history frameworks we can understand the interaction between organisms and their environment and the effects of anthropogenic actions such as food supplementation of birds.

Acknowledgements

My research was funded by the Natural Environmental Research Council (NERC) through studentship NE/G001553/1 and by CJ Wildlife Ltd. through a CASE studentship award. The analysis of my doubly-labelled water samples was funded by the Swedish Research Council (grant number 621-2008-5349). My research would not have been possible without the provision of nestboxes, bird feeders and food by CJ Wildlife Ltd. and their support, enthusiasm and advice (in particular that of Chris Whittles, Peter Deans and Martin George) have been invaluable. I would like to thank the Leverhulme Trade Charities Trust for providing me with extra funding during my write-up period, and The Society for Experimental Biology and the Company of Biologists for a travel grant which enabled me to present some of my results at the European Ornithological Union meeting in Riga in 2011. Thanks are also due to Trovan IDUK for the supply of Passive Integrated Transponder (PIT) tags and readers, AC Hughes for the supply of colour rings and Livefoods Direct Ltd., Sheffield, Yorkshire for the supply of mealworms (*Tenebrio molitor*). I am extremely grateful to The Worcestershire Wildlife Trust for their permission to conduct research in Chaddesley Woods National Nature Reserve, Worcs., and for their continuing media support and enthusiasm for the project. My thanks also go to the Home Office, Natural England and the British Trust for Ornithology (BTO) for granting me licenses to perform my research.

My study would not have been possible without the aid of a large contingent of field assistants and volunteers. Firstly, I need to thank the fantastic volunteers of the Worcestershire Wildlife Trust for their aid with caterpillar sampling, nestbox maintenance and cutting net rides. In particular, I am indebted to Rose and Mervyn Needham, for their endless assistance, humour and an inexhaustible supply of tea. Thanks also go to Peter Beasley for providing some beautiful photographs of Chaddesley birds on his feeders and to Brian Rickett for his continuing support of the project. I am extremely grateful to the members of Birmingham University Ringing Group, especially Michael Barstow, Phil Ireland, Leigh Kelly, Tony Kelly, Andrew and Karen Moss, and Dan and Jane Potter, for their enthusiasm, dedication and for the supply of cake and sausage sandwiches. I would also like to thank those who helped with fieldwork during the breeding season, as my study would not have happened without them, namely, Michael Barstow, Dr Adam Bates, Helen Bates, Joe Berry, Dr Rebecca Boulton, Dr Phill Cassey, Emily Challis, Neil Croton, Camille Duval, Professor Michael Griesser, Laine Hart, Leigh and Tony Kelly, Kirsten Miller, Chris Nicholls, Dan Potter, Dr Jolyon Troscianko, Tom Wallace, and Alan West. In addition I would like to thank our many British Council students (Ishmail Bah, Livia Berti, Ivana Budinski, Marija Dundovic, Andrea Grimmer, Vedran Lucic, Marija Majer and Petra Svoboda), who have worked so hard every year helping with caterpillar sample processing and a multitude of other essential tasks.

My first field season in 2008 was spent working with Professor Marcel Visser's team at The Netherlands Institute of Ecology (NIOO) in Heteren. I will be eternally indebted to Marcel, Dr Andrey Bushuev, Timur Dimaz, Dr Luc te Marvelde, Dr Franciso Pulido, and Dr Sonja Schaper in particular for teaching me so much about how to study my study species, how to use the doubly-labelled water technique, for providing inspiration and continuing friendship, and for teaching me how to ride a bicycle whilst carrying a ladder. I also wish to thank the staff at the Centre for Isotope Research at the University of

Groningen in The Netherlands, in particular Professor Gertjan van Dijk and Berthe Verstappen-Dumoulin for such rapid processing of my samples and for technical advice.

I am profoundly grateful to Professor Michael Griesser (University of Bern) for providing financial support, without which the energetics aspect of this study would not have been possible and to Michael and Katharine Bowgen for their immense enthusiasm in helping me to think through some of my results. There were many people who provided advice regarding statistics and experimental design, including Dr Phill Cassey, Dr Will Cresswell, Dr James Grecian, Dr Steve Portugal, Emma Rosenfeld, Dr Jon Sadler, and Dr Jolyon Troscianko, all of whom helped me make sense of my challenging ecological data. For help in developing research ideas during the early part of my study, I am much obliged to the Supplementary Feeding Working Group: Professor Stuart Bearhop (University of Exeter), Dr Jon Blount (University of Exeter), Nick Carter (Gardman Ltd.), Dr Dan Chamberlain (University of Turin), Dr Dave Leech (BTO), Dr Kate Plummer (University of Exeter) and colleagues from Birmingham.

I cannot thank enough my Chaddesley co-workers, Kaat Brulez, Dr Tim Harrison, and Dr Jen Smith for being fantastic fieldworkers and friends. Without their endless passion for the birds, nestboxes, random events and teacakes of Chaddesley, the fieldwork would have been a lot less fun. Thanks also go to the wonderful group of researchers at Birmingham to whom I owe huge thanks for their support, ideas and friendship, including Dr Adam Bates, Dr Rebecca Boulton, Dr Phill Cassey, Dr Jackie Chappell, Zoe Demery, Camille Duval, Laine Hart, Dr Katy Jones, Kirsten Manduell, Dr Golo Maurer, Dr Julia Myatt, Dr Abi Phillips, Dr Steve Portugal, Emma Rosenfeld, Dr Susannah Thorpe, Emma Tecwyn, and Dr Jolyon Troscianko. I would also like to thank my ringing trainer Terry Coombs for teaching me so much about ringing, and Ben Roberts, Richard Facey, Adele

Powell and Dr Claire Salisbury for sharing motivational thoughts for the duration of my PhD.

My wonderful housemates during my time in Birmingham, Eleni Mouatsou, Maria Antonopoulou, Tara Millar, Anupa Parmar, Nicole Thomson and Evgenia Andreopoulou, deserve a special mention. Their tolerance of the early mornings and the mud, their supply of food, laughter and amazing friendship made the Birdhouse into my Birmingham family. Thanks in particular go to Tara Millar for taking some truly inspired photographs of my fieldwork.

I owe an immense debt to my supervisors, Dr Jim Reynolds and Professor Graham Martin, whose patience, dedication and encouragement have helped me immeasurably over the years. I sincerely hope that this thesis is a reflection of the amount of time you have devoted both to my PhD study and to the project as a whole, and cannot thank you enough for the support, creativity and stimulating discussions that have helped me to navigate through the choppy waters of avian ecology.

Finally, I would like to thank my sleepy field assistant, Neil for being a constant inspiration, and my family, without all of whom I would never have achieved all that I have over the last four years. The ultimate mention, however, goes to the resilient Chaddesley Blue and Great Tits, for being such endlessly fascinating study species.

Table of Contents

List of Figures

List of Tables

Chapter One

General Introduction

1.1. Life history theory

Life history theory is a framework to explain the complex diversity of reproductive strategies and life cycles evident in both the animal and plant kingdoms (Stearns 1992). The overarching theory has predominantly been employed to interpret how evolutionary natural selection can result in different life cycles at the inter-specific scale (Stearns 1977, 1992). Life history theory is also useful in understanding intra-specific variation in reproductive strategies (Partridge & Harvey 1988, Lessells 1991) and the ecological and reproductive strategies employed by different organisms to ensure maximal Darwinian fitness (Darwin 1859, Spencer 1864). Such understanding enables us to make predictions of how species and individuals will respond to fluctuating environmental conditions such as rapid climate change (Winkler *et al.* 2002, Visser *et al.* 2006) or decreased food availability (Pascual & Peris 1992).

1.1.1. Life history traits and trade-offs

The basis of life history theory is that a series of life history traits (e.g. adult body size, age at first breeding attempt, number of offspring produced, lifespan) characterise the life cycle of an organism (Stearns 1992). These traits are ineluctably linked to each other by trade-offs between, for example, current reproduction and survival, current and future reproduction and the number, size and sex of offspring produced (Stearns 1989a). The keystone of life history trade-off theory is the assumption that organisms function within a finite pool of resources (e.g. time, energy) and that allocation of a subset of those resources to one trait necessarily diminishes the residual resource available for allocation to another trait (van Noordwijk & de Jong 1986). Fluctuating food availability, in particular, has been acknowledged (e.g. Martin 1987) to play a pivotal role in determining which life history strategies are most successful in fitness terms. This relationship is evident both at the inter-specific level as embodied in *r/K* selection theory (Pianka 1970, Stearns 1976), and also at the intra-specific population level, as embodied in bet-hedging theory (Olofsson *et al.* 2009). Bet-hedging is a means of defining strategies for lifetime reproductive success with these strategies varying in response to temporal environmental variation (reviewed in Partridge & Harvey 1988, Simons 2011). The terms *r*- and *K*-selection may have a broad range of interpretations (Parry 1981) and the use of the terminology and the principles has been much questioned (Stearns 1992), but the application of the theory to some modern life history evolution studies (e.g. Reznick *et al.* 2002), has aided in interpreting their results. The ecological meaning of *r*- and *K*-selection is commonly defined as follows: *r*-selection is associated with earlier maturity, more and smaller young, larger reproductive effort within each breeding attempt and shorter lifespan, while *K*-selection is associated with the inverse (see Table 1 in Pianka 1970, Stearns 1977). Selection as viewed in this manner is expected to be a continuum rather than a dichotomous categorisation (Pianka 1970).

Avian species can further be divided into ‘capital’ and ‘income’ breeders (Drent & Daan 1980), although again this may not be a dichotomous distinction and, used intra-specifically, breeding females may be able to shift strategically along the capital-income axis (Meijer & Drent 1999, Houston *et al.* 2007a). Those species that sequester fat and micronutrient reserves for depletion during breeding, such as Anseriform species (e.g. Alisauskas & Ankney 1994), are defined as ‘capital’ breeders, and those that do not store nutrients but are reliant on extrinsic resources (such as Blue Tits, *Cyanistes caeruleus* and Great Tits, *Parus major*) are defined as ‘income’ breeders (Drent & Daan 1980, Meijer & Drent 1999).

1.1.2. Measuring current reproductive value in birds

These theoretical life history frameworks can greatly aid our understanding of observed intra-specific variation in life history traits within varying environmental conditions (Drent & Daan 1980, Martin 1987, Lessells 1991). Of particular interest for those studying reproductive biology are the theoretical trade-offs between current and future reproductive investment, and the resultant number and size (or quality) of offspring produced (Lessells 1991, Bennett & Owens 2002). In avian species a number of parameters are commonly used to characterise reproductive success in the current breeding attempt. These include the date of clutch initiation (laying date – e.g. Verhulst & Tinbergen 1991), the number of eggs laid (clutch size – e.g. Pettifor *et al.* 1988), the size of eggs laid (egg size or mass – e.g. Christians 2002), the number of nestlings (brood size – e.g. Tinbergen 1987) or size of nestlings (nestling size or mass – e.g. Both *et al.* 1999) that hatch and survive to fledging, and the number or size of offspring that survive to independence (post-fledging survival – e.g. Naef-Daenzer *et al.* 2001) or become part of the breeding population (offspring recruitment – e.g. Verboven & Visser 1998). Directional selection has been found to shape certain of these parameters or life history traits (e.g. for larger clutch sizes – Boyce & Perrins 1987, for heavier nestlings – Lindén *et al.* 1992, for earlier laying dates – Visser *et al.* 1998), although the optimal value for these traits can vary between years (e.g. Boyce

& Perrins 1987). This identifies these reproductive parameters as key contributory components of an individual's lifetime reproductive success, and to the persistence of the expression of that life history trait within the population.

Although selection is seen to favour certain expressions of these parameters, there exists large intraspecific variance in each of them in some species (e.g. Blue Tit clutch size – Nur 1986, e.g. Great Tit egg size – Perrins 1996, e.g. Great Tit laying date – Visser *et al.* 2011a). For example, although there are selective advantages for early breeding in many avian species (reviewed in Perrins 1970, Daan *et al.* 1989), variation in laying date is maintained. Similarly, although larger clutch and brood sizes usually produce the most surviving offspring (demonstrated experimentally by Tinbergen & Sanz 2004), lower clutch and brood sizes are maintained. Such variation supports life history trade-off theory, as extrinsic circumstances may represent constraints which 'prudent parents' (Drent & Daan 1980) are unable or unwilling to overcome, and as environmental stochasticity maintains variability in optimal values for life history traits (e.g. of clutch size – Boyce & Perrins 1987). In life history terms one key determining factor which limits the number of offspring produced by an individual in a current reproductive attempt is the probability of survival of that individual to a subsequent reproductive attempt. This is because over-investment in the current breeding attempt is predicted by life history theory to influence negatively the chances of adult survival to a subsequent breeding attempt (Stearns 1992), although the possible physiological mechanisms through which this happens remain contentious (Zera & Harshman 2001).

1.1.3. Residual reproductive value

The combined value to the individual of survival beyond a current breeding attempt, and the possibility of a future reproductive attempt is termed ‘residual reproductive value’ (Williams 1966). The trade-off between the level of parental investment in a current breeding attempt and that remaining for residual reproductive value is the basis of the theorised cost of reproduction (Williams 1966, Partridge & Harvey 1985, Newton 1989). This trade-off predicts a predominantly negative relationship between current reproductive effort (e.g. in clutch size) and the probability of future survival (Williams 1966, Stearns 1989a). Much evidence of this negative correlation has been garnered, both at the inter-specific (reviewed in Martin 1995) and intra-specific (e.g. Bryant 1979) level. Positive correlations between such life history traits as fecundity and survival are theorised to exist, however, in conditions of higher resource availability (van Noordwijk & de Jong 1986) when the relative cost of both fecundity and survival are reduced. The concept of a trade-off between the quantity and quality of offspring has been given empirical support by repeated studies demonstrating that the largest clutch or brood sizes are not always selected for and may not improve an individual’s lifetime reproductive success (Kluijver 1951, Boyce & Perrins 1987, Pettifor *et al.* 1988). However, such evidence (e.g. Boyce & Perrins 1987) has also been used to provide evidence of a conservative bet-hedging strategy wherein parents reduce their investment in a year of low food availability to reduce the likelihood of total brood failure (Partridge & Harvey 1988).

1.1.4. Experimental techniques for increasing reproductive costs

Life history theorists (e.g. Partridge & Harvey 1985, Nur 1988) have advocated the investigation of reproductive life history trade-offs through experimental manipulation, to avoid the possible confounding effects of adult body condition and structural size. A multitude of experimental techniques have been used to increase the cost to adults of breeding attempts with the expectation of finding downstream negative repercussions. These techniques have included increasing clutch or brood size (e.g. Visser & Lessells 2001), partner removal (e.g. Saetre *et al.* 1995), feather cutting to increase flight costs (e.g. Slagsvold & Lifjeld 1988) and playback of nestling begging calls (e.g. Hinde 2006). Conversely, some experimental approaches have tried to reduce the cost to adults of breeding attempts by reducing clutch or brood size (e.g. Verhulst & Tinbergen 1997), heating nestboxes to reduce thermal costs (e.g. Bryan & Bryant 1999), direct feeding of nestlings (e.g. de Neve *et al.* 2004) and through food supplementation of adults (e.g. Moreno *et al.* 1999). The results from such studies have been equivocal, particularly when seeking evidence of inter-annual trade-offs. Although some studies (e.g. Nur 1984b, Visser & Lessells 2001) have supported the prediction of an inverse relationship between reproductive effort and survival or future fecundity, others (e.g. Moreno *et al.* 1999, Tinbergen & Sanz 2004) have reported no effect at all, or even a positive relationship (Hõrak 2003). Such positive relationships (e.g. between the number of young raised and adult survival) are more commonly found in un-manipulated populations, as in a correlative study of Song Sparrows (*Melospiza melodia*) (Smith 1981). In longer-lived species such as Eurasian Magpies (*Pica pica*), positive relationships (e.g. between clutch size and adult survival) could be related to increased fecundity in older more experienced adults (Hogstedt 1981). One of the key differentiating factors between these studies, which may explain conflicting results, is the metric used to quantify adult investment in the current reproductive attempt.

1.2. Metrics of parental investment

The concept of trading off investment in current reproduction against that of future breeding attempts necessitates quantification of the former. Many experimental life history studies are predicated upon a theorised cost of reproduction (reviewed in Dijkstra *et al.* 1990, Golet *et al.* 1998), so evidence of over- or under-investment in the current reproductive attempt is expected to be balanced respectively by decreased or increased survival to a future reproductive attempt. There have been a variety of metrics used to examine how much of their intrinsic or extrinsic resources parents are allocating to the current reproductive attempt. Most experimental studies have used breeding parameters such as egg size (e.g. Williams 2001a) or adult or offspring body mass trajectories (e.g. Nur 1984b, Both *et al.* 1999) to quantify parental investment in the current reproductive attempt. Gaining additional metrics of parental investment such as the work rate of brood-rearing adults (e.g. Moreno *et al.* 1999), time budgets (e.g. Enoksson 1990) or energy expenditure (Tinbergen & Dietz 1994) can clarify how much effort a parent is devoting to a specific activity or phase of the reproductive period. These metrics allow studies to compensate for individual variation in localised resource availability or parental quality, which may affect the timing of reproduction (e.g. Verhulst *et al.* 1995) or how many young are produced (e.g. Tremblay *et al.* 2003), The metrics give a measurement of how much energy, time or effort parents are allocating to the breeding attempt as well as quantifying the end product (e.g. clutch size). Such information can subsequently provide evidence of energetic bottlenecks, when energy availability may limit reproductive effort, such as during incubation in Blue Tits (Nilsson 1994) or during brood rearing in Northern Wheatears *Oenanthe oenanthe* (Moreno & Hillström 1992).

1.2.1. Measuring parental investment using provisioning rates

One of the most commonly used metrics of parental investment in altricial species is the provisioning rate of parents feeding nestlings (e.g. Gibb 1955, Moreno *et al.* 1995, Weimerskirch *et al.* 2000, Grieco 2002a). Provisioning rates have been measured by visual observation at nests (e.g. Henderson & Hart 1993), by automated mechanical recorders (e.g. Gibb 1955) or balances (e.g. Jones 1987), using video recordings (e.g. Moreno *et al.* 1995) or using electronic recording equipment (e.g. Rose 2009, Wilkin *et al.* 2009a). They have been used predominantly to explore foraging ecology (e.g. Jones 1987, Tremblay *et al.* 2005) by measuring the number of visits a nestling receives. They have also been used in a broader context to investigate the balance between nestling demands and parental effort (e.g. parent-offspring conflict – Ottosson *et al.* 1997) and to examine the division of labour between parents (e.g. in testing game-theoretical models – Johnstone & Hinde 2006). There is large inter-pair variation in provisioning rates (e.g. Rose 2009), which has been linked to greater parental effort as feeding rates increase with nestling age (e.g. Barba *et al.* 2009), lower prey availability (e.g. Naef-Daenzer *et al.* 2000), and larger brood sizes (e.g. Wright *et al.* 1998).

There have been questions raised as to the efficacy of all measures of reproductive investment; for example, loss of body mass in adults may be an adaptive strategy to increase flight efficiency, rather than an indication of a cost being incurred (e.g. Cavitt & Thompson 1997). However, provisioning rates of brood-rearing adults have been questioned as a measure of parental effort from the very earliest studies of adult feeding rates (e.g. Gibb 1955). This is because they may not necessarily reflect the quality of delivered food and, hence, give little indication of prey selection and foraging effort (Grieco 2002b, Mock *et al.* 2009). Provisioning rates do not increase linearly with brood size, and large broods receive fewer visits per nestling than small broods (e.g. Royama 1966, Wright *et al.* 1998). This does not indicate that parents are investing less, however, as parents may alter their foraging strategy or increase the load being delivered to nestlings to maintain an equivalent food intake per nestling (e.g. Wright *et al.* 1998). Therefore, additional metrics of parental effort or prey quality are required to support provisioning rate data when examining parental investment.

1.2.2. Measuring parental investment using energy expenditure

Integrating provisioning rate measures with additional metrics such as Daily Energy Expenditure (DEE) or metabolic rates (e.g. Basal Metabolic Rate [BMR], Field Metabolic Rate [FMR]) could give a much more valid picture of investment by an adult in a current breeding attempt (e.g. Nilsson 2002, Ricklefs & Wikelski 2002, te Marvelde *et al.* 2011b). However, there is significant unexplained inter-individual variation in rates of energy expenditure (reviewed in Williams 2001b), and some studies (e.g. Verhulst & Tinbergen 1997, Welcker *et al.* 2009) have produced evidence of no relationship between current energetic reproductive investment and residual reproductive value. Therefore, studies incorporating a variety of metrics may be preferable to gain the most insight into parental investment (Bryant 1988, Williams 2005).

Energy expenditure or metabolic rate is most commonly measured in three ways, through monitoring respiration directly using respirometry (e.g. de Heij *et al.* 2007) to calculate BMR, or indirectly through heart-rate monitoring (e.g. Green *et al.* 2009) to calculate oxygen consumption (converted to FMR) or through the doubly-labelled water (DLW) technique (e.g. Bryant & Westerterp 1983b) to calculate carbon dioxide consumption (converted to DEE). Respirometry measurements from captive animals can attribute an energetic price to a specific activity such as diving in duck species (e.g. Stephenson 1994) or the energetic demands of flight in passerine species (e.g. Ward *et al.* 2001). Heart rate monitoring and DLW measurements have been used more widely in field studies to gain insights into the metabolic demands of behavioural stages such as incubation (e.g. Weimerskirch *et al.* 2002) or feeding nestlings (e.g. Tinbergen & Dietz 1994).

Due to the size of heart rate loggers and radio transmitters required for heart rate telemetry (Butler *et al.* 2004), the DLW technique is more appropriate for use with small passerines. DEE obtained from DLW measurements is an integrated measure of energy expended over a 24 hour period and so is useful for studies examining the energetic investment in all activities at a specified point in the annual cycle (e.g. Bryant & Tatner 1991). In investment terms this would equate to attributing an energetic cost (or energetic price – Zera & Harshman 2001) to the egg laying phase rather than to the cost of producing one egg (Williams 2005). When using the DLW technique, stable isotopes of oxygen (^{18}O) and hydrogen (^{2}H, deuterium) are administered to the focal animal. The slope of the washout rate of the isotopes between an initial and final tissue sample (usually blood) is then calculated to ascertain the elimination rate of isotopes from the body as the ^{18}O is diluted by inhaled oxygen (O_2) or expelled either as waste water (H_2O) or exhaled carbon dioxide (CO_2) (Lifson & McClintock 1966, Speakman 1997). Deuterium is only flushed out with the body water and so the differential between the turnover rate of ^{18}O and ^{2}H allows the exhaled CO_2 to be calculated and then converted into DEE.

The DLW technique has been criticised for the unexplained inter-individual variation remaining after other variables have been accounted for (Butler *et al.* 2004). Indeed studies of DEE and manipulated brood size demonstrate conflicting results in passerines: for example, increased DEE was found with experimentally increased Great Tit brood size – (Sanz & Tinbergen 1999), compared to no increase in DEE with experimentally increased Pied Flycatcher (*Ficedula hypoleuca*) brood size (Moreno *et al.* 1997). The inter-individual variation in DEE could account for conflicting results in studies using energy expenditure to quantify parental investment and could be why increased DEE has not been linked consistently to increased risk of mortality (but see Deerenberg *et al.* 1995). Alternatively brood size manipulations in particular could result in non-optimal responses by parents as the perception of the value of the brood to the parent is altered (reviewed in Lessells 1993). A key variable which is missing from many DEE studies, however, is food availability (e.g. Moreno *et al.* 1997, Sanz & Tinbergen 1999), which may play a critical role in how much energy parents are able to invest in their offspring (e.g. te Marvelde *et al.* 2011b). It is not clear, however, whether individuals raise their DEE when food availability is higher (e.g. Bryant & Tatner 1991), or whether lower food availability demands higher DEE because foraging conditions are more difficult (e.g. te Marvelde *et al.* 2011b). Clarification on the role that resource availability plays in contributing to energy expenditure could be gained through manipulation of food supply, but very few studies have achieved this.

1.3. Food availability and reproduction

From some of the earliest modern studies of avian ecology (e.g. Lack 1947, Gibb 1950), resource availability has been acknowledged as playing a key role in enabling or constraining reproductive success. David Lack's seminal work introduced the concept that the number of offspring a parent produces is determined by the number of nestlings that they can successfully provision, which, in turn, is determined by food availability during the nestling phase (Lack 1947, 1954). Perrins (1970) then suggested that food availability acted as a constraining factor on the onset of egg production but not on the number of eggs produced. There is, however, little information on the diet of insectivorous bird species pre-egg laying to explore Perrins' assertion (but see Eeva *et al.* 2000 for an study of arthropod abundance throughout the breeding season of passerines). For example, there have been no studies of the diet of Great Tits immediately before egg laying until very recently (te Marvelde 2012). Studies of food availability during egg laying could inform of how the nutritional demands of egg production are met or constrained by natural food availability and the paucity of such studies is surprising. Logistical difficulties in quantifying individual consumption of invertebrate species may be the primary reason for this. In a recent study, te Marvelde *et al.* (2012) linked sudden increases in key high-protein arthropod species such as the larvae of *Coleophora laricella* and *Diptera* spp. to the onset of egg laying in Great Tits, and demonstrated with radio-tracking that females would travel considerable distances to gain specific prey items. Prior to this study, however, the primary means by which studies have investigated the importance of energy, protein or micro-nutrient availability before egg production has been through food supplementation experiments, which have tested the effect of specific nutrients on egg laying females (e.g. with high-energy or high-protein supplements Nager *et al.* 1997).

It has long been maintained (Gibb 1950, Perrins 1965) that many avian species adjust their timing of reproduction so that maximum nestling demand coincides with seasonal peaks in natural food availability, and that the accuracy of such timing may be a selective pressure on offspring recruitment (van Noordwijk *et al.* 1995). This selective pressure may be determined by the emergence or availability of key prey species such as the Winter Moth (*Operophtera brumata*) or *Tortrix spp.* caterpillars for Blue and Great Tits (e.g Betts 1955, van Noordwijk *et al.* 1995), or the Lesser Sandeel (*Ammodytes marinus*) for seabird species (e.g. Rindorf *et al.* 2000). Such prey species can constitute up to 72% of the diet of passerine nestlings (e.g. Lepidoptera larvae or pupae for Blue Tit nestlings of which 58% of larvae were *Tortricoid* or Winter Moths) (Betts 1955), or up to 100% of the diet of seabird nestlings (e.g. Lesser Sandeels for Black-legged Kittiwake (*Rissa tridactyla*) and European Shag (*Phalacrocorax aristotelis*) nestlings) (Rindorf *et al.* 2000). There may be flexibility in the constitution of nestling diet, however, even in conditions of high abundance of key prey species (e.g. in Great Tits Royama 1970). Using an index such as the availability of key prey groups such as caterpillar species to Great Tits has provided insights into the cost and success of well-timed reproductive attempts (e.g te Marvelde *et al.* 2011b), however, and such indices may be used as predictors of a species' response to rapid environmental change (Visser *et al.* 1998, Both *et al.* 2009).

Although the timing of breeding attempts is undoubtedly critical to reproductive success, it may be constrained by the availability of food (Perrins 1970) or micronutrient resources such as calcium (Reynolds & Perrins 2010) prior to egg laying. From the first food supplementation experiments (e.g. Källander 1974, Yom-Tov 1974), it was found that supplementation advanced the laying date of breeding birds and this has proven to be one of the most consistent effects of subsequent food supplementation studies (summarised in Boutin 1990, Nager *et al.* 1997, Robb *et al.* 2008a). Food availability may, therefore, not only act as a factor constraining the onset of reproduction (Perrins 1970), but also as a cue to indicate the timing of peaks in food availability later in the season (Schultz 1991, Källander & Karlsson 1993). Very few food supplementation studies have monitored natural food availability (but see Grieco *et al.* 2002, Bourgault *et al.* 2009), however, to examine whether removing a theorised energetic constraint that prevented earlier egg laying enabled more of the population to match the timing of their breeding attempt to peak prey abundance.

1.3.1. Food supplementation and reproduction

The issue of whether food availability limits the onset of reproduction or the number of offspring produced has been empirically tested using food supplementation experiments (reviewed in Boutin 1990, Robb *et al.* 2008a). Food supplementation has the advantage over monitoring natural food availability of testing the effect of increased food resources at specific points in the reproductive cycle, and of testing for the removal of constraints imposed by the need to acquire particular nutrients. Food supplementation also circumvents the problem of high inter-territory variability in the timing of peak arthropod availability (e.g. Tikkanen & Julkunen-Tiitto 2003, Tremblay *et al.* 2003). Results from food supplementation experiments have varied considerably, however (summarised in Table 1.1, reviewed in Boutin 1990, Nager *et al.* 1997, Robb *et al.* 2008a).

The provision of supplementary food resources increases clutch size in many species such as Great Tits (e.g. Nager *et al.* 1997), but clutch size may also remain unaffected by supplementation in species such as Red-winged Blackbirds (*Agelaius Phoeniceus*) (Ewald & Rohwer 1982), or even decrease, as was found in Great and Blue Tits (Harrison *et al.* 2010). This variation in response could be partly due to variation in natural food availability; the effect of food supplementation on clutch size varies considerably inter-annually (Nager *et al.* 1997), and supplementation may have a more profoundly positive effect in years of low natural food availability (Schultz 1991, Nager *et al.* 1997). Alternatively, the timing or nutritional composition of food supplementation

Table 1.1. Summary of the percentage of studies of avian species showing positive, negative or no effect of food supplementation on the specified parameters (updated from Table 2: Robb *et al.* 2008a). The number of studies (*N*) representing the direction or each effect are in the adjacent column. Only statistically significant results are reported. For references of studies included please see Appendix One.

	Response to Food Supplementation					
Breeding parameter	Positive effect (%)	*N*	Negative effect (%)	*N*	No effect (%)	*N*
Advanced Laying Date	61.0	42	1.0	1	38.0	26
Clutch Size	44.4	32	3.0	2	53.0	38
Egg Size/Quality	42.0	17	0	0	59.0	24
Fledging Success	58.4	31	0	0	41.0	22

may dictate how a study population responds to additional food resources. For example, providing a food supplement after egg laying has begun may not have any effect on clutch size (e.g. Nilsson & Svensson 1993). Similarly, it is not known how resource availability affects the physiological processes involved in egg formation (reviewed in Williams 2005), so determining at what point an increase in food availability should trigger the onset of laying, or whether it could influence the number of eggs produced, is problematic. The laying date of species such as Great Tits (e.g. Visser *et al.* 2009) is inextricably linked to an increase in ambient temperature, so food supplementation may not advance breeding if ambient temperatures are low. To examine the effect of food supplementation on laying date and clutch size, it would be advisable to begin supplementation far in advance of laying, but not so early as to give potential overwinter survival benefits (e.g. Brittingham & Temple 1988).

Conflicting results from food supplementation studies (Table 1.1) may also be attributable to differences in individual intake of supplements. Harrison (2010) concluded from Stable Isotope Analysis that a high energy (peanut cake) and high protein (live mealworms) supplement formed a very small proportion of the diet of breeding Blue and Great Tits. The peanut cake supplement formed 10 % of the adult diet and 13 % of the nestling diet in both species, and the mealworm supplement formed 1 % of the adult diet and 3 % of the nestling diet in both species. Variation in individual consumption, however, led to significant increases in productivity (e.g. larger clutch size with increased intake of peanut cake – Harrison 2010) and on the timing of breeding (e.g. greater advance in laying date with increased intake of peanut cake in Blue but not Great Tits – Harrison 2010).

Food supplementation studies have also investigated the role of food availability later in the breeding season (reviewed in Martin 1987). They have shown that increased food availability may accelerate incubation (e.g. Harrison 2010), increase hatching or fledging success (e.g. Nilsson & Smith 1988), increase nestling body mass (e.g. von Brömssen & Jansson 1980, Arcese & Smith 1988), and improve offspring survival prospects (e.g. Verhulst 1994, Reynolds *et al.* 2003b). Food supplementation experiments have generally supported life history trade-off theory (Kacelnik & Cuthill 1990), with increases in food availability commonly associated with increased fecundity. Although positive covariance of life history traits such as fecundity and survival can be predicted from life history models in response to increased food availability (van Noordwijk & de Jong 1986), there is a conspicuous lack of empirical evidence from multi-generational long-term food supplementation studies to test the theory. However, food-supplemented adult Song Sparrows have been found to have lower survival prospects following increased reproductive investment (Arcese & Smith 1988). In another study of European Pied Flycatchers (Verhulst 1994), female survival prospects were significantly improved when mealworms were provided, although there was no significant increase in clutch size. Clearly there is a need for food supplementation studies that monitor metrics of parental investment that can then be related to both adult and offspring survival.

1.3.2. Variation in nutritional value of food supplements

Food supplementation is a tool not only for studying the effect of food quantity on reproductive parameters, but also for investigating which macro- and micronutrients constrain reproduction (reviewed in Nager *et al.* 1997, Harrison 2010). Common food supplements that are supplied by the general public feeding birds may contain a wide variety of nutrients (Lin 2005), although supplements that are high in energy rather than protein tend to be most popular (e.g. sunflower seeds, peanuts – Lin 2005). Energy-rich supplements may be useful to isolate whether females are constrained by energy specifically prior to or during egg laying, but such high-energy supplements should be low in other nutrients such as protein (e.g. Harrison *et al.* 2010). Supplements that are high in energy have been used to investigate whether there is an energetic constraint on the timing of egg laying or on the size and number of eggs laid (Nager *et al.* 1997, Reynolds *et al.* 2003a). A high-energy supplement advanced laying but did not increase clutch size independently in Florida Scrub-Jays (*Aphelocoma coerulescens*) (Reynolds *et al.* 2003a), but did increase the clutch size of Great Tits (Nager *et al.* 1997). In the latter case, however, the high-energy supplement included sunflower seeds which have a protein component (Kaffka *et al.* 1982).

The availability of protein or specific amino acids has been suggested to limit egg production (e.g. Murphy 1994), rather than energy, and the provision of high protein food sources to egg laying birds has been demonstrated to increase egg size (e.g. Ramsay &

Houston 1997, Reynolds *et al.* 2003a). In contrast, a low protein diet in captive birds such as Zebra Finches (*Taeniopygia guttata*) has been shown to have longer term effects, reducing maternal body mass and the fecundity of offspring (Gorman & Nager 2004). However, the Nager (1997) study of Great Tits found no difference in clutch or egg size when females were supplemented with a high-protein as opposed to a high-energy diet. The high-energy supplement (sunflower seeds) did have a protein component though, as previously discussed. The nature of the high-protein supplement used in each of these studies of wild birds varied widely, however, and included boiled chicken egg (Ramsay & Houston 1997), mealworms (Nager *et al.* 1997) and pre-formed high-protein pellets (Reynolds *et al.* 2003a). The choice of supplement should be determined by the nature of the investigation; mealworms are higher in protein at up to 28% (Ghaly & Alkoaik 2009) than are sunflower seeds at 19% (Kaffka *et al.* 1982), but they appear not to affect clutch size across a number of studies (reviewed in Harrison 2010). Similarly peanuts contain a similar amount of protein to mealworms at 28.5% (Hoffpauir 1953), but both peanuts and mealworms may not contain the specific amino acids required for egg formation (Murphy 1994). To address the question of whether females are energy limited rather than protein limited during egg laying definitively would require measurement of energy expenditure in conjunction with food supplementation, as has been conducted on captive birds (Vézina *et al.* 2006).

Food supplementation studies (e.g. Moreno *et al.* 1999, Jodice *et al.* 2002) conducted during the nestling phase have focussed predominantly on the provision of food which is suitable for nestlings, to reduce the cost of brood provisioning. Although adults consume these supplements (e.g. Cucco & Malacarne 1997), studies which differentiate between a supplement which parents would use purely for self-feeding and one which can be fed to nestlings are rare (but see Harrison 2010). An experimental design with distinct food supplements for adult and nestling feeding would aid in the interpretation of results from a life history perspective; one supplement would reduce somatic maintenance costs, and the other the cost of investment in offspring.

1.3.3. Food supplementation – a nationwide experiment

It is estimated that up to 60% of UK households (DEFRA 2002) and 47% of US households (USFWS 2006) regularly provide food for wild birds in gardens and backyards, spending annually approximately £200 million and $3,350 million, respectively (BTO 2006, USFWS 2006). In an increasingly urbanized society (United Nations 2009) these figures are set to rise, as people seek to benefit from closer contact with nature (Jones & Reynolds 2008, Jones 2011), and the global bird seed market is estimated to be growing by 4% per year (Lin 2005). Following recommendations on when to feed birds from ornithological organisations such as the BTO and the Royal Society for the Protection of Birds (RSPB) (Toms & Sterry 2008), between 40% (Cowie & Hinsley 1988) and 81% (Nicholls & Reynolds 2011) of the UK households involved in feeding birds now provide food for birds during the summer as well as the winter. This represents an unprecedented nationwide food resource which is now available during the breeding season as well as overwinter.

The impact of a protracted increase in food resources during the breeding season has been little explored and indeed may counter-intuitively reduce the reproductive output of species such as Blue and Great Tits (Harrison *et al.* 2010). Given the enthusiasm with which the UK public now feed garden birds with the hope of ‘giving something back’ to nature (Jones & Reynolds 2008), the potential for widespread negative repercussions of extensive food supplementation is disquieting. In particular, of great concern is the aforementioned lack of clarity regarding how food availability affects key reproductive parameters, avian physiology, and trophic interactions with natural food availability. Such a widespread increase in resources of varying nutritional composition (Lin 2005) could have profound implications for individuals and populations.

1.4. Food supplementation and reproductive investment

The availability of food or other resources may have a dramatic effect on shaping the cost of reproductive attempts (Verhulst 1994). Although a multitude of experimental studies have increased the cost of reproduction with the expectation of reduced residual reproductive value (summarised in Table 6 Dijkstra *et al.* 1990, Lessells 1993), far fewer studies have reduced the cost of reproductive attempts and then monitored the downstream survival consequences (but see Verhulst & Tinbergen 1997). Food supplementation provides an effective way of underwriting the costs of reproductive attempts in birds (Martin 1987). Not only does the supplement itself constitute a predictable source of nutrients and calories, foraging time may be reduced (Kacelnik & Cuthill 1990), permitting more time and energy for other activities.

1.4.1 Food supplementation and seasonal timing

The consistent advance of laying date in food-supplemented female birds (e.g. Källander 1974, Nilsson & Svensson 1993) may provide significant advantages for lifetime reproductive success in terms of increased recruitment of offspring into the breeding population (Richner 1992) or improved adult survival (Verhulst *et al.* 1995). In addition, many breeding parameters such as clutch size and nestling body mass show seasonal declines in magnitude (Verhulst *et al.* 1995, García-Navas & Sanz 2011) so early breeding could be advantageous within the current breeding attempt. However, very few food supplementation studies have been conducted of sufficient duration to determine lifetime reproductive success, so the potential advantages of food supplementation are based on inference rather than empirical evidence. Significant disadvantages may also exist, i.e. breeding too early may be disadvantageous (Nilsson 1994) through the disruption of seasonal interactions with peak prey availability. Again, there are few food supplementation studies (but see Bourgault *et al.* 2009) that have monitored natural food availability in supplemented and unsupplemented parents, so there is a paucity of information on whether food supplementation can provoke seasonal mismatches between predator and prey.

1.4.2. Food supplementation and maternal investment during egg laying

The most common investment metrics used to measure the effects of food supplementation during egg laying are clutch size and egg volume or mass. Although the clutch sizes of supplemented females have been found to increase or be unchanged in almost all food supplementation studies (Table 1.1, Robb *et al.* 2008a), a more recent study has illustrated that food supplementation may decrease clutch size (Harrison *et al.* 2010). Less than 50% of supplementation studies measuring egg size found that food supplementation significantly increased egg size (Christians 2002), but supplemented Great Tits can lay heavier eggs (Harrison 2010). Using an additional metric of adult investment such as DEE in tandem with egg size and clutch size may resolve these conflicting results (Vézina *et al.* 2006), but such measurements are difficult to obtain, due to the sensitivity of egg-laying females to disturbance, and have never been achieved within a food supplementation context.

1.4.3. Food supplementation and parental investment during the nestling phase

Food availability significantly affects parental effort during brood rearing (e.g. Thomas *et al.* 2001, te Marvelde *et al.* 2011b). Jodice *et al.* (2002) found lower DEE in food-supplemented Black-legged Kittiwake parents, but in a study of European Pied Flycatchers (Moreno *et al.* 1999), the DEE of females was unaffected by food supplementation. Studies of the effects of food supplementation on provisioning rates of brood-rearing adults have tended to focus on longer-lived, more *K*-selected species such as American Kestrels (*Falco sparverius*), with females reducing provisioning rates at food-supplemented nests (Dawson & Bortolotti 2002). In contrast, the provisioning rate of female Black Redstarts (*Phoenicurus ochruros*) increased as a result of food supplementation as males spent more time defending their territories (Cucco & Malacarne 1997). Therefore, there may be sex-specific responses of parental effort in food-supplemented pairs, but few studies of food supplementation and provisioning rates in passerine species exist.

1.4.4. Food supplementation and parental fitness

The ability of parents to time their breeding attempts to seasonal peaks in food availability may have a direct influence on their lifetime reproductive success (e.g. van Noordwijk *et al.* 1995). There is also a significant selective advantage in early breeding, with both offspring recruitment rates (Verboven & Visser 1998) and adult survival rates (Verhulst *et* *al.* 1995) declining over the course of the breeding season. Advanced breeding as a result of food supplementation may, therefore, confer selective advantages on supplemented parents, although there are few long-term food supplementation studies that have tested this. Food supplementation may, however, increase current reproductive output and adult survival rates as was found by Davis *et al.* (2005) in Parasitic Jaegers (*Stercorarius parasiticus*), or it may increase current reproductive output to the detriment of adult survival (e.g. in Song Sparrows – Arcese & Smith 1988), or decrease current reproductive output and increase adult survival (e.g. in American Kestrels – Dawson & Bortolotti 2002). Including additional measures of investment in studies may have enhanced the interpretation of the effects of food supplementation on the lifetime reproductive success of individuals and the ensuing consequences for population sizes (Zera & Harshman 2001, Ricklefs & Wikelski 2002).

1.5. Aims of the thesis

The main aim of my thesis was to conduct an integrative study of the effects of food supplementation on parental investment and key life history traits of breeding birds. The species selected were a specialised arboreal forager (i.e. Blue Tits) and a more generalist forager (i.e. Great Tits) (Betts 1955, Slagsvold & Wiebe 2007). Due to their differing foraging techniques, Blue and Great Tits were expected to respond differently to a food supplement, as Blue Tits may be more sensitive to the consumption of specific food items during the breeding season (Betts 1955, Bourgault *et al.* 2006). In addition, studying short-lived species enables life history trajectories to be followed over a shorter time frame, and investment in offspring can be easier to determine than in long-lived species as breeding seasons are unlikely to be skipped (e.g. in seabirds Golet *et al.* 1998).

The study incorporated measurement of breeding parameters, parental provisioning rates and female energy expenditure of Blue and Great Tits to assess parental investment in the current reproductive attempt. The quantification of the latter was then compared with metrics of residual reproductive value for evidence of life history trade-offs, using adult survival until the following breeding season and offspring recruitment into the breeding population. The study was conducted within a six-year food supplementation research programme and so the assessment of parental effort was further examined within a life history context by quantifying reproductive investment over multiple breeding attempts. A secondary aim was to gauge whether food supplementation affected trophic interactions with peak prey availability and had subsequent life history consequences.

To address the aims of this thesis, data were used from a field experiment that was conducted from 2006 to 2011 using a population of Blue and Great Tits breeding in nestboxes in Chaddesley Woods National Nature Reserve, Worcs., UK (Figure 1.1). The

Figure 1.1. Chaddesley Woods National Nature Reserve in Worcs., UK. (Photo: S.Webber)

field site was divided into three areas and assigned to one of three food supplementation treatments: control (unsupplemented), supplemented with peanut cake (composed of 50% beef tallow and 50% peanut flour), or supplemented with peanut cake and additionally mealworms during the nestling phase. This experimental design was intended to isolate differences in reproductive investment between parents supplemented with a high-energy food resource which they would use for self-feeding (i.e. peanut cake) and one which could also be used for feeding nestlings (i.e. mealworms). Reproductive output was monitored across all years through measurement of laying date, clutch size, hatch date, brood size and nestling mortality. In a subset of years, additional measurements of egg size, female energy expenditure during egg laying and brood rearing, male and female provisioning rates, nestling body mass, adult survival and offspring recruitment into the breeding population were also obtained. A number of females bred in two consecutive years and so intra-individual variation in parameters such as timing of breeding, clutch size and nestling mortality could be examined for evidence of inter-seasonal trade-offs.

The metric used to quantify female effort during egg laying was DEE, which was measured using the doubly-labelled water (DLW) method (Lifson & McClintock 1966, Tatner & Bryant 1987). The DLW method is currently the only technique suitable for measuring DEE or FMR in free-living passerines and DEE has never been measured during egg laying as part of a food supplementation study. Higher food intake in captive Zebra Finches has been related to higher DEE and larger clutches (Vézina *et al.* 2006). These results suggest that females with access to higher food availability may be able to elevate their DEE and invest more in reproduction. However, there may be energetic demands related to predator vigilance, or thermoregulation as a result of ambient temperature fluctuation in free-living birds which were not accounted for. The metrics used to analyse parental effort during the nestling phase were DEE of females (measured using the DLW technique) and provisioning rates of females and males (measured using PIT tag readers). An unequivocal effect of food supplementation on provisioning rates had not been found previously, and they may increase (Cucco & Malacarne 1997) or decrease (Eldegard & Sonerud 2010) in response to supplementary food. Measures of DEE may clarify this relationship (but see Moreno *et al.* 1999) and supplemented parents may reduce DEE and hence reproductive costs when food supplemented (e.g. Jodice *et al.* 2002).

1.6. Structure of the thesis

In **Chapter Two** I examine the effects of food supplementation on laying date and matching to peak natural prey availability at both the population and intra-individual levels. In **Chapter Three** I investigate how food supplementation affects egg parameters such as clutch size, egg size and mass with additional intra-individual analyses and DEE measurements. In **Chapter Four** I explore how food supplementation and natural food availability are related to nestling mortality, brood size and parental effort by measuring pair brood provisioning rates and female DEE. In **Chapter Five** the energetic and reproductive consequences of a mismatch between the timing of peak nestling nutritional demand and the peak in natural food availability are presented. The mismatch occurred as a result of food supplementation and provided evidence of life history trade-offs. In **Chapter Six** I examine the long-term effects of food supplementation on parent survival and offspring recruitment rates. Finally, in **Chapter Seven** I discuss my results within a life history context and provide directions for future research.

Chapter Two

The effects of food supplementation on the timing of reproduction in Blue and Great Tits relative to peak prey availability

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2.1. Abstract

Food availability is incontrovertibly linked to reproductive timing and success in seasonally breeding birds, with selection for accuracy of timing to seasonal peaks in food availability evident in some species. The timing of peak availability of key food types varies inter-annually and so individuals that display phenotypic plasticity in their timing of breeding are often more successful. It is acknowledged that food supplementation advances breeding attempts and so could confer selective advantages in populations with strong selection for early breeding. A high-energy food supplement was provided to a UK population of Blue and Great Tits between 2007 and 2011 to examine whether food supplementation affected the ability of females to match maximum nestling demand and peak caterpillar availability. Food supplementation advanced breeding, but led to phenological mismatch between the timing of peak nutritional demand of Great Tit nestlings and that of the availability of their caterpillar prey. The phenotypic plasticity of individual Great Tit laying dates was significantly negatively affected by food supplementation. Blue Tits maintained their phenotypic plasticity in laying date, matching their breeding attempts to peaks in caterpillar availability. Blue and Great Tits appear to use different cues in initiating breeding and Great Tits are more susceptible to mistiming.

2.2. Introduction

It has long been recognised that the timing of reproductive attempts in many avian species is inextricably linked to seasonal peaks in food availability (Kluijver 1950, Perrins 1965). Synchrony of breeding attempts with a peak in food availability (e.g. of prey species such as caterpillar larvae) has been long postulated as one of the key drivers of reproductive success (Lack 1947, Kluijver 1951, van Balen 1973). There is a seasonal decline in reproductive output in many bird species (e.g. Great Tit - Perrins 1970, Verhulst *et al.* 1995, e.g. Common Guillemot [*Uria aalge*], Votier *et al.* 2009) and early breeding is strongly selected for in some populations (e.g. Verboven & Visser 1998, Charmantier *et al.* 2008). The advantages of breeding early are compounded by advancing prey phenology[1] as a result of warming spring temperatures (Visser *et al.* 1998, Both *et al.* 2009).

It has only been identified more recently, however, that selection pressure can also act as a mechanism to favour individuals that are able to time breeding attempts to coincide with peak prey availability (van Noordwijk *et al.* 1995). The seasonal peak in availability of caterpillar prey for Blue Tits and Great Tits, for example, has been demonstrated to vary significantly between years in Dutch (Tinbergen 1960), Swiss (Nager & van Noordwijk 1995) and UK (Perrins 1991) populations. Certain individuals are able to track these inter-annual shifts in timing, and indeed, in some populations, such flexibility in timing is heritable and selected for (Nussey *et al.* 2005). The provision of supplementary foods to breeding birds is associated with a variation in the timing of breeding of most avian species (reviewed in Robb *et al.* 2008a) and so could have

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[1] Although the word ‘phenology’ strictly should refer to the act of studying seasonal events and interactions between animals and plants, I will be using the word in its broader and commonly used sense meaning ‘relating to seasonal timing’. Hence, a phenological mismatch is a mismatch in timing between two species, such as a predator and its seasonally occurring prey.

profound effects on the synchrony of breeding birds with the peak availability of their main prey resource and, hence, may influence their reproductive success.

2.2.1. Food availability as a constraint on egg laying

The precise role of food availability in determining the timing of breeding in birds, and in particular the start of egg laying (clutch initiation or laying date), has been much discussed. Resource availability was identified in early modern studies of the breeding ecology of birds as being a limiting factor in the timing and success of breeding attempts (Kluijver 1951) through the need to provide 'food for the brood' (Lack 1947). This interpretation of the importance of food has since been refined and food availability has been proposed in the 'constraint hypothesis' (Perrins 1970) to be an important constraining factor in preventing earlier laying. High variation in laying dates within a population are thus maintained, due to differences in localised food availability (Perrins 1965, 1970). Females could either be limited energetically at this stage in the breeding cycle (Perrins 1996, Vézina & Williams 2002), or nutritionally limited by the availability of macronutrients such as protein (Schoech *et al.* 2004) or of micronutrients such as calcium for eggshell formation (Reynolds & Perrins 2010). These limitations would be particularly evident in 'income' breeders such as Blue and Great Tits (Meijer & Drent 1999) that are unable to store large amounts of fat which can be metabolised at a later breeding stage. The initiation of the clutch could, therefore, be postponed until a food availability threshold is reached.

2.2.2. Food supplementation – food availability as a cue for initiating laying

Food supplementation studies during the breeding season of birds consistently report a responsive advance of laying, irrespective of the nature of the food supplement (see reviews by Boutin 1990, Robb *et al.* 2008a). This has led to the development of a further hypothesis relating food availability to timing of laying of birds – the so-called '*cue hypothesis*' (Källander 1974, Visser *et al.* 2011a). This hypothesis posits that an increase in temperature (Visser *et al.* 2009, 2011a) or in food (Källander 1974) in the period before egg laying could act as a cue for the breeding female giving an indication of the timing of the caterpillar peak later in the season (Perrins 1991, Harrison *et al.* 2010). In some instances food supplementation has called the constraint hypothesis into question through removing energetic and macronutrient limitations pre-laying, without increasing clutch size (Nager & van Noordwijk 1992, Nager *et al.* 1997). Much remains unknown about the details of nutritional and physiological requirements of a female that is laying eggs (reviewed in Williams 2005), however, so the constraint hypothesis cannot be entirely dismissed.

Although breeding in species such as Blue and Great Tits is timed to a peak in caterpillar availability, laying starts approximately 37 days before this peak (Perrins 1991), so reproductive events are initiated before the maximum availability of caterpillars. Preparation for egg laying also requires significant hormonal changes (Williams 2005) and a recrudescence of reproductive organs such as the oviduct (Vézina & Williams 2003), which require time to complete, so cues relating to the best timing for breeding must be interpreted accurately by the female. Clues as to the timing of the caterpillar peak within a given year could also be provided by tree budburst (Bourgault *et al.* 2010), ambient temperature (Visser *et al.* 2009) or the appearance of the earliest (and smallest) caterpillars when they hatch (Perrins 1991), around 40 days before peak availability.

Whether food availability during pre-laying acts to remove energetic constraints or provides cues that predict future environmental conditions, it is certain that anthropogenic food supplements for birds could have dramatic repercussions for their breeding biology. An advance in laying as a result of food supplementation could give selective advantages to birds through earlier breeding (Verboven & Visser 1998). It could also cause birds to become mistimed from their natural food resource upon which they rely to feed their nestlings (Norris 1993), a phenomenon known as phenological mismatch . Feeding birds in gardens and backyards has now become a widespread and year-round activity (Chapter One), representing an unprecedented food resource for breeding birds. The interpretation of the effects of food supplementation on the accuracy of seasonal timing in breeding birds is not, therefore, merely an improvement of the mechanistic understanding of how birds time their breeding attempts, but also of critical applied ecological importance.

2.2.3. Phenotypic plasticity and phenological mismatch

Phenotypic plasticity is the ability of a genotype to alter its phenotype in response to a changing environment (Stearns 1989b). More specifically, in a sub-category of phenotypic plasticity described as phenotypic flexibility or life history staging (Piersma & Drent 2003), morphological or behavioural traits (such as laying date) can vary within one individual as they track environmental cues (Ricklefs 1991, Piersma & van Gils 2011). Theoretically, this plasticity should allow individuals and consequently populations to adjust to stochastic variation in food availability and is inextricably linked to ecological conditions (Piersma & van Gils 2011). The provision of a stable food resource to breeding birds, therefore, could affect not only the timing of breeding but also the ability of individuals to track stochasticity in natural food resources between years. Food supplementation has been used to miscue females by simulating a false food peak early in one season, disrupting the egg-laying female's ability to time her breeding attempt in a subsequent year (Grieco *et al.* 2002, Gienapp & Visser 2006). It has not been investigated whether protracted food supplementation could have a similar effect on plasticity by giving misleading cues as to peak food availability in the first of two years, however.

2.2.4. Protracted food supplementation during the breeding season

In this chapter I address some of the unanswered questions raised above. A food supplementation experiment was conducted between 2006 and 2011 using Blue and Great Tits to examine not only the population-level effects of the provision of a stable anthropogenic food resource during the breeding season, but also the effects on the accuracy of seasonal timing within individual females. Natural food availability, in the form of geometrid caterpillars, was also monitored. Food supplementation studies that also monitor natural food availability are rare and I investigated whether the advance in breeding associated with food supplementation provided potential fitness benefits by improving phenological matching (e.g van Noordwijk *et al.* 1995). Improved synchrony with the caterpillar peak should manifest in a reduction in the variance of matching to peak caterpillar availability, as individuals that would otherwise have initiated laying later are able to advance their breeding attempt. I also examined whether population-level responses to food supplementation reflected intra-individual responses and improved or reduced phenotypic plasticity in laying date. I tested predictions from a number of working hypotheses based on the premise that a predictable food supplement should cue earlier laying (e.g. Källander 1974) and potentially remove energetic constraints that may prevent more accurate timing of breeding. I predicted that food supplementation of both species should: significantly advance laying and hatch dates; allow more accurate (phenological) matching of breeding attempts to peaks in caterpillar availability; reduce variance in laying dates with all birds responding by advancing laying; and increase female phenotypic plasticity in laying date as energetic constraints on egg production are relaxed.

2.3. Methods

2.3.1. Nestbox study

The study was conducted over six breeding seasons between 2006 and 2011 in Chaddesley Woods National Nature Reserve in Worcestershire, UK (52°36’N, 2°14’W, Fig. 2.1a), a predominantly oak (*Quercus* spp.) broadleaved woodland. The study site formed an area of 48 hectares within this woodland with 288 identical plywood nestboxes (Fig. 2.1b), later replaced by plastic nestboxes (Fig. 2.1c). The change in nestbox construction was to alleviate multi-seasonal damage to the plywood nestboxes by Grey Squirrels (*Sciurus carolinensis*). The study area was divided into three woodland blocks of 96 nestboxes each (Fig. 2.1d). From 2006 to 2009 all three of the woodland blocks were used (Fig 2.1d), but in 2010 and 2011 only 192 of the nestboxes in two of the woodland blocks were studied. This reduction in study area was due to the incorporation of an additional food supplementation regime, which does not form part of this thesis. Nestboxes were approximately 2 m above the ground facing north-east, away from the south-westerly direction of prevailing winds. They had a 32 mm entrance hole. The spacing of nestboxes was based on a grid system with 40 m spacing between rows and columns within a block

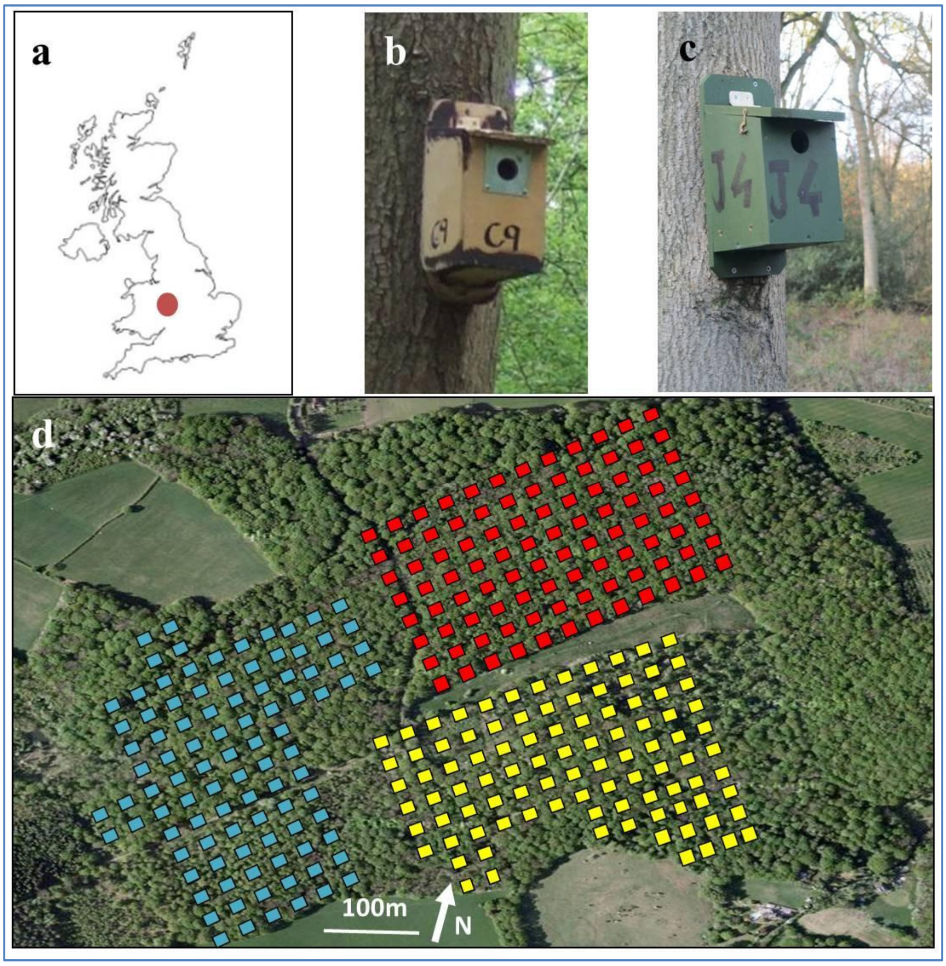

Figure 2.1.(a) The location of Chaddesley Woods National Nature Reserve in the UK, (b) and (c) plywood and plastic nestboxes, respectively, in which Blue and Great Tits bred between 2006 and 2011, and (d) schematic diagram showing the nestbox arrangement within each of the woodland blocks (see text for details) (Photos: S. Webber & K. Brulez).

and with a buffer zone of width 90 m between woodland blocks (Fig. 2.1d). The resulting density was 7.79 nestboxes per hectare. The nestboxes were occupied almost exclusively by Great Tits and Blue Tits that are intensely territorial during the reproductive period (Perrins 1979). They were not expected to cross the buffer zone to forage because of such territoriality (but see Wilkin *et al.* 2009b, te Marvelde *et al.* 2011c). Video recordings of the feeders were conducted during the egg-laying period in 2010 which corroborate this in the fact that 74% of the colour-ringed birds visiting the feeders were from the same woodland block. Although there were visits to feeders from birds breeding in the control area, the increased distance that parents from the control area would need to cover to reach a feeder was expected to make any benefits negligible compared to supplemented parents.

Nestbox monitoring

In most years (2008 to 2011, inclusive), nestboxes were checked at least every other day for signs of nest building from mid-March and then daily once the nest was half completed until the appearance of the first egg (laying date). In 2006 and 2007 the nestboxes were checked at least once a week (Harrison 2010), and the laying date was determined by back counting based on the number of eggs in the clutch. From 2006 to 2008, eggs were checked for hatching 10 days after egg laying ceased (i.e. after clutch completion date). From 2009 to 2011the onset of incubation was determined after the female was observed sitting tightly on the eggs for two consecutive days, or after the eggs were found uncovered and warm two days in a row, at which point nest checking ceased. Nestboxes were checked daily for signs of hatching after day 10 of incubation (counting the day of onset of incubation as day 0). The day of hatching of the first egg was designated as day 0, and hereafter is referred to as hatch date. From 2007 onwards adults were captured at various points of the brood-rearing phase, as part of other protocols, and identified with uniquely numbered BTO metal rings and aged according to moult limits (Svensson 1992).

2.3.2. Focal species

The two focal species used for this study were Blue Tits (Fig. 2.2a) and Great Tits (Fig. 2.2b), both of which are small cavity-nesting passerines which will readily use nestboxes. They are also two of the most common visitors to gardens and frequently use bird feeders (Soper 2006, Toms & Sterry 2008). The importance of matching of their breeding

Figure 2.2. The two small passerine species (a) Blue Tit and (b) Great Tit studied in Chaddesley Woods National Nature Reserve Worcs., UK, to investigate the effects of food supplementation on the timing of their breeding. (Photos: Peter Beasley).

attempts to the peak availability of their key prey species (e.g. Winter Moths) has been well studied in both species (van Noordwijk *et al.* 1995, Thomas *et al.* 2001) and, as such, it makes them ideal candidates to examine the effects of anthropogenic food supplementation on phenological interactions.

2.3.3. Food supplementation

Each woodland block in the study site was allocated to a food supplementation treatment group (Fig. 2.3a, b and c). From 2006 to 2009 two food supplements were provided during the breeding season (peanut cake and live mealworms), and in 2010 and 2011 one food supplement was provided (peanut cake). The 500 g cylindrical blocks of peanut cake consist of 50% peanut flour and 50% beef tallow (composition: 70.5% fat, 17.1% protein – CJ Wildlife Ltd. pers. comm.) and are known to be consistently consumed by Blue and Great Tits (Harrison *et al.* 2010, Smith 2011). The peanut cake feeders (Fig. 2.3d) were placed at the centre of four nestboxes (Fig. 2.3b and c), approximately 28 m from each of the four nestboxes surrounding it, to replicate the shared nature of garden bird food

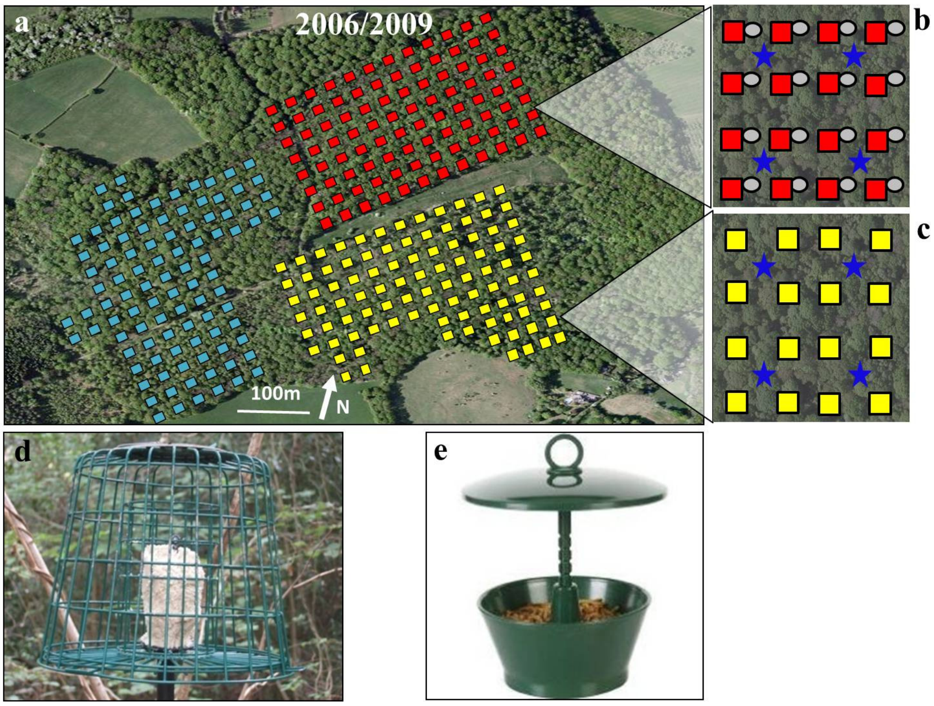

Figure 2.3. (a). Map illustrating allocation of dietary treatments to each block of nestboxes in 2006 and 2009: nestboxes allocated to the control treatment group are highlighted in blue, those allocated to the peanut cake treatment are in yellow and those allocated to the peanut cake and mealworms treatment are in red. (b) represents the positioning of peanut cake feeders (blue star) between four nestboxes (red boxes) and mealworm feeders (grey ellipse). (c) represents the positioning of the peanut cake feeders (blue star) between four nestboxes (yellow boxes). (d) is a peanut cake feeder in the wood, and (e) is a mealworm feeder. (Photos: S. Webber & CJ Wildlife Ltd.).

resources. In addition, in one of the supplemented woodland blocks, live mealworms were provided in pole-mounted plastic feeders (Fig. 2.3e) placed approximately 5-8 m from individual nestboxes (Fig. 2.3b) upon the hatching of the nestlings. Within the three woodland blocks, food supplements were rotated between years (Figs 2.3a and 2.4) in the same sequence, with one block always receiving no supplement and acting as a control. The data in this chapter were taken for the period before mealworm supplementation so the two treatment groups are considered as either unsupplemented (i.e. control) or

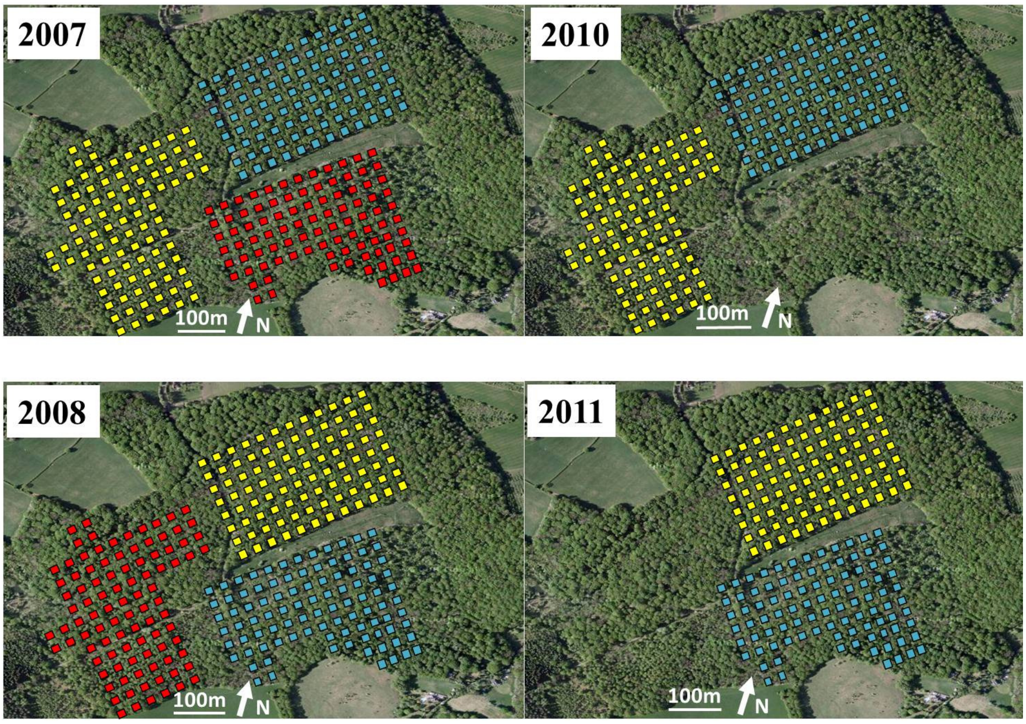

Figure 2.4. Maps illustrating the allocation of food supplementation treatment to each block of nestboxes in the indicated year at Chaddesley Woods National Nature Reserve, Worcs., UK where the effects of food supplementation on Blue and Great Tits were studied. Control nestboxes are represented in blue, those supplemented with peanut cake are in yellow and those supplemented with both peanut cake and mealworms are in red. The map for 2006 and 2009 can be found in Fig. 2.3a). The maps for 2010 and 2011 demonstrate that only two woodland blocks of nestboxes were used in these years (see text for details).

supplemented with peanut cake. In 2010 and 2011 the peanut cake supplement was provided in only one woodland block (Fig. 2.4) and no mealworms were supplied, leading to an experimental design using only two woodland blocks, one unsupplemented (i.e. control) and one supplemented with peanut cake. In all years the peanut cake supplement was provided *ad libitum* from 7th March to 28th July (i.e. from pre-laying to post-fledging). It was not anticipated that the peanut cake supplement would be fed to nestlings (but see Harrison 2010) so the peanut cake supplemented-area was used to test for direct benefits of feeding to the breeding adults rather than to their offspring.

Food supplementation has been demonstrated to increase the density of breeding pairs (reviewed in Boutin 1990), and to affect the response of breeding females to high densities of conspecifics by increasing the number of offspring produced (Arcese & Smith 1988). Although territories should have been long established by the time food supplementation started (e.g. in the Great Tit – Kluijver 1951), immigrants to the population that breed later (e.g. Nager & van Noordwijk 1995) may be affected in their choice of nesting site by food availability or territory quality (Gosler 1993). In order to verify that there was no effect of food supplementation on breeding density, I tested for variation in occupancy of nestboxes between food treatment areas (Appendix Three) and found that there was no significant difference in occupancy between food treatment areas in either Blue or Great Tits. Density-dependence did then not need to be taken into account in subsequent models as density did not vary between treatments and the focus in all analyses was on difference between treatment areas.

2.3.4. Estimating caterpillar biomass

Peak caterpillar availability was sampled in 2007, 2008, 2010 and 2011. Due to problems with the storage of caterpillar samples from 2009, they could not be used to estimate caterpillar availability. In 2007 quartets, and in all other years pairs, of water-filled plastic trays (Fig. 2.5a) were placed under 10 oak trees in each woodland block to catch the caterpillars as they dropped to the ground to pupate (Zandt 1994, Chamberlain *et al.* 1999). The location of the trays remained consistent between years. Samples were collected three times per week and frozen (Harrison 2010), then later defrosted, sorted, dried to constant mass at 60ºC in an oven (Electrolux, Sweden) and weighed to the nearest 0.0001g on a Sartorius electronic balance (Harrison 2010). The dried caterpillar biomass was then

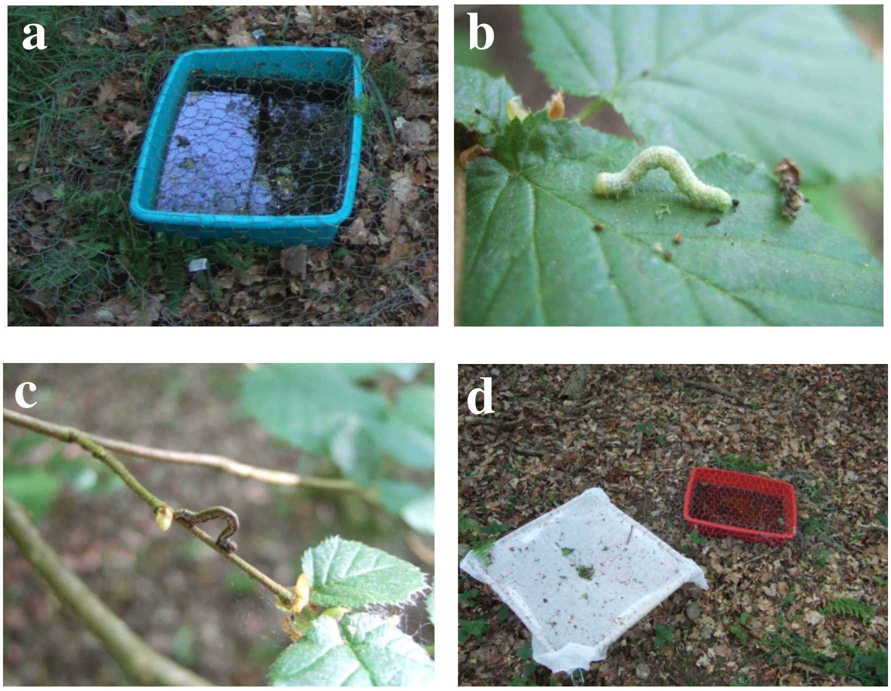

Figure 2.5. Estimation of caterpillar availability at Chaddesley Woods National Nature Reserve, Worcs., UK where the effects of food supplementation were studied in breeding Great and Blue Tits: (a) a water-filled plastic tray into which caterpillars drop; (b) and (c) two species that form the diet of breeding tits, namely a Winter Moth caterpillar and a species of geometrid caterpillar respectively; and (d) a frass collector. (Photos: S. Webber)

averaged across woodland blocks, divided by the number of days represented in the sample, and the grammes of biomass per m^2 of collection (tray) area calculated. The 'half-fall' date could then be ascertained as the point at which half the total biomass had fallen, and the midpoint between the two collection days was used as the half-fall date (Perrins 1991) which is commonly used to determine the date of peak caterpillar availability. Although the diet of Great Tit nestlings is predominantly geometrid moths (Betts 1955), it is not exclusively so and *Operophtera brumata* (Fig 2.5b) can form a low proportion of the species of caterpillars which are fed to nestlings (Kluijver 1950, Cramp 1993, Nour *et al.* 1998). For this reason my analyses include all the caterpillar species found and not just the *Operophtera brumata* or other Geometridae species (e.g. Fig 2.5c).

Frass collection

Many studies of the ecological interactions of Blue and Great Tits with the seasonal peak in caterpillar availability (e.g. van Noordwijk *et al.* 1995, Nour *et al.* 1998, Seki & Takano 1998, Naef-Daenzer & Keller 1999, Thomas *et al.* 2001, Smith *et al.* 2011) use frass production as a method of estimating caterpillar biomass. To determine whether there was a timing difference between the peak in caterpillar availability estimated using pupating caterpillars *versus* that estimated using frass fall, I sampled the latter in 2011. Frass collectors (Fig. 2.5d) were placed next to the water trays in 12 locations across the whole study site. The collectors were constructed using weighted butter muslin stretched across a 0.25 m^2 bamboo frame (Tinbergen 1960) and were emptied three times a week on the same days as the caterpillar water trays. The frass was then dried for three days at 60℃ to constant mass and weighed to the nearest 0.0001 g on a Sartorius electronic balance. Raw values were then doubled to give a value in grammes per m^2 and averaged across one woodland block. Midpoint dates were calculated as per the tray sampling method.

2.3.5. Video recording of feeders

In 2010 a roost catch was conducted in February to enable each individual to be marked with a coloured plastic ring to denote in which woodland block it had been captured. Birds caught during the roost catch were expected to breed in the woodland block in which they were caught (van Balen 1980). The roost catch was conducted over five nights by visiting each nestbox, removing and identifying each occupying bird and attaching a coloured plastic ring to its leg and replacing it in the nestbox. Unfortunately, only five Blue Tits were caught so most of the data gathered related to Great Tits of which 134 were captured and colour ringed. Of these, 82 were recaptured during breeding, and these individuals constituted 31% of the breeding population that year. A Sony Handycam DCR-SR90E video recorder mounted on a tripod was then used to record birds visiting peanut cake feeders in the supplemented area for 90 mins approximately one hour after dawn. These videos were captured during the nest construction and egg-laying periods to determine whether birds from outside of the peanut cake treatment block were visiting the feeders. The videos were then analysed for appearances of colour-ringed birds.

2.3.6. Data filtering

Only first breeding attempts within each year were considered and of necessity only broods which survived to ringing age were included in analyses so that the synchrony between nestling demand and caterpillar availability could be quantified. Known and probable second attempts were excluded by removing any broods which hatched more than 15 days after the mean hatch date within each year. Second clutches are known to be smaller (Perrins 1965) and the additional delay caused by initiating a second attempt could reduce adult investment in the breeding attempt (Goutte *et al.* 2011). The excluded data equated to 6% of Great Tit breeding attempts and 4% of Blue Tit breeding attempts averaged across the five years of the analysis. Blue and Great Tit datasets were analysed separately. Data were analysed only from broods in which the breeding adults had been identified, to account for the effects of individual variation as certain individuals were known to breed in multiple years. This excludes data from 2006 as no adults were captured in this year. Analyses of data from the full six year dataset are presented in Appendix Two.

For intra-individual laying date analyses only those females that provided two consecutive years of breeding records were included to examine the potential carry-over effects from one year to the next (Harrison *et al.* 2011). Third and fourth breeding attempts were discounted from these analyses as there were not enough examples within the six year study.

2.3.7. Statistical analyses

All statistical analyses were conducted using Generalised Linear Mixed Models (GLMMs), Generalised Linear Models (GLMs) or Generalised Least Squares (GLS) regression in R version 2.11.1 (R Development Core Team 2011). Following non-significance of the Shapiro-Wilk test for normality (Crawley 2007), all data were checked against a Poisson diagnostic distribution plot and a Poisson error structure for skewed count data applied unless otherwise stated. Poisson analyses were checked for under- and overdispersion and corrected as required. All interactions between explanatory variables were tested using backward stepwise regression to find the minimal adequate model. This model simplification was conducted using Chi-squared (i.e. Poisson distribution) or F tests (i.e. normal distribution) to compare the residual deviance of models with and without terms and interactions (Crawley 2007). An alpha level of 0.05 was used throughout. The fit of all models was checked using plots of residual deviance against fitted values to verify that none of the assumptions of the model in use were being violated. Pseudo R^2 values were calculated where possible for GLMMs, to illustrate model fit, using the R^2 value from a linear model of the correlation between fitted and observed values. The amount of variance in laying dates between individuals was examined using a Levene's Test for homogeneity of variance with separate tests for each year.

Mismatch between maximum nestling demand and peak caterpillar availability

When analysing the mismatch between peaks in nestling demand and caterpillar availability, the response variable was transformed to make all values positive for ease of analysis. Two caterpillar biomass GLS regressions were conducted, using a Compound Symmetry correlation structure to correct for the longitudinal temporal autocorrelation of the caterpillar sampling method (Zuur *et al.* 2009a). In order to examine the difference in total caterpillar biomass between years, the raw biomasses per woodland block were used. The average daily biomass was calculated by dividing the total biomass by the number of days between each collection and this total was used to examine differences in the size of the daily caterpillar peak between years. The duration or 'width' of the peak in caterpillar biomass was calculated by counting the number of days during which the caterpillar biomass exceeded 5% of the mean daily biomass for that year.

Intra-individual analyses

Two analyses were conducted to investigate patterns in the ability of individual females to time their breeding attempts accurately across years. The first analysis was a GLMM examining the difference between laying date in the first and second of two consecutive years to investigate individual female responses to being matched or mismatched from the peak in caterpillar availability in the first year (Gienapp & Visser 2006). This analysis controlled for the effect of year and dietary treatment in year two, to investigate carry-over effects of dietary treatment and phenological mismatch. The second analysis was a GLM residual regression of the difference from the population mean of the laying date in the second year. This analysis was designed to identify whether food supplementation in the first year had an effect on the laying date in year two. The residuals were calculated from a regression of the laying date in the second year on that of the first year.

2.4. Results

2.4.1. The effects of food supplementation on laying and hatch date

Food supplementation significantly advanced laying date in Great but not Blue Tits across all years of the study (Fig. 2.6, Table 2.1). The advance in laying date was 3.2 days for

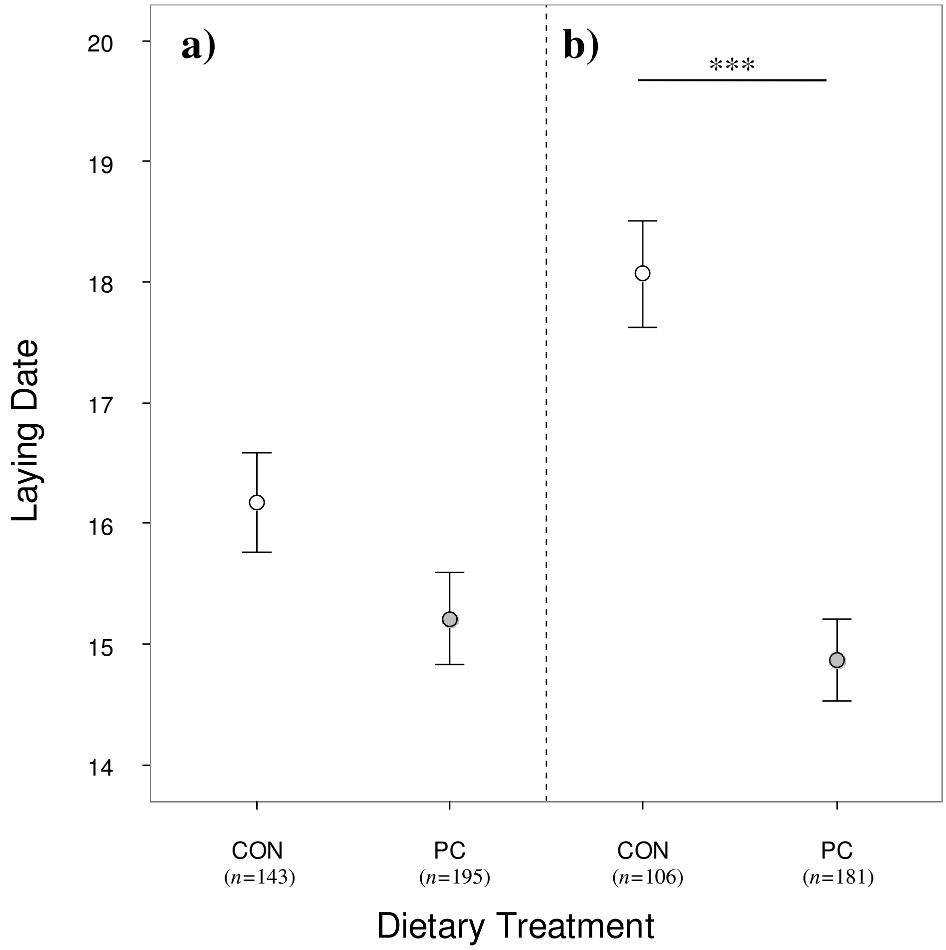

Figure 2.6. Laying date (mean ± 1 SE from model estimates) of (a) Blue and (b) Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011. Laying date is in April Days where 1 = 1st April. Females breeding in the control treatment area (CON) are represented by open circles, and those in the peanut cake treatment area (PC) by grey-filled circles. *** = $P < 0.001$. Number of females is shown in parentheses below each axis label. See Table 2.1 for statistical tests and text for details.

Table 2.1. Results from simplified GLMM analysis of laying date and hatch date of female Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011 regressed on significant explanatory variables (Fixed Effect). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 2.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	***R*^{2}**
Laying Date 2007-2011	BT	Intercept	16.175	0.060				0.73
		Dietary Treatment: PC	15.212	0.034	3.3	0.07		
		Year			123.3	**< 0.001**	2009: earliest year 2010: latest year	
		Female Age	16.399	0.087	31.3	0.87		
		Year:2011 × Female Age			11.8	**< 0.05**	Older females able to lay earlier in 2011	
Laying Date 2007-2011	GT	Intercept	18.067	0.053				0.60
		Dietary Treatment: PC	14.868	0.032	34.6	**< 0.001**	Earlier laying date in PC treatment	
		Year			114.1	**< 0.001**	2009: earliest year 2010: latest year	
		Female Age	17.107	0.032	15.6	0.09		
Hatch Date 2007-2011	BT	Intercept	41.940	0.035				0.44
		Dietary Treatment: PC	40.121	0.012	14.6	**< 0.01**	Earlier hatching in PC treatment	
		Year			255.3	**< 0.001**	2011: earliest year 2010: latest year	
		Clutch Size	-0.304	0.003	6.0	**< 0.05**	Earlier hatching with smaller clutch sizes	
Hatch Date 2007-2011	GT	Intercept	35.246	0.058				0.60
		Dietary Treatment: PC	31.157	0.020	36.5	**< 0.001**	Earlier hatching in PC treatment	
		Year			94.3	**< 0.001**	2009: earliest year 2010: latest year	
		Clutch Size	0.419	0.005	4.9	**< 0.05**	Later hatching with larger clutch size	
		Female Age	34.588	0.020	5.4	0.35		

Random effects were nestbox and female ID in all models. Tested fixed effects were dietary treatment (Intercept = control and PC = peanut cake), year and female age and only significant contributors to each model are displayed. All analyses used a Poisson error distribution.

Great Tits, controlling for inter-annual variation and female age (Fig. 2.6, Table 2.1). When female identity was not included in the model and all six years of available data were used, there was a significant 1.6 day advance in Blue Tit laying dates in response to food supplementation (see Appendix Table A.2.1: mean ± SE for: CON 26.0 ± 0.02; PC 24.4± 0.02, $\chi^2 = 8.8$, $P < 0.01$). There was significant inter-annual variation in laying dates in both species (Table 2.1), with the earliest laying dates occurring in 2009 and the latest in 2010.

Female age had a significant effect on laying date only in 2011 for Blue Tits, when older females initiated laying earlier (Table 2.1). Blue Tits exhibited significantly more variance in laying dates in the supplemented area in 2007 (Levene's Test: $F = 6.90$, $P < 0.05$) and 2008 (Levene's Test: $F = 5.39$, $P < 0.05$), but there were no significant differences in variance in any other years. Great Tits exhibited significantly higher variance in laying dates in the supplemented area in 2007 (Levene's Test: $F = 4.80$, $P < 0.05$) and 2009 (Levene's Test: $F = 4.00$, $P < 0.05$), which were both comparatively early years, but there were no significant differences in variance in any other years. There was a significant advance of mean hatch date in response to food supplementation in Blue and Great Tits (Fig. 2.7, Table 2.1). The advance in hatch date was 1.8 days for Blue Tits and 4.1 days for Great Tits, controlling for inter-annual variation and clutch size (Fig. 2.7, Table 2.1). The relationship between clutch size and hatch date was reversed between Blue and Great Tits, with smaller clutches being associated with later hatching in Blue Tits (Table 2.1), but larger clutches being associated with later hatching in Great Tits (Table 2.1). There was significant inter-annual variation in hatch date in both species, with the earliest hatch dates occurring in 2011 in Blue Tits and 2009 in Great Tits, and the latest hatch dates occurring in 2010 in both species.

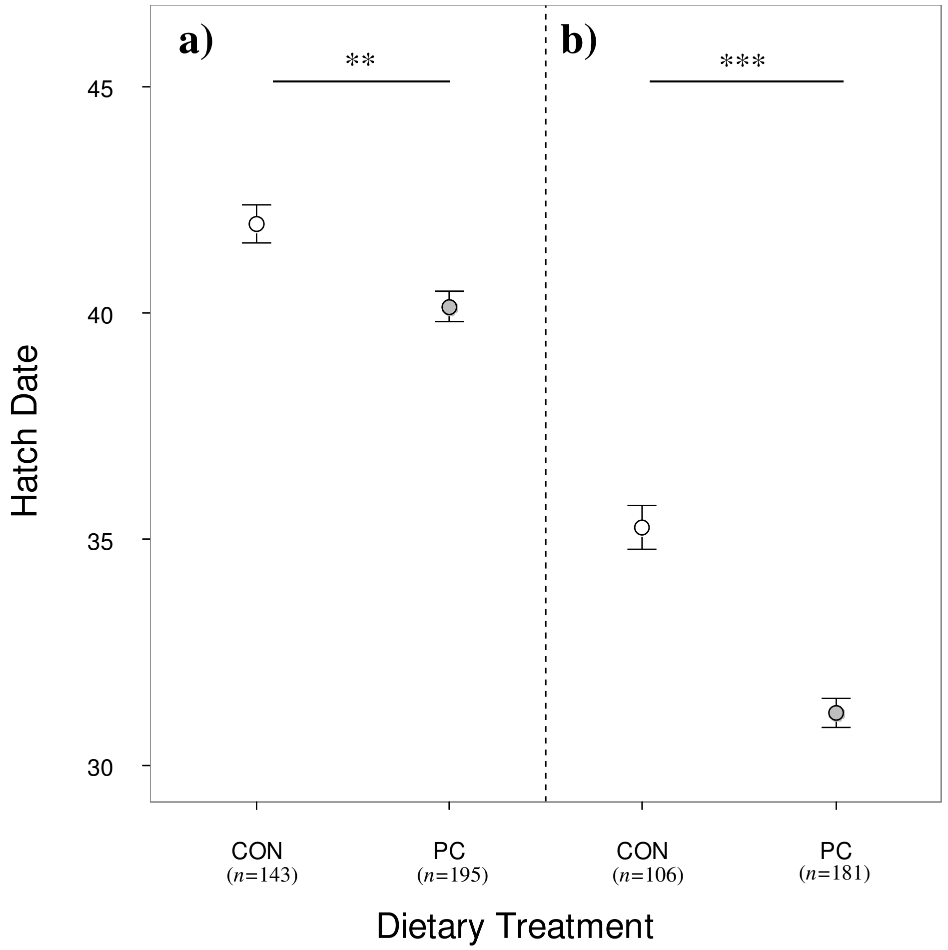

Figure 2.7. Hatch date (mean ± 1 SE from model estimates) of (a) Blue and (b) Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011. Hatch date is in April Days where 1 = 1st April. Females breeding in the control treatment area (CON) are represented by open circles, and those breeding in the peanut cake treatment area (PC) by grey-filled circles. *** = $P < 0.001$ and ** = $P < 0.01$. Number of females is shown in parentheses below each axis label. See Table 2.1 for statistical tests and text for details.

Female age was not significantly related to hatch date in either species.

2.4.2. The effects of food supplementation on matching to peak caterpillar availability

Food supplementation significantly increased the mismatch between the timing of peak nestling nutritional demand (at nestling day 10) and the peak in caterpillar availability of

Great, but not, Blue Tits (Fig. 2.8, Table 2.2). The mean increase in Great Tit mismatch was 1 day (Fig. 2.8, Table 2.2), controlling for inter-annual variation (Table 2.2).

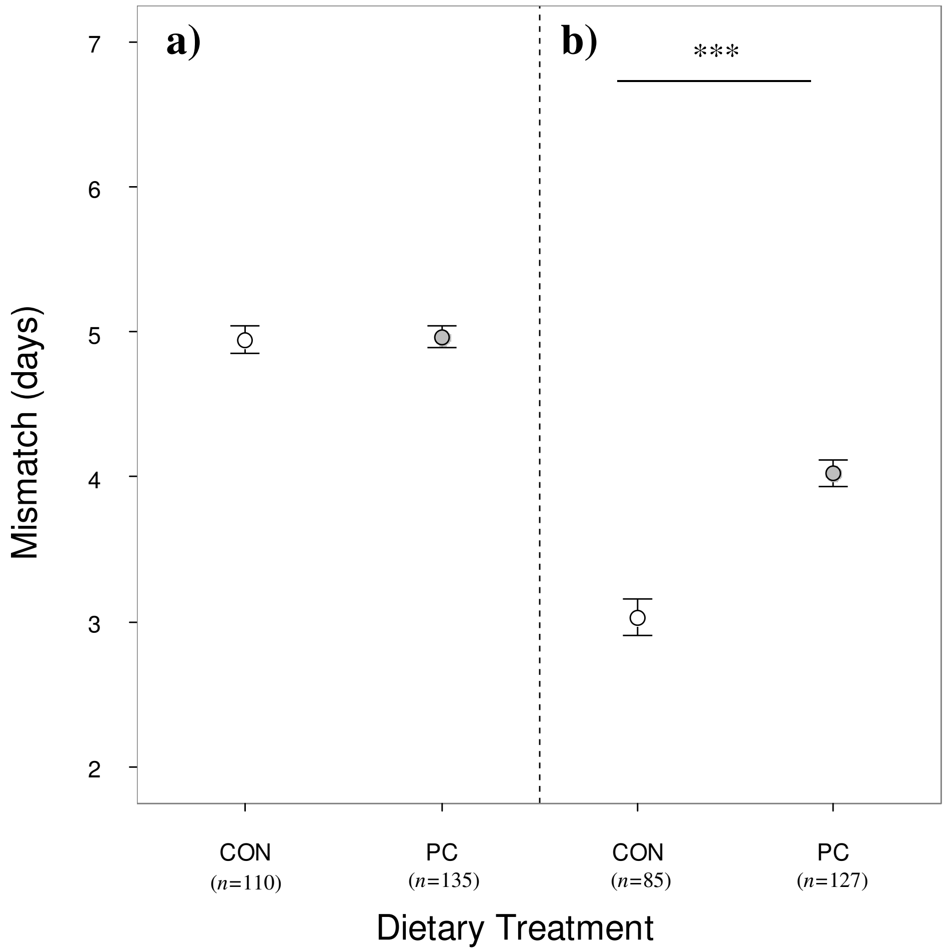

Figure 2.8. Mismatch of peak nutritional demand of chicks from peak caterpillar availability (mean ± 1 SE from model estimates) of (a) Blue and (b) Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011. Hatch date is in April Days where 1 = 1^{st} April. Females breeding in the control treatment area (CON) are represented by open circles, and those breeding in the peanut cake treatment area (PC) by grey-filled circles. *** = $P < 0.001$. Number of females is shown in parentheses below each axis label. See Table 2.2 for statistical tests and text for details.

There was significant inter-annual variation in the accuracy of matching peak nestling nutritional demand to peak caterpillar availability in both species, with the most accurate

Table 2.2. Results from simplified GLMM analysis of the mismatch between peak nestling nutritional demand (at 10 days) and the seasonal peak in caterpillar availability of female Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011. The response variable was regressed on significant explanatory variables (Fixed Effect). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 2.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	$\mathbf{R^2}$
Mismatch	BT	Intercept	4.957	0.086				0.84
		Year			78.84	**< 0.001**	2008: lowest 2011: highest	
2007,2008 2010,2011		Female Age	4.725	0.072	12.259	0.51		
Mismatch	GT	Intercept	3.030	0.125				0.92
		Dietary Treatment: PC	4.023	0.086	10.79	**< 0.001**	More mismatch in PC treatment	
2007,2008, 2010,2011		Year			22.029	**< 0.001**	2007: lowest 2011: highest	

Random effects were nestbox and female ID in all models. Tested fixed effects were dietary treatment (Intercept = control and PC = peanut cake), year and female age and only significant contributors to each model are displayed. All analyses used a Poisson error distribution.

matching in 2008 for Blue Tits and 2007 for Great Tits, and the least accurate matching in 2011 for both species (Figs 2.9 and 2.10, Table 2.2). Female age was not a significant predictor of matching to peak caterpillar availability in either species (Table 2.2). Laying and hatch dates were not included in this analysis due to high collinearity with the mismatch variable.

There was a significant difference in variance of mismatch between dietary treatment groups only in Blue Tits in 2007 when there was significantly higher variance in mismatch in supplemented than unsupplemented Blue Tits (Levene's Test: $F = 5.19$, $P<0.01$).

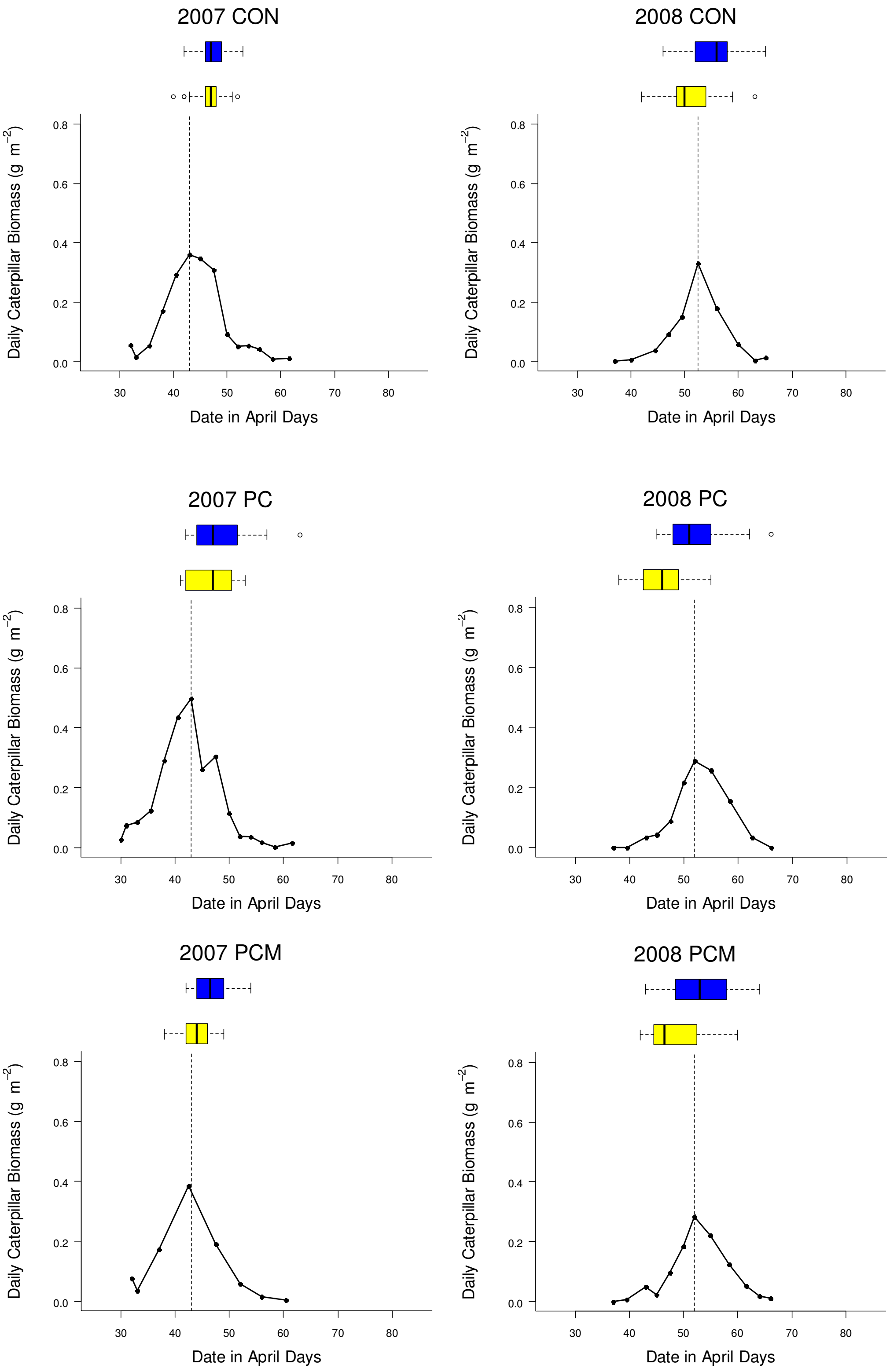

Figure 2.9. Seasonal peaks of mean daily dried caterpillar biomass in each dietary treatment area in Chaddesley Woods National Nature Reserve Worcs., UK, in years 2007 and 2008. Coloured box and whisker plots represent the date of peak Blue (in blue) and Great (in yellow) Tit nestling demands. The line within the box and whisker plots represents the median value and the box edges the first and third quartile ranges. Dates are in April Days where 1 = 1st April. The dashed line represents the caterpillar half-fall date.

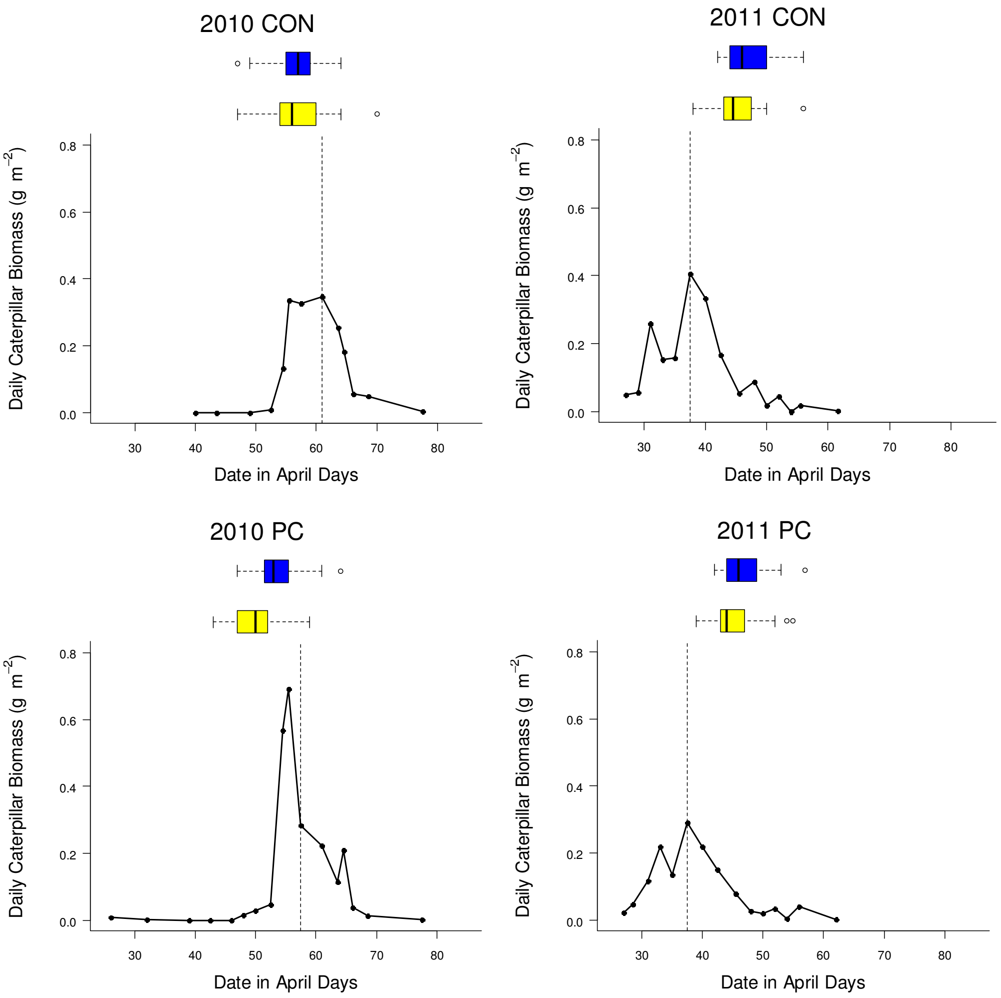

Figure 2.10. Seasonal peaks of mean daily dried caterpillar biomass in each dietary treatment area in Chaddesley Woods National Nature Reserve Worcs., UK, in years 2010 and 2011. Coloured box and whisker plots represent the date of peak Blue (in blue) and Great (in yellow) Tit nestling demands. The line within the box and whisker plots represents the median value and the box edges the first and third quartile ranges. Dates are in April Days where 1 = 1st April. The dashed line represents the caterpillar half-fall date.

2.4.3. Inter-annual variation in the timing of peak caterpillar availability

There were 23.5 days difference between the earliest half-fall date in the years studied (2011) and the latest half-fall date (2010), (Fig. 2.11). The mean half-fall date was day 48, 18th May. It was not possible to perform statistical analyses on the mean values of the timing of the caterpillar peak between years due to the low number of year replicates.

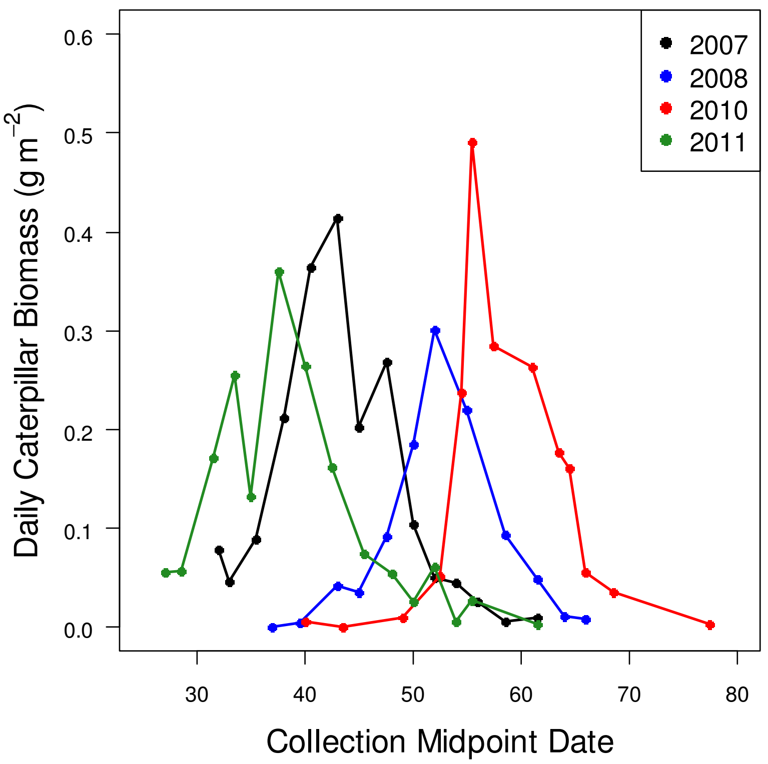

Figure 2.11. The seasonal peaks of mean daily dried caterpillar biomass in Chaddesley Woods National Nature Reserve, Worcs., UK, in years 2007, 2008, 2010 and 2011 in relation to the collection midpoint dates (see text for details). Collection midpoint dates are in April Days where 1 = 1st April.

2.4.4. Inter-annual variation in the amount of caterpillar biomass

There was significant inter-annual variation in the total amount of mean dried caterpillar biomass collected (Fig. 2.12, Table 2.3) but not in the mean daily dried caterpillar biomass (Table 2.3). Hence, although the amplitude of the daily peak did not vary between years, the amount of caterpillar biomass available to the breeding birds did. In both biomass analyses there was a significant year × midpoint collection date interaction (Table 2.3), indicating that there was a significant difference in the timing of caterpillar peak between years. The width or duration of the peak in biomass varied from 8.5 days (2011) to 14

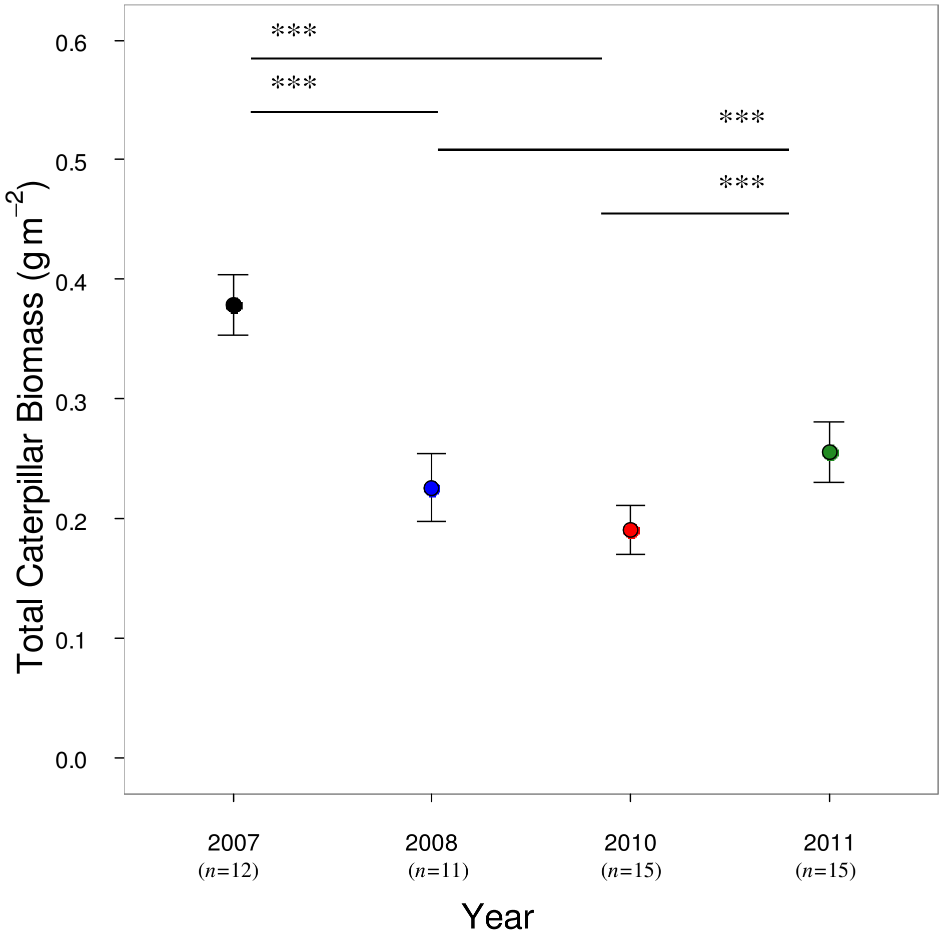

Figure 2.12. Total caterpillar biomass (mean ± 1 SE from model estimates) collected from Chaddesley Woods National Nature Reserve in Worcs., UK in 2007, 2008, 2010 and 2011. *** = $P < 0.001$. Number of dried caterpillar samples is shown in parentheses below axis labels. See Table 2.3 for statistical tests and text for details.

days (2008) although it was not possible to conduct statistical analyses on these data due to having only four replicates.

2.4.5. Comparing the timing of peak frass fall to peak pupation half-fall date

There was a difference of 5.5 days between the half-fall date as calculated by frass fall and that calculated using the caterpillar biomass falling to pupate (Fig. 2.13). This difference

Table 2.3. Results from simplified GLS analysis (correcting for temporal autocorrelation) of dried caterpillar biomass collected from Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011 regressed on significant explanatory variables (Fixed Effect). *F* test results (*F*) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 2.3.7.

Response	**Fixed Effect**	**Estimate**	**SE**	***F***	***P***	**Direction**	R^2
Total Caterpillar Biomass	Year			4.8629	**< 0.01**	Lowest: 2008 Highest: 2007	0.09
	Midpoint	0.0026	0.0011	0.0223	**< 0.05**		
2007,2008, 2010,2011	Year × Midpoint			9.6904	**< 0.001**	Earliest: 2011 Latest: 2008	
Daily Average Caterpillar Biomass	Year			1.2198	0.30	Highest: 2011 Lowest: 2008	0.09
	Midpoint	-0.0012	0.0005	0.0922	**< 0.05**		
2007,2008 2010,2011	Year × Midpoint			9.9580	**< 0.001**	Earliest: 2011 Latest: 2008	

Random effect was collection date in all models and biomass was averaged across woodland blocks to counter spatial autocorrelation. Tested fixed effects were year and collection date and only significant contributors to each model are displayed.

was driven predominantly by a slower decline in the pupating caterpillar biomass (Fig. 2.13) although the timing of the peak as measured from the raw data values in both frass and pupating caterpillars was only 2 days apart.

2.4.6. Within-female adjustments of seasonal timing

The difference in laying dates of female Blue Tits breeding in two consecutive years was significantly affected by the dietary treatment in year 1 and by the mismatch from the caterpillar peak in year 1, but not by the interaction between these two variables (Fig. 2.14,

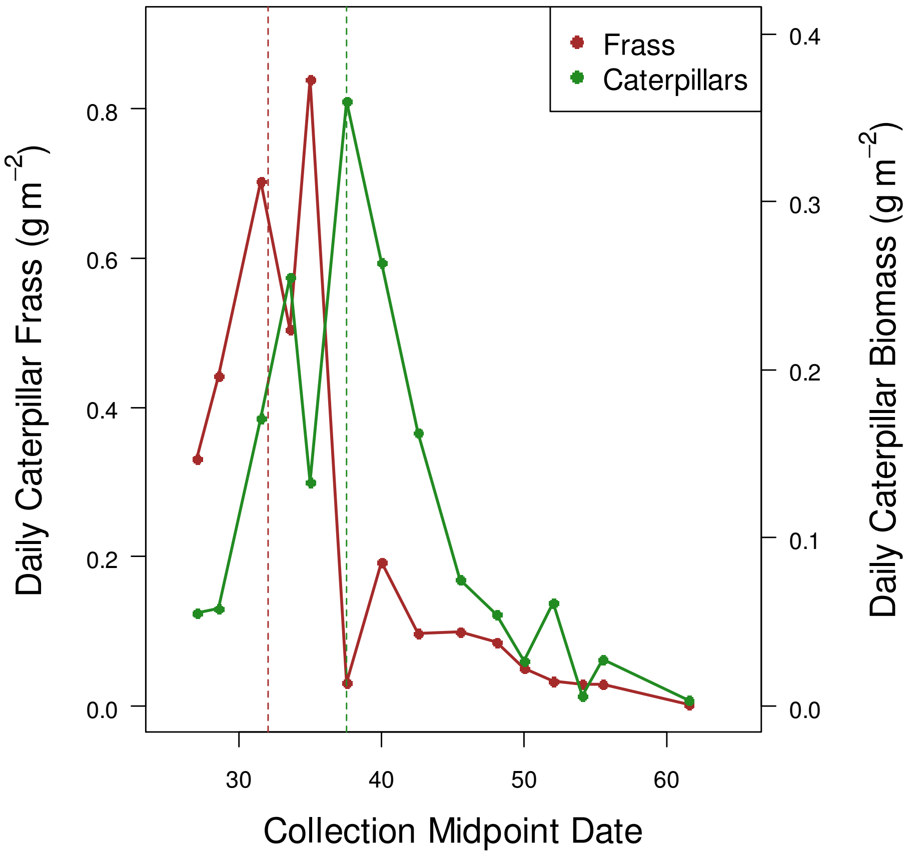

Figure 2.13. Caterpillar availability as measured using daily dried frass (brown line) and daily dried biomass from water-filled plastic trays (green line) collected in Chaddesley Woods National Nature Reserve, Worcs., UK in years 2007, 2008, 2010 and 2011 as a function of the collection midpoint date. Collection midpoint date is represented in April Days where 1 = 1st April. The dashed lines represent the half-fall date of caterpillar frass (in brown) and water tray biomass (in green).

Table 2.4). Although the interaction was not significant, the effect of mismatch in year 1 on the adjustment of laying date between year 1 and year 2 was in the opposite direction from the control area (Fig. 2.14).

In Great Tits there was a similar effect of dietary treatment in year 1 on the difference in laying dates in two consecutive years (Fig 2.15, Table 2.4). The interaction

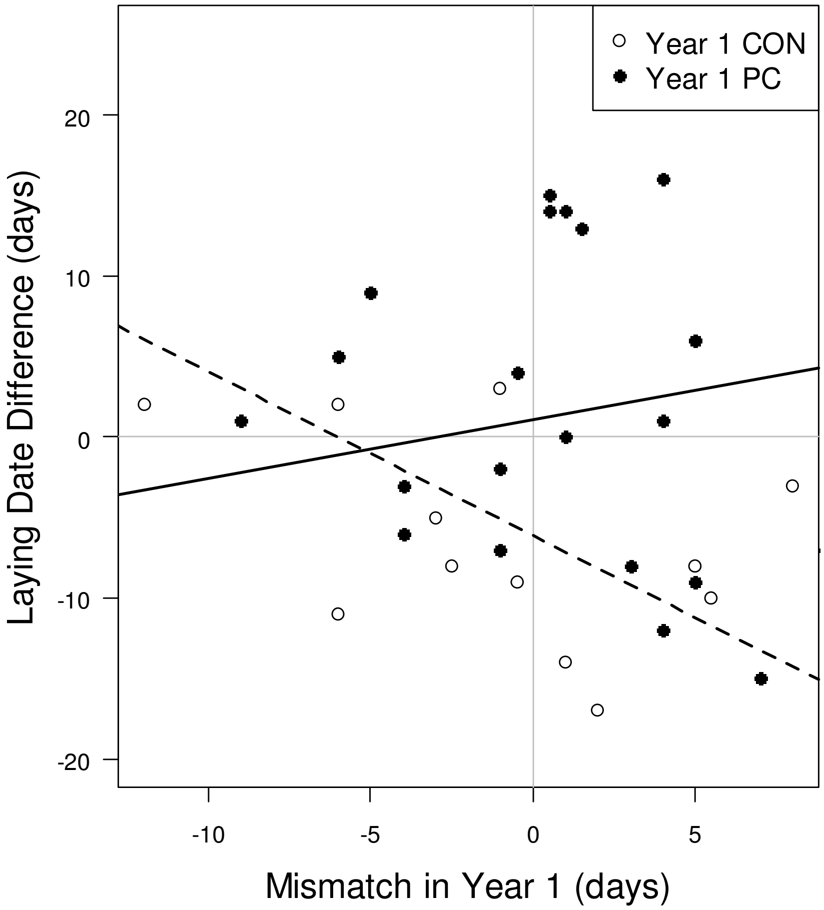

Figure 2.14. Difference between laying dates of female Blue Tits breeding in control (CON, open circle, dashed line) and peanut cake-supplemented (PC, filled circle, solid line) areas in the first of two consecutive years in Chaddesley Woods National Nature Reserve in Worcs., UK, between 2007 and 2011 against the mismatch of their timing from peak caterpillar availability in the first year. Regression lines (non-significant relationship) are predicted from model estimates correcting for annual variation. See Table 2.4 for statistical tests and text for details.

between dietary treatment in year 1 and mismatch in year 1 was nearing significance (Fig. 2.15, Table 2.4). When female Blue and Great Tits bred in the control area in the first year, they responded to asynchrony with the caterpillars by adjusting their laying date in the second year; females that bred too early in the first year delayed their laying date in the

Table 2.4. Results from simplified GLMM and GLM analysis of the intra-individual variation in laying date of female Blue (BT) and Great (GT) Tits breeding for two consecutive years in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011. The response variables were the difference in laying dates between the two years, and the deviation from the population mean in laying date in the second year. Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo R^2 value is presented. For details of statistical tests applied see section 2.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	R^2
Intra-individual Difference in Laying Dates 2007-2011	BT	Intercept	-6.136	4.741				0.66
		Dietary Treatment Year 1: PC	7.208	2.670	10.4	**< 0.01**	Earlier laying in PC area	
		Mismatch Year 1	-1.016	0.367	124.1	**< 0.001**	Lower laying date difference	
		Dietary Treatment Year 1: PC × Mismatch Year 1	0.366	0.420	0.7	0.41		
Intra-individual Difference in Laying Dates 2007-2011	GT	Intercept	-5.688	4.149				0.73
		Dietary Treatment Year 1: PC	4.198	1.823	6.0	**0.05**	Earlier laying in PC area	
		Mismatch1	-0.612	0.214	124.5	**< 0.001**	Lower laying date difference	
		Dietary Treatment Year 1: PC × Mismatch Year 1	0.423	0.244	3.3	0.07	Higher laying date difference in birds breeding in PC area in year 1 with increasing mismatch	
Laying Date Year 2 Residual Regression 2007-2011	BT	Intercept	-0.5851	0.517				0.05
		Dietary Treatment Year 1	0.6399	0.455	3.2	0.14		
		Dietary Treatment Year 2	0.1678	0.426	0.2	0.49		
Laying Date Year 2 Residual Regression 2007-2011	GT	Intercept	1.580	0.588				0.31
		Dietary Treatment Year 1: PC	-0.993	0.621	2.2	0.08		
		Dietary Treatment Year 2: PC	-2.035	0.615	13.0	**< 0.001**	Earlier laying in PC area	
		Dietary Treatment Year 1 × Dietary Treatment Year 2	1.120	0.676	1.9	0.1		

Random effect was year 1 in the first model and year 1 and dietary treatment in the second model. Tested fixed effects were dietary treatment in year 1 (Intercept = control, 'PC' = peanut cake) and mismatch from peak caterpillar availability in the first models, and dietary treatment in years 1 and 2 in the second models. Only significant contributors to each model are displayed.

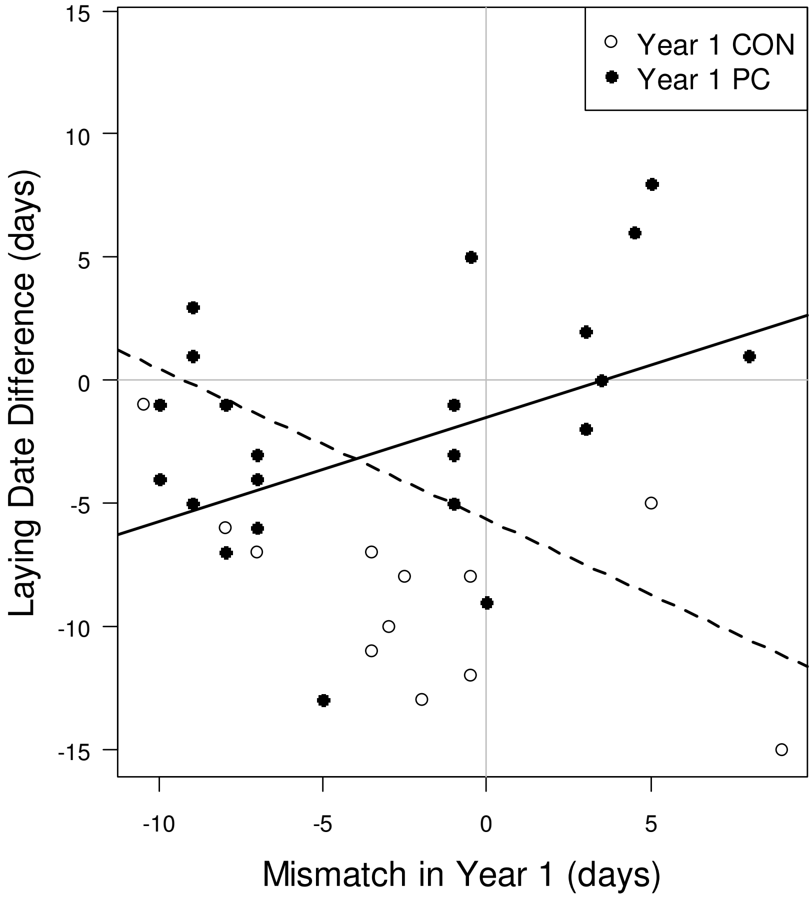

Figure 2.15. Difference between laying dates of female Great Tits breeding in control (CON, open circle, dashed line) and peanut-cake supplemented (PC, filled circle, solid line) areas in the first of two consecutive years in Chaddesley Woods National Nature Reserve in Worcs., UK, between 2007 and 2011 against the mismatch of their timing from peak caterpillar availability in the first year. Regression lines show a near-significant relationship predicted from model estimates correcting for annual variation and the dietary treatment in the second year. See Table 2.4 for statistical tests and text for details.

second year and females that bred too late in the first year advanced their laying in the second year (Fig. 2.15). When female Great Tits bred in the supplemented area first, however, the females advanced their laying date when they had laid too early in the previous year and delayed their laying date when they had laid too late (Fig. 2.15). When the deviance of laying date from the population response by individual females was examined, again there appeared to be little effect of any combination of food supplementation on Blue Tits (Table 2.4). In Great Tits, however, there was a significant effect of combinations of food supplementation treatments on the accuracy of the female's ability to match the population mean (Fig. 2.16, Table 2.4). Tukey post-hoc analysis

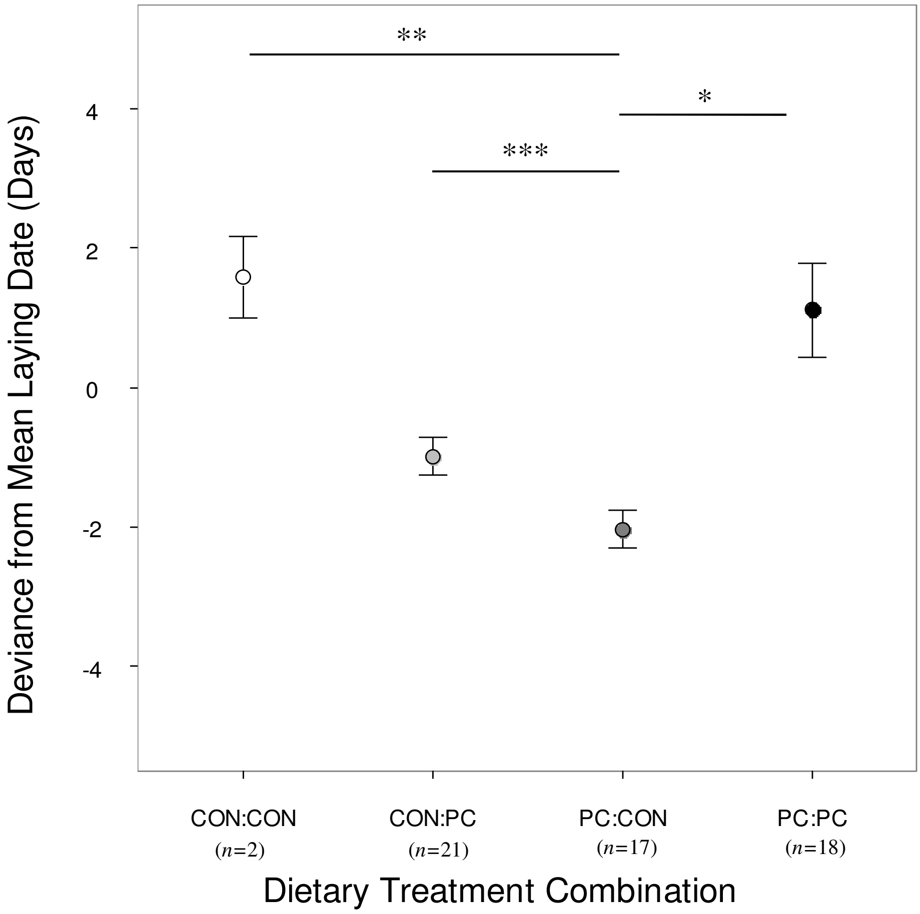

Figure 2.16. Deviance from the population mean for laying date in the second of two consecutive years (mean ± SE) of individual Great Tit females breeding in Chaddesley Woods National Nature Reserve in Worcs., UK, between years 2007 and 2011. Dietary treatment combinations are represented for females breeding in an unsupplemented area in both years (CON:CON, open circles), those breeding in a supplemented area in the first year and an unsupplemented area in the second (CON:PC, light grey circles), those breeding in a supplemented area in the first year and an unsupplemented area in the second year (PC:CON, dark grey circles), and those breeding in a supplemented area in both years (PC:PC, black circles). *** = $P < 0.001$, ** = $P < 0.01$ and * = $P < 0.05$. The number of females in each treatment combination is represented in parentheses below each axis label. See Table 2.4 for statistical tests and text for details.

revealed that females breeding first in the supplemented area and subsequently in the control area were significantly further from the population mean for the lay date in year 2 than all other combinations although there were few replicates for females breeding in the control group in both years. Females breeding in a food supplemented area for two consecutive years had positive residual deviance of a similar value to females breeding in a control area in both years.

2.5. Discussion

Food supplementation led to a significant seasonal advance of laying dates in Great, but not Blue, Tits (Fig. 2.6, Table 2.1) and to an advance of hatch dates in both species (Fig. 2.7, Table 2.1). Food supplementation increased the variance of laying dates in both Blue and Great Tits in certain years. There was a significant increase in mismatching between peak nestling nutritional demand and caterpillar availability in food-supplemented Great, but not Blue, Tits (Fig. 2.8, Table 2.2). There was significant inter-annual variation in laying dates, hatch dates and mismatching with peak caterpillar availability. Analyses of caterpillar biomass data revealed significant inter-annual differences in both the timing (Fig. 2.11, Table 2.3) and abundance (Fig. 2.12, Table 2.3) of caterpillars available to breeding birds. There was a 5.5 day difference in caterpillar biomass as measured using pupating caterpillars, and that measured using frass production (Fig. 2.13). When the matching of female seasonal timing to the peak in caterpillar availability was examined at the intra-individual level, food supplementation in the first of two consecutive years was found to have a negative effect on the accuracy of timing in the second year in Great, but not Blue, Tits (Figs 2.14 and 2.15, Table 2.4). Female Great Tits breeding in a supplemented area in one year but an unsupplemented area in the other had significantly earlier laying than those breeding in a supplemented area in both years or those breeding in an unsupplemented area in both years (Fig. 2.16, Table 2.4).

2.5.1. The effects of food supplementation on laying and hatch dates

An advance in laying date associated with food supplementation has also been found in many other studies (reviewed in Boutin 1990, Robb *et al.* 2008a) and earlier years of investigation in this population (Harrison *et al.* 2010). This advance was not significant in Blue Tits, however, which is unusual as Blue Tits commonly advance their laying date in response to food supplementation (Clamens & Isenmann 1989, Nilsson & Svensson 1993, but see Nager *et al.* 1997). This phenological shift in breeding was then increased by the time hatching occurred to a mean of 1.8 days in Blue Tits and 4.1 days in Great Tits, potentially as a result of lower clutch sizes and reduced incubation duration in response to food supplementation (Harrison 2010).

In analyses of lifetime reproductive success of Great Tits in a Dutch population, the probability of a fledgling recruiting into the breeding population has been calculated to decline by -0.082 recruits per day (Verhulst *et al.* 1995). This translates into an increase of 0.34 recruits for a 4 day advance in hatching. The fact that food supplementation studies advance laying to only a small degree, when compared to the spread of laying dates in a population, is commonly considered evidence that food availability does not constrain the onset of egg production (Meijer & Drent 1999). However, although the shift in Great Tit hatch dates in my study population seems quite low, the extra recruitment advantage of a 4-day advance is considerable. Given that there would be expected to be a shift towards a greater degree of synchrony between broods at the time of hatching as population-level variation in timing declines (Cresswell & McCleery 2003), this increase in disparity between the two dietary treatment groups from that exhibited during egg laying is particularly striking.

I predicted a decrease in variance of laying dates in response to food supplementation. However, where there was a difference in the homogeneity of variance of laying dates in response to food supplementation, it was an increase and not a decrease. This is an intriguing result as it suggests that responses to food supplementation occur at the level of the individual; certain females advance their laying date in response to supplementation by a larger amount than others, and this increases the disparity between the clutch initiation dates of supplemented individuals. For this reason the 'cue' hypothesis (Källander 1974, Visser *et al.* 2011a) seems to be supported in the Chaddesley population. Had there been an energetic constraint on the initiation of egg laying, this would have been removed by supplementation and the whole population in the supplemented area would be expected to advance. There could be other micro- (e.g. calcium: Reynolds & Perrins 2010) or macronutrients (e.g. protein: Schoech *et al.* 2004) necessary for egg production, however, which peanut cake does not provide. Thus, a nutritional constraint on egg laying may still exist and so the *constraint hypothesis* cannot be dismissed by these data (but see Nager *et al.* 1997, Stevenson & Bryant 2000, te Marvelde *et al.* 2011c).

2.5.2. The effects of food supplementation on phenological matching

Contrary to my predictions, the advance in laying and hatch dates did not equate to more accurate timing in relation to peak caterpillar availability. Indeed, although the seasonal timing of Blue Tits was neither significantly positively nor negatively affected by supplementation, the matching of Great Tits to the caterpillar food peak was highly negatively affected. When the raw mismatch values were examined it became apparent that this difference was driven by the Great Tits breeding too early, rather than too late.

Both species struggled to advance their breeding attempts to the degree necessary to match the caterpillar peak in 2011, indicating that the population may be unable to track very early caterpillar peaks. This is similar to the phenology of Dutch populations that are approximately 10 days behind the caterpillar peak (Visser *et al.* 1998). In the phenologically early years the advanced laying and hatch dates in the supplemented area could confer fitness benefits in terms of more offspring being recruited into the population (Verboven & Visser 1998). Mistiming in the phenologically late years could have negative consequences for adults and offspring due to the reliance on non-optimal prey items in the absence of caterpillars (Pascual & Peris 1992, Nilsson 1994). Food supplementation could, therefore, have dramatically different effects under varying natural food availability conditions. This reflects other studies that have found that the effects of food supplementation vary under different ecological contexts (Clamens & Isenmann 1989, Svensson & Nilsson 1995). Food supplementation can, therefore, lead to substantial phenological mismatch between breeding birds and the seasonal availability of their key foods. The fitness consequences of phenological matching and mismatching within this population could have significant demographic consequences (Miller-Rushing *et al.* 2010). Very few food supplementation studies monitor natural food availability and certainly not over a number of years (but see Grieco *et al.* 2002, Gienapp & Visser 2006, Bourgault *et al.* 2009), and this is the first time that an examination of the various effects of food supplementation under different timing of natural food availability has been possible due to the fact that the Chaddesley study has been conducted over multiple years.

With increasing selection for early breeding in Great Tits as caterpillar emergence advances in response to warming spring temperatures (Visser *et al.* 1998, Both *et al.* 2009), phenological mismatches are becoming more common in some parid populations (e.g. in the Hoge Veluwe, The Netherlands: Visser *et al.* 2006) but more scarce in others (e.g. Wytham Woods, Oxford, UK: Cresswell & McCleery 2003). Such mismatches could be an adaptive response to changing environmental conditions (Visser *et al.* 2011b) if the fitness *benefits* of early breeding are outweighed by the fitness *costs* of earlier egg laying and incubating in cooler temperatures (Stevenson & Bryant 2000, de Heij *et al.* 2007). Having underwritten the costs of such potential increased energetic or thermal demands by providing a high calorie food supplement, I predicted that supplementation would improve phenological matching within my population by reducing any physiological constraints, thus rendering this phenological mismatch non-adaptive. Here, I have examined the intra-individual phenological responses of breeding Blue and Great Tits to supplementation.

2.5.3. Inter-annual variation in timing and magnitude of caterpillar availability

Both the timing and the magnitude of the peak in caterpillar biomass varied significantly between years, in common with other longer-term studies of inter-annual caterpillar variation (e.g. Tinbergen 1960, Nager & van Noordwijk 1995, Charmantier *et al.* 2008). I found only a 23.5 day difference, however, between the earliest year in terms of half-fall date and the latest. This compares with a study in Wytham Woods which records a difference of up to 30 days, although the latest years were 1951 and 1962 (Perrins & McCleery 1989) and there have been significant effects of warming spring temperatures on caterpillar phenology since then (Visser *et al.* 2006). A more recent study in The Netherlands found a reduced 21 day difference, in conjunction with a persistent advance in peak caterpillar half-fall date since 1985 (Visser *et al.* 2006). There were no emerging patterns in the 'width' of the caterpillar peak; one study found that a 'late' season should be associated with a higher and sharper peak (Visser *et al.* 2006) and although 2010 exhibited this tendency (Fig. 2.11), 2008 did not and it had the longest peak of 14 days. There was no significant difference in the magnitude of caterpillar peaks across years in my study, however, although this could be attributable to a low number of replicates.

One of the omissions of my study was not accounting for the variation in the phenology of peak caterpillar availability between trees. There is substantial spatial variation in the timing of peak caterpillar availability (Tremblay *et al.* 2003) due to significant asynchrony being maintained between the budburst of individual oak trees (Tikkanen & Julkunen-Tiitto 2003). However, it would require many more years of study at the field site before it is possible to analyse fine-scale individual responses of birds to spatial variation in addition to broad-scale temporal variation in such parameters. There were no significant differences in total caterpillar biomass between the woodland blocks in Chaddesley, however, and given the extent of variation from one tree to another it would require sampling underneath trees adjacent to the nestboxes rather than at centralized points.

The half-fall date, as measured by collecting the pupating caterpillars in water-filled trays, differed from that measured by frass collection by 5.5 days. The comparative accuracy of both techniques as a means of recording biomass has been verified (Zandt 1994, Fischbacher *et al.* 1998), and timing has been compared between techniques in two locations (Lack 1955). My data appear to be the first time that the difference in measurement of the timing of the peak in biomass has been investigated in the same location. In a previous study by Lack (1955) the timing of the peak biomass was compared by using the day of the highest frass measurement against the half-fall date of the pupating caterpillars, a difference of 2.5 days in my study. It has been found that the proportion of caterpillars in the nestling diet increases as the number of caterpillars collected from the tree canopy decreases (Naef-Daenzer *et al.* 2000). Given that the frass collectors in my study area registered the majority of biomass occurring before the peak in pupating caterpillars, and that there was an advance of 2.5 days compared to the tray method, using the frass collection method could result in the peak being estimated earlier. This has important implications for the accurate assessment of phenological mismatch as a result of global climate change (Both *et al.* 2009), particularly in populations where Great Tits are estimated to be breeding behind the caterpillar peak (Visser *et al.* 2006). Further years of data collection are required, however, before this could be confirmed unequivocally. It may be imperative that all such studies use a standard methodology to estimate such natural food availabilities before inter-population conclusions can be drawn (Visser & Both 2005).

2.5.4. Female phenotypic plasticity in lay date and food supplementation

Studies of Blue Tits (e.g. Grieco *et al.* 2002) and Great Tits (e.g. Nager & van Noordwijk 1995, Gienapp & Visser 2006) have shown that laying date can be influenced by food availability in the previous year and that phenologically mismatched birds should correct themselves in the second year of breeding (Nager & van Noordwijk 1995). Two of these studies (Grieco *et al.* 2002, Gienapp & Visser 2006) are based, however, on creating an artificial food peak late in the season to manipulate the perception of timing in the first year of the study without a commensurate artificial peak provided in the second year. Findings of such studies seem to indicate that the females are able to ‘learn’ from mistakes made in the previous year and adjust their timing, even when supplemented pre-laying (Grieco *et al.* 2002). Such phenotypic plasticity is suggested as an adaptive response to energetic or thermal constraints on earlier egg laying (Visser *et al.* 2011b).

I designed this study to investigate whether individual females modulated their inter-annual timing differently in response to food supplementation, and whether they exhibited carry-over effects (Harrison *et al.* 2011) from the supplementation regime in the previous year. Contrary to my prediction of increased plasticity and improved matching in the supplemented females, the opposite was true. Although there were small sample sizes for both Blue and Great Tit analyses of within-individual responses to food supplementation, some intriguing results emerged for Great Tits in particular. Because of missing data from 2009, there were insufficient data to examine in detail the effects of the combination of food supplementation treatments on the mismatch in year 2 in both species. However, when the difference in laying dates between years was used as a response variable, to illustrate directional shifts in clutch initiation date from one year to the next, there was a striking effect of food supplementation in Great Tits and a tendency towards a similar relationship in Blue Tits.

The relationship between the difference in laying dates and the degree of phenological synchrony in the first year is expected to be a negative slope, as females that laid too early in the first year correct themselves by laying later in the second year (e.g. Grieco *et al.* 2002, Gienapp & Visser 2006). Within my study population the direction of this linear relationship in the CON:PC treatment combination was the same as for previous studies (Figs 2.14 and 2.15), but the intercept was lower in both species as females in the supplemented area advanced their laying date in the second year. When female Great Tits were food supplemented in the first year, however, the direction of the relationship changed, and females that had bred too early in the first year laid even earlier in the second year. Females that bred too late in the first year initiated laying even later in the second. Protracted food supplementation in the first year appeared to disrupt the precision of the female's plastic response to natural food availability and misdirect and exacerbate the extent of the mistiming from the first year. These carry-over effects are unique as the previous phenotypic plasticity studies involving food supplementation of Blue Tits (e.g. Grieco *et al.* 2002) and Great Tits (e.g. Gienapp & Visser 2006) suggested that short-term supplementation both prior to egg laying and during the incubation period did not alter the direction of the relationship between synchrony and change in laying dates, but merely shifted the intercept higher (Grieco *et al.* 2002) or lower (Gienapp & Visser 2006).

When female Great Tits were supplemented in both years of the intra-individual study their deviance from the mean population difference in laying dates was lower than females that were supplemented in only one year of the study (Fig. 2.16). Again, females whose dietary treatment changed from supplemented to control (i.e. PC:CON) exhibited higher deviance from the population mean than females whose treatment changed from control to supplemented (i.e. CON:PC). There appears to be some evidence, therefore, that it is the shift from supplemented to control that decouples the timing of Great Tit breeding attempts from natural resource availability and that persistent supplementation allows the females to re-calibrate their timing. This could be because the shift from supplemented to control represents the loss of a stable energy resource from one year to the next and that environmental conditions are, therefore, more unstable.

Protracted food supplementation during the breeding season could, therefore, have concerning consequences for the long-term reproductive success of individuals by disrupting synchrony with natural food resources. Should inter-annual variation in the timing of the peak in caterpillar availability be reduced as a result of increasing spring temperatures, however, there could be a reduction of selection on plasticity in laying date and increased selection on early breeding. If this is the case, protracted food supplementation during the breeding season in repeated years could enable birds to track the advance of peaks in their natural food resources through earlier breeding.

2.5.5. Inter-specific differences in responses to food supplementation

There was a marked difference between Blue and Great Tits in both their population- and individual-level responses to food supplementation. The phenological matching of Great Tits was significantly adversely affected by supplementation whereas the same was not true of Blue Tits. These two species are considered to have similar *r*-selected life history strategies, albeit with Great Tits being further along the *r/K* selection continuum, exhibiting lower fecundity and a higher age of reproductive senescence (Bennett & Owens 2002). The inter-specific variation in response to food supplementation of such important ecological fitness traits as laying date and phenological matching is therefore surprising. Blue Tits appear to be able to maintain their phenotypic plasticity even when provided with cues which may mislead them, and can advance their breeding without becoming mismatched from key food resources.

Blue Tits and Great Tits have distinct foraging niches at certain times of the year (Suhonen *et al.* 1994), particularly in March and April when egg laying begins (Gibb 1954). Gibb (1954) observed Great Tits spending more time foraging on the ground at this time of year, whereas Blue Tits were foraging in terminal branches of the trees. Even accounting for the advance in spring phenology of 8 to 14 days (Crick 1997, Visser *et al.* 1998, 2006) in the time since Gibb (1954), this separation of foraging location would still be valid at the time that supplementation within my study system began. I propose, therefore, that Blue and Great Tits may respond to different phenological cues in timing their breeding attempts, potentially through the consumption of different foods at this point in the breeding season (e.g. by consuming tree buds: Bourgault *et al.* 2006). Great Tits are more generalist foragers (Slagsvold & Wiebe 2007), and so the appearance of a stable food resource prior to the breeding season could stimulate them to advance laying more consistently at the population level than Blue Tits. There is evidence within the existing literature to support this in the form of conclusions from disparate studies, indicating that Blue Tits may use bud formation in trees as a cue to initiate laying (Bourgault *et al.* 2010), whereas Great Tits use neither buds nor the visual appearance of caterpillars (Schaper *et al.* 2011). To my knowledge, this is the first time that persuasive comparative data have indicated this might be the case (but see Clamens & Isenmann 1989 for a study where Blue but not Great Tits advanced laying date in response to food supplementation).

In the next chapter I will investigate the effects of protracted food supplementation on clutch size and egg size, at both the population and individual level and use measures of energy expenditure to determine whether there is evidence of an energetic constraint on egg laying.

Chapter Three

The effects of food supplementation on maternal investment during egg laying

3.1. Abstract

Avian clutch size is one of the most widely researched aspects of life history theory. Intra-specific variation in clutch size has led to the suggestion that producing eggs may be energetically costly and limited by the availability of exogenous reserves. Food supplementation may have profound, if seemingly contradictory, effects on the reproductive output of birds and has been reported to increase, decrease or have no effect on clutch size. One method of resolving these conflicting results and exploring the cost of egg laying is to measure the physiological cost of parental investment. Energy expenditure can be used as a form of currency to measure maternal investment, but this has never been achieved within a food supplementation study. In a novel experiment, I measured the Daily Energy Expenditure (DEE) of egg-laying Blue Tits and Great Tits during a food supplementation study to determine whether supplementation reduced the costs of the egg laying period. Females of both species reduced DEE in response to food supplementation without reallocating this energetic saving into increasing clutch or egg size. There was a seasonal decline in DEE in Great Tits associated with females in high body condition and a seasonal increase of DEE of females in low body condition. These results confirm for the first time that breeding birds may make energetic savings from having a supplementary food resource, but that the translation of this saving into reproductive effort is complex and condition-dependent.

3.2. Introduction

From early studies of the evolution of avian clutch size (Lack 1947), there has been much contention about what determines the quantity and quality of eggs laid (reviewed in Cody 1966, Godfray 1991). Avian eggs require considerable extrinsic resources to be obtained by the female in the production of her clutch which may even exceed her own body mass (Perrins 1970). The availability of exogenous resources is particularly important for ‘income breeders’, that diminish little of their own endogenous reserves to form eggs (Drent & Daan 1980, Meijer & Drent 1999). When exogenous resources are limited, it is hypothesised that a trade-off will exist between the number and the quality of eggs that a female will produce (Stearns 1977, van Noordwijk & de Jong 1986). Food availability is hence widely accepted to be intricately related to the production of eggs by income breeders, although the mechanisms through which food acts either to constrain or cue breeding females are unclear (Martin 1987, Nager 2006). Egg-laying females also demonstrate specialised foraging for micronutrients (e.g. Reynolds & Perrins 2010), in order to meet some of the specific physiological requirements of egg formation (Graveland & Berends 1997). The provision of anthropogenic food sources may, therefore, affect the number and quality of eggs laid by a female. Food supplementation may not only reduce the energetic costs incurred during the egg laying period by presenting a female with a predictable source of energy to fuel physiological processes, but it may also reduce the proportion of foraging time devoted to finding such sources, thereby allowing more time to seek specific nutrients such as calcium.

3.2.1. Clutch size theory in a life history framework

The dramatic inter- and intra-specific variation in avian clutch size has long fascinated life history theorists (Lack 1947, 1954). Continuing Lack's early work which determined that food availability when rearing young limits the number of offspring a breeding bird will produce (Lack 1947), Perrins suggested that females may also be energy-limited at the time of egg laying (Perrins 1970). It is, however, considered unlikely that energy will restrict the number of eggs that they can produce (Lack 1966, Perrins 1970). In a refinement of his original theory on clutch size determination, Lack (1947) identified that the average clutch size should be the one that results in the highest number of surviving young (Lack 1954, 1966). It was thought to be the ability of the parents to provide food for the nestlings that determined this optimal clutch size (Lack 1954). This premise has been tested many times, predominantly through experiments that manipulate clutch or brood size; some studies found support for the principle (e.g. Cronmiller & Thompson 1980, Pettifor *et al.* 1988) and others did not (summarised in Vanderwerf 1992). The Lack (1947) hypothesis was rejected by the majority of studies in a meta-analysis of 42 brood enlargement experiments (Vanderwerf 1992) on the basis that parents rearing enlarged broods produced more young. However, in the meta-analysis studies conducted over multiple years demonstrated that this increase in one year did not translate into more young raised overall. This could be either due to a downstream cost associated with increased parental effort (i.e. reduced adult survival or reduced future reproductive output) or because parents optimised the number of young across multiple seasons to reduce the detrimental effect of 'bad years' (e.g. Boyce & Perrins 1987).

Two overarching theories have been developed from the doubt surrounding the Lack hypothesis. These hypotheses aim to explain why birds do not lay larger clutches given that the ‘optimal’ clutch size (in terms of production of recruits into the breeding population) is often larger than the observed average clutch size (Klomp 1970, Lessells 1986) and that the largest clutches and broods within a population almost always produce more recruits (Gustafsson & Sutherland 1988, Perrins & McCleery 1989). The first explanatory theory is that of life history trade-offs (Charnov & Krebs 1974). This states that egg production may be costly either as a process in its own right (Carey 1996, Monaghan *et al.* 1998), or because larger clutches and broods impose penalties for the adult. These penalties may manifest in terms of reduced ‘residual reproductive value’ (Gustafsson & Sutherland 1988, Visser & Lessells 2001), in other words a reduction in the combined value of adult survival and future reproductive potential (Williams 1966).

The second theory is the ‘individual optimization hypothesis’, developed from Lack’s studies, which posits that each individual lays the number of eggs that will maximise their reproductive output within the current season (Perrins & Moss 1975). Under this hypothesis reducing the clutch size of an individual will have equally as detrimental an effect on the number of recruits produced (or overall fitness) as increasing clutch size. Although the hypothesis has been demonstrated to apply in certain populations of species such as the Great Tit (Pettifor *et al.* 1988), in other populations of the same species its viability has been questioned (Both *et al.* 1998, Tinbergen & Both 1999) as the principles of the hypothesis were upheld in some years but not others. This lack of evidence for the individual optimization hypothesis in the latter studies suggests that other constraints may be in operation during egg laying and that they may vary inter-annually. Such constraints may prevent the laying of larger clutches but not affect parental ability to raise offspring later in the breeding season (Tinbergen & Both 1999). Indeed the difference between the two populations studied in terms of energy limitation during egg laying are manifested in the ability of one and the inability of the other to track advancing prey phenology (reviewed in Charmantier *et al.* 2008).

In spite of much theoretical and experimental work, the physiological and life history costs of increasing investment in egg production remain unclear (Williams 2005). One of the key unanswered questions is whether increased egg production is costly and without resolving this issue it is difficult to assess the relative role of egg production in determining life history traits.

3.2.2. Clutch and egg size, and food supplementation

Many studies have concluded that food availability should not limit clutch size (reviewed in Meijer & Drent 1999), even if it represents a threshold which constrains the timing of breeding. Although food availability is intuitively related to the number of eggs a female can produce based on the increase in resource requirements particularly for smaller species, results from food supplementation studies in passerines have been equivocal (reviewed in Boutin 1990, Nager 2006, Robb *et al.* 2008a). By way of example, food supplementation has been shown to increase (Nager *et al.* 1997, Ramsay & Houston 1998), have no effect on (Clamens & Isenmann 1989), or even decrease (Harrison *et al.* 2010) clutch size of Blue and Great Tits. Such inconsistent results may be attributable to the nutritional composition of the food supplement, as females may be constrained by specific nutrients such as protein during egg laying, rather than by energy availability (e.g. Reynolds *et al.* 2003a). The Nager (1997) study tested for protein limitation in Great Tits but found no difference in clutch sizes between high-protein and high-energy food treatments, however, as both increased clutch size. In the Harrison *et al.* (2010) study of Blue and Great Tits testing the effect of a high-energy supplement, the supplement was found to decrease clutch size. Although both studies used supplements described as 'high-energy', there may have been micro-nutrient differences between them (Nager *et al.* 1997 - coconut fat and sunflower seeds, Harrison *et al.* 2010 – beef tallow and peanut flour) which had an unforeseen effect on clutch size.

Food supplementation has, therefore, thus far failed to clarify how food availability affects the number of offspring a female can produce, possibly due to the discrepancy between the nutritional value of supplements and that of natural diet (te Marvelde 2012). Alternatively it may be that food availability does not limit egg production energetically, and other factors such as parental condition or quality take precedence (e.g. Slagsvold & Lifjeld 1990). With the provision of food supplements by members of the public increasing (Chapter One), it is important that more clarity is achieved. Food availability may also be subject to shifts in timing or abundance as a result of climate change (Visser *et al.* 2006, Both *et al.* 2009) and the ability to predict the implications of such shifts in food availability for breeding birds may require a thorough understanding of the role that food availability plays in determining the timing and reproductive success of birds (Houston *et al.* 2007b, Schoech *et al.* 2008).

One consistent finding of egg-laying studies is that within seasonal habitats, there is a decline in clutch size as the season progresses (Lack 1947, Kluijver 1951), although often there is an increase in egg mass (Perrins 1970). This is explained by later breeding females benefitting from more favourable conditions later in the egg-laying period. This indicates the potential for a trade-off between the quantity and quality (e.g. egg size) of eggs that are produced as the season progresses (Williams 2001a). Egg size has been found to increase in response to food supplementation (Harrison 2010), particularly when the supplement contains a high protein content (Ramsay & Houston 1997, Reynolds *et al.*

2003a), although the majority of studies find no effect (reviewed in Christians 2002). The role of food availability is, therefore, also unclear in terms of the quality and absolute size of eggs that a female may produce.

3.2.3. The energetic cost of egg production

Given the discrepancy between studies in the effects of food supplementation on clutch and egg size, attempting to elucidate how the quantity and quality of food available to egg-laying females affects the cost of reproduction becomes problematic. One of the most fundamental factors which has been poorly explored, however, is the role that physiological processes play in egg production (Williams 2005). Analysing the energy requirements of egg-laying females has provided new insights into the energetic costs of egg production. These have shown that ambient temperature is negatively correlated, and egg mass positively correlated with DEE and Resting Metabolic Rate (RMR) (Stevenson & Bryant 2000, Nilsson & Råberg 2001). Field studies have not been able to relate the energy expenditure of a reproductive female to seasonal timing of egg laying or to clutch size, however (Ward 1996, te Marvelde *et al.* 2011c). Therefore, it remains unclear whether it is beneficial for females to pay a higher energetic cost to overcome low temperatures or low food availability early in the egg-laying phase and breed earlier.

Recent studies on captive birds have demonstrated that the costs of egg laying may be relatively low, at 22-24% above Basal Metabolic Rate (BMR) (e.g. Vézina & Williams 2002, Salvante *et al.* 2010). Furthermore, females forced to cover increased energetic costs, such as low temperatures, may reallocate energy between different activities in order to meet the total energetic requirements, rather than increasing metabolic rate (Salvante *et al.* 2010). A study of RMR in captive European Starlings (*Sturnus vulgaris*) (Vézina &

Williams 2003) succeeded in attributing an energetic cost to one of the physiological changes required for egg production; the mass of the oviduct increased 22-fold prior to egg laying and this explained 18% of the variance in RMR. In a study of captive Zebra Finches, RMR was not related to the number and the mass of eggs laid but was highly repeatable within individuals (Vézina & Williams 2005). This indicates that the profound physiological changes and associated energetic costs experienced by an egg-laying female may not be visible in life history parameters such as clutch size. Indeed, within Zebra Finches complex energy management strategies have been revealed, wherein females reduced their locomotor activity rather than increased food consumption to cover the costs of egg production (Houston *et al.* 1995, Vézina *et al.* 2006). The latter study also found that females exhibiting the highest DEE whilst laying their first egg laid larger and heavier clutches (Vézina *et al.* 2006). Although these captive studies have provided critical new insights into the inter-relationship between egg production, resource availability and locomotor activity, there exists a paucity of data on the energy budgets of free-living egg-laying females, particularly in relation to food availability.

In order to address some of these questions, in 2009 and 2010 I investigated the DEE of free-living Blue and Great Tits that were receiving *ad libitum* food with a high-energy supplement, using the DLW technique (Speakman 1997). I aimed to examine the role food availability plays in both the energy budgets and reproductive decisions of egg-laying females by testing whether energy availability, rather than nutrient availability, limited clutch size. This study represents a crucial missing link in the current literature between the energetic costs of egg laying in relation to food availability in free-living birds (Vézina *et al.* 2006, Salvante *et al.* 2010), and how this cost may influence key life history parameters. It is the first time that energy expenditure during egg laying has been measured in a food supplementation study. Based on the theoretical positive covariance between life history traits in conditions of higher resource availability (van Noordwijk & de Jong 1986), I predicted that food supplementation should increase clutch size at both the population and individual levels (e.g. Nager *et al.* 1997) and increase egg size and mass (e.g. Christians 2002). I also predicted that food supplementation would reduce the DEE of egg-laying females by providing easier foraging at a predictable food resource and lowering foraging costs (as increased food availability has been found to reduce DEE during the nestling phase – te Marvelde *et al.* 2011b). I anticipated that food-supplemented females would advance their laying date (e.g. Svensson & Nilsson 1995) and lay larger eggs (Reynolds *et al.* 2003a) without any concomitant rise in DEE. I also predicted that DEE would decrease with female body condition and in unsupplemented (control) females with laying date, as temperatures increased and natural food resources became more plentiful (e.g. Salvante *et al.* 2010).

3.3. Methods

3.3.1. Nestbox study

Details of the study site and routine monitoring of breeding attempts between 2006 and 2011 are provided in Chapter Two.

3.3.2. Food supplementation

Details of the food supplementation regime are provided in Chapter Two. The food supplementation treatment blocks were combined for analyses as all parameters examined were related to the egg-laying phase, when woodland areas were either unsupplemented (Control, CON) or supplemented with peanut cake (PC).

3.3.3. Egg measurements

In all clutches laid in 2009, 2010 and 2011 the first six eggs were numbered using an insoluble permanent marker to determine the laying sequence (Visser & Lessells 2001). The length and breadth (at widest point) of eggs 1 to 6 were measured in years 2009 and 2011 using a dialMax 0.1mm analogue calliper in a subset of the breeding population of Blue and Great Tits (Fig. 3.1). Egg measurements were taken on the day each egg was laid. In 2010 only eggs 4 and 5 in each clutch were measured. In years 2009 to 2011 eggs 4 and 5 were also weighed to the nearest 0.0001 g on a Sartorius electronic balance in the laboratory following removal of the eggs as part of the DLW protocol under Natural England Licences (20091351, 20100857 and 20110924). Egg volume was calculated from the equation of Hoyt (1979):

Figure 3.1. Measuring the breadth of a Blue Tit egg using analogue callipers. (Photo: K. Brulez).

$$\text{Volume (mm}^3\text{)} = 0.51*LB^2$$

where L is the length and B the breadth of the egg.

3.3.4. Measurement of DEE

The DEE of egg-laying female Blue and Great Tits was measured using the DLW technique (Lifson & McClintock 1966). Although a sequence of blood or urine samples is usually used to determine the isotope turnover rate, egg albumen can also be used as a body water sample (e.g. Stevenson & Bryant 2000, te Marvelde *et al.* 2011c). This refinement of the DLW technique minimizes handling time at a particularly sensitive part of the reproductive cycle (Kania 1989) and reduces the risk of desertion (te Marvelde *et al.* 2011c).

DLW field protocol

Female Blue (in 2009 only) and Great Tits (in 2009 and 2010) were caught at sunrise as they left the nestbox after laying their third egg using a mist net box trap (te Marvelde *et al.* 2011a). The trap consisted of a 40 cm × 40 cm × 40 cm (0.064 m^3) bamboo frame with mist net stretched loosely over the outside to form catching pockets (Fig. 3.2a). The birds were removed within 5 mins of capture and injected intra-peritoneally with either 100 μL (Blue Tits) or 200 μL (Great Tits) of DLW (composition mix in all years: 66% ^{18}O; 2009: 16.5026 g 98.23% ^{18}O, 8.5024 g 99.9% ^{2}H; 2010: 13.2162 g 98.44 % ^{18}O, 6.8111 g 99.9 % ^{2}H) using a BD Micro-Fine™ 0.3ml 30G 8 mm insulin syringe (Fig. 3.2b).

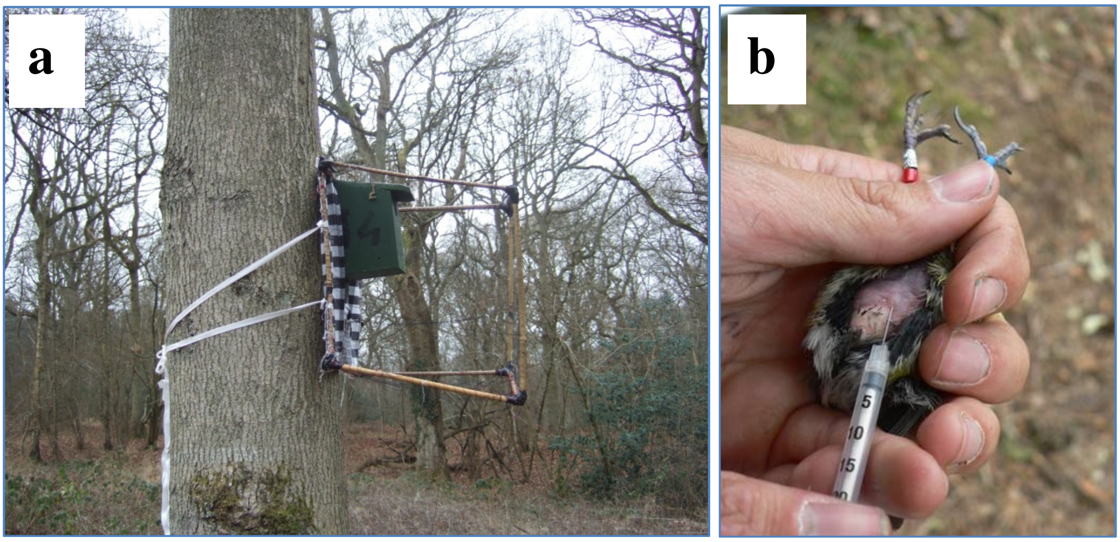

Figure 3.2. (a) Mist net box trap in position around a nestbox on a tree and (b) administration of an intra-peritoneal injection of DLW into a female Great Tit. (Photos: S. Webber & L. te Marvelde).

All procedures were carried out under Home Office Project Licence (PPL 40/2926) and all egg samples were collected under the aforementioned Natural England Licences. The syringes had been filled and weighed to the nearest 0.0001 g prior to injection to determine the exact dose administered (Speakman 1997). Following the injection, the female was weighed to the nearest 0.1 g on a SATRUE-500 electronic balance, ringed with a uniquely numbered BTO metal ring (if the bird was not already ringed) and released. In 2010 coloured plastic leg rings were used as area markers (Chapter Two) and these were attached to the bird's leg if there was not one already present. Female tarsus length (used to represent the size of the bird) was not measured during egg laying but was ascertained upon capture during the nestling phase using methods described by Svensson (1992).

Egg processing

On the day following the injection (i.e. 24 hour sample), the first egg sample (egg number 4) was removed from the nest and transported back to the laboratory. A second egg sample (egg number 5) (i.e. the 48 hour sample) was taken on the next day. Occasionally, a laying break occurred between injection and the laying of either egg 4 or 5, in which case corrections were made during the calculation of DEE. On four occasions the female had not laid an egg on leaving the nestbox on day 3, and so the DEE of the female was measured whilst producing egg 4. However, DEE has been shown not to vary within the egg-laying sequence (te Marvelde *et al.* 2011c), and, therefore, these females are referred to with the others as having laid egg 4 for the first sample and egg 5 for the second sample. Each egg's dimensions were measured and it was weighed to the nearest 0.0001 g before being bisected longitudinally and the shell removed. The albumen was separated from the yolk using fine tweezers, weighed to the nearest 0.0001 g and passed into 3 × 25 µl Vitrex non-heparinized pre-calibrated capillary tubes and flame-sealed immediately with a butane gas torch to preserve the sample. In 2010 the albumen samples were frozen at -80ºC following weighing and later defrosted and passed into flame-sealed capillary tubes. A further egg sample was taken from a nest (also under Natural England Licence) where the female had not been injected with DLW to provide background isotope levels.

Calculation of Daily Energy Expenditure

Mass spectrometry analysis of the DLW samples was conducted at the University of Groningen Centre for Isotope Research in The Netherlands using methods described in detail elsewhere (Visser *et al.* 2000, Van Trigt *et al.* 2002). DEE was calculated from the raw delta values using equation number 7.17 for single pool applications (Speakman 1997):

$$r\mathrm{CO}_2 = (N / 2.078).(k_{18\mathrm{O}} - k_{2\mathrm{H}}) - 0.0062 k_{2\mathrm{H}} . N$$

where N is the Total Body Water percentage (TBW) and k_{18O} and k_{2H} are the rates at which ^{18}O and ^{2}H, respectively, are eliminated from the body per hour (log decay divided by the time difference between the initial and final sample). TBW can differ between the initial and final sample due to variation in body mass, so I calculated an average pool size as suggested by Lifson and McClintock (1966), by multiplying the average mass of the individual females by the percentage of TBW. An average TBW of 66% was assumed based on body composition of dried Great Tit carcasses (Mertens 1987). An energy equivalence of 27.8 kJ L^{-1} CO_2 produced was used (adapted for a protein-rich diet of an insectivorous bird) to convert the amount of CO_2 produced per hour into kJ (Gessaman & Nagy 1988, te Marvelde *et al.* 2011b), after which it was multiplied by 24 (i.e. the number of hours in a day) to obtain DEE.

The Speakman (1997) equation updates the original Lifson *et al.* (1949) equation to allow for fractionation, (i.e. where the H_2O remaining in the body has a different isotopic composition from that of the H_2O or CO_2 leaving the body). The updated equation of Lifson and McClintock (1966) is thus adapted to correct the fractionated water loss assumption to 25%. For comparative purposes the DEE measures were divided by BMR to gain measures of energetic effort as multiples of BMR. BMR (or post-absorptive rate of metabolism at rest) was calculated from an equation provided by Bryant and Tatner (1991) for passerines:

$$\text{BMR (kJ hour}^{-1}\text{)} = 0.1326\ M^{0.734}$$

where M is mean individual body mass of the population (in this case 12.56 g for Blue Tits and 20.90 g for Great Tits). BMR was then multiplied by 24 to obtain the daily BMR and the DEE was divided by the BMR day^{-1} to obtain the DEE as a multiple of BMR.

3.3.5. Measuring ambient temperature

Ambient temperatures were recorded by placing Thermochron® DS1921G iButton® loggers (Maxim Integrated Products., Sunnyvale, CA, USA) on the inside of the nestbox, just under the lid two days before the capture of the female. The iButton® loggers were programmed to record the temperature every 15 mins using proprietary OneWireViewer® software (Maxim Integrated Products., Sunnyvale, CA, USA). The iButton® loggers were removed upon completion of the DLW sampling period and the data downloaded using Blue Dot Receptors. I then calculated mean daily temperature from the recorded data for the DLW sample period. In the instances where iButton® logger data were not available, the temperature recordings from a nearby nestbox were used.

3.3.6. Data filtering

In all instances only first breeding attempts were used and suspected or known second or replacement clutches were excluded from analyses (Chapter Two). In order to maintain consistency with earlier analyses of the timing of breeding (Chapter Two), only clutches which resulted in successful hatching and survival to nestling day 10 were used for the clutch size analyses. When considering egg size and DEE analyses, however, completed clutches were used regardless of whether the nestlings hatched. Within-individual variation in clutch size was examined by analysing the relationship between clutch size in the second of two consecutive years and that of clutch size in the first year. One outlying data point from error in DEE measurement was excluded from a female where the albumen samples spanned three days. Female body condition was calculated by regressing female body mass during egg laying on tarsus length, as measured later in the breeding season.

3.3.7. Statistical analyses

All statistical analyses were conducted using Generalized Linear Models (GLMs – glm function) or Generalized Linear Mixed Models (GLMMs – glmer function in lme4 package or glmmPQL in MASS package) in R version 2.11.1 (R Development Core Team 2011). Following non-significance of the Shapiro-Wilk test for normality (Crawley 2007), all data were checked for a Poisson error structure using a diagnostic distribution plot. All response variables were then checked for over- or underdispersion and corrected where necessary using a quasi-likelihood error structure. All interactions between explanatory variables were tested using backward stepwise regression to find the minimal adequate model, and quadratic terms tested where appropriate. This model simplification was conducted using Akaike's Information Criterion (AIC) via likelihood ratio tests to compare models with and without terms and interactions (Crawley 2007). An alpha level of 0.05 was used throughout. Pseudo R^2 values were calculated where possible for GLMMs, to illustrate model fit, using the R^2 value from a linear model of the correlation between fitted and observed values. Pseudo R^2 values for GLMs were calculated from the proportion of residual deviance in the model. The amount of variance between individuals in clutch sizes was examined using a Levene's Test for homogeneity of variance.

3.4. Results

A total of 552 Blue Tit clutches and 447 Great Tit clutches laid between 2006 and 2011were analysed for variation in relation to a number of parameters, including food supplementation treatment. Of these clutches 338 Blue Tit clutches and 287 Great Tit clutches were examined in a more detailed analysis to account for individual variation in clutch size. Intra-individual analyses of variation in clutch size were conducted for 55 Blue Tit females and 58 Great Tit females. Analyses of individual egg sizes and mass were conducted on 156 clutches of Blue Tits and 158 clutches of Great Tits and measures of female DEE were gained for 29 Blue Tits and 59 Great Tits.

3.4.1. Clutch size

Food supplementation significantly reduced clutch size in Great Tits by a mean of 1.05 eggs (Fig. 3.3, Table 3.1) but the effect of supplementation on mean clutch size in Blue Tits was nearing significance, reducing clutch size by a mean of 0.54 eggs (Fig. 3.3, Table 3.1). There was a significant decline in clutch size with later laying dates in both species, although this effect was more significant in Blue Tits than Great Tits (Fig. 3.4, Table 3.1). Egg removal as part of the DLW protocol led to a mean reduction in clutch size of 1.4 eggs and 1.9 eggs in Blue and Great Tits, respectively (Table 3.1) indicating that removed eggs were not replaced (unlike a previous study of Great Tits – Oppliger & Christe 1996). For this reason, clutch size was defined as the number of eggs that remained after egg removal, to account for any variation in later parameters as a result of reduced incubation costs through smaller clutch sizes (de Heij *et al.* 2007). Egg removal was tested in all analyses including clutch size to check if reduced clutch size had a significant effect on response

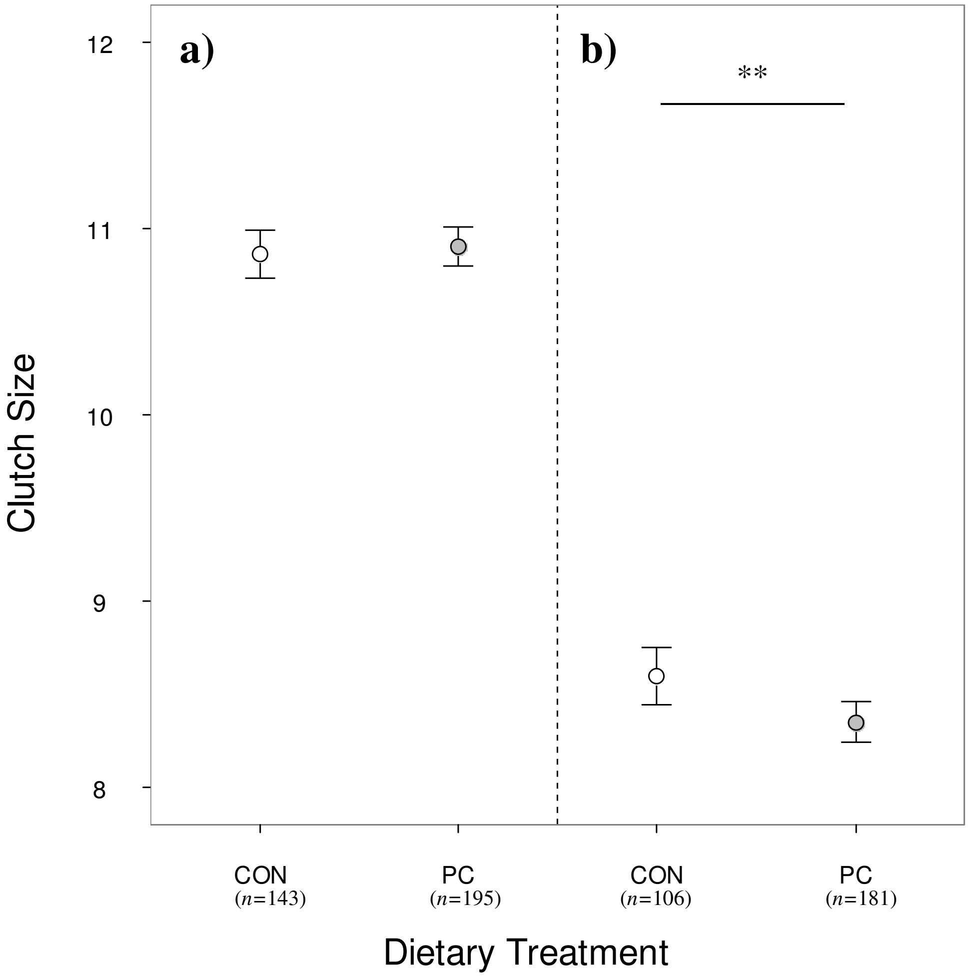

Figure 3.3. Clutch size (mean ± 1 SE from raw data) of (a) Blue and (b) Great Tits breeding in unsupplemented (CON) and food supplemented (PC) areas in Chaddesley Woods National Nature Reserve in Worcs., UK, from 2007 to 2011. ** = $P < 0.01$. Number of clutches is shown in parentheses below each axis label. See Table 3.1 for statistical tests and text for details.

variables. There was also a highly significant amount of inter-annual variation in clutch size in Blue Tits but less inter-annual variation in Great Tits (Table 3.1). Female age was not a significant predictor of clutch size in either species (Table 3.1). There were no differences between the analysis of the full six year dataset (Appendix Two, Table A.2.1), and the subset of data including known female identities (Table 3.1) in terms of the

Table 3.1. Results from simplified GLMM regression analysis of clutch size (CS) of female Blue (BT) and Great Tits (GT) breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011, regressed on significant explanatory variables (Fixed Effect). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 3.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	R^2
Clutch Size	BT	Intercept	14.576					0.63
		Dietary Treatment: PC	14.040	0.019	4.0	0.08		
		Laying Date	-0.231	0.002	66.0	**< 0.001**	Lower CS	
		Year			79.7	**< 0.001**		
		Egg Removal	-1.370	0.020	25.6	**< 0.01**	Lower CS	
2007-2011		Female Age	14.549	0.019	0.01	0.92	Lower CS	
Clutch Size	GT	Intercept	10.328					0.91
		Dietary Treatment: PC	9.278	0.028	14.9	**< 0.01**	Lower CS	
		Laying Date	-0.111	0.003	12.5	**< 0.01**	Lower CS	
		Year			12.7	**< 0.05**		
		Egg Removal	-1.925	0.027	61.0	**< 0.001**	Lower CS	
2007-2011		Female Age	9.976	0.026	2.0	0.19		

Random effects were nestbox and female identity in all GLMM models. Tested fixed effects were dietary treatment (‘Intercept’ = control, ‘PC’ = peanut cake), laying date, year, egg removal and female age and only significant contributors to each model are displayed. All analyses used a Poisson error distribution with correction for underdispersion.

variables which were significant predictors of mean clutch size. In Great Tits the effect of dietary treatment on mean clutch size was less significant in the six year dataset, however, as was the same for Blue Tits (Appendix Two, Table A.2.1).

Intra-individual variation in clutch size was low in both Blue and Great Tits and clutch size in the second of two consecutive years was not related to any other variable

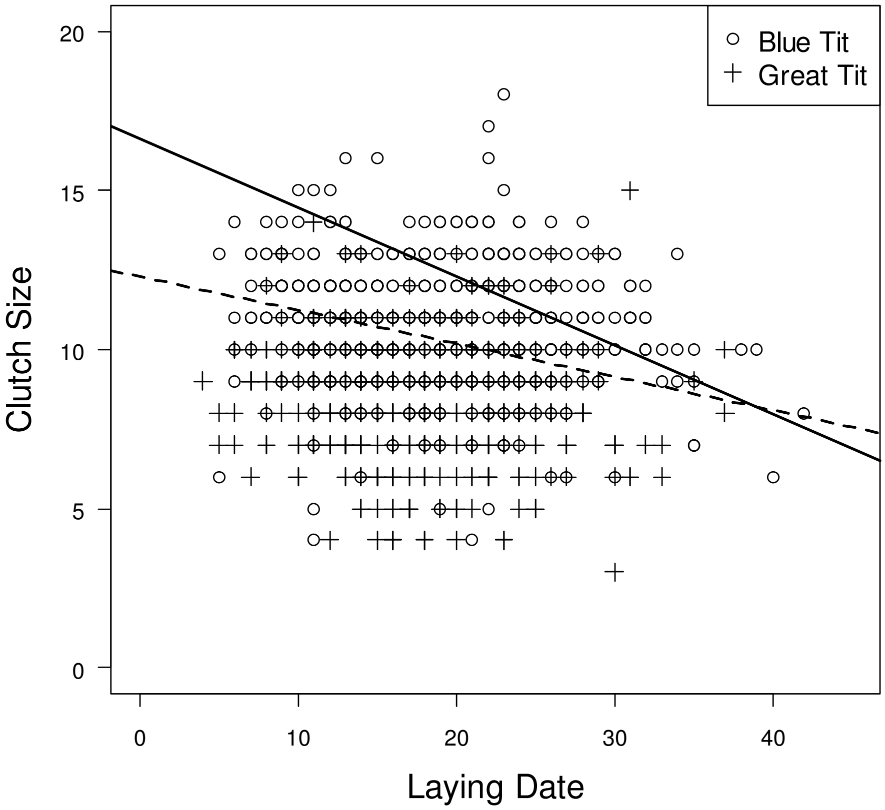

Figure 3.4. Clutch sizes of Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011in relation to laying date. Dates are in April Days, where 1=1st April. Blue Tits are represented by open circles and Great Tits by saltire crosses. Regression lines are predicted from model estimates. The solid line represents the seasonal decline of clutch size in Blue Tits and the dashed line that of Great Tits. See Table 3.1 for statistical tests and text for details.

except the clutch size in the preceding year (Fig. 3.5, Table 3.2). There was no effect of dietary treatment in either the year under examination or in the preceding year.

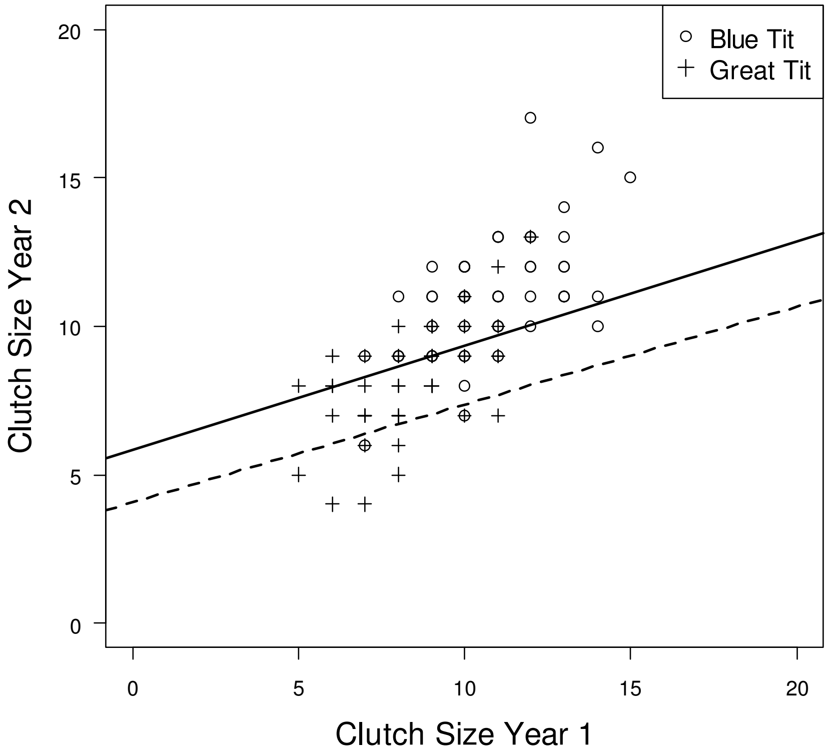

Figure 3.5. Clutch sizes of Blue and Great Tits breeding in the second of two consecutive years in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011in relation to clutch size in the preceding year. Blue Tits are represented by open circles and Great Tits by saltire crosses. Regression lines are predicted from model estimates. The solid line represents the increase of clutch size in Blue Tits and the dashed line that in Great Tits. See Table 3.2 for statistical tests and text for details.

Table 3.2. Results from simplified GLM regression analysis of clutch size in the second of two consecutive years (Response) of female Blue (BT) and Great Tits (GT) breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2008 and 2011, regressed on significant explanatory variables (Fixed Effect). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 3.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	R^2
Clutch Size Year Two	BT	Intercept	5.845					0.36
		Clutch Size	0.351	0.022	5.901	**< 0.01**	Higher clutch size	
2008-2011								
Clutch Size Year Two	GT	Intercept	4.085					0.37
		Clutch Size	0.328	0.014	36.5	**< 0.001**	Higher clutch size	
2008-2011								

Tested fixed effects were dietary treatment, laying date, year, egg removal and female age for both years and only significant contributors to each model are displayed. All analyses used a Poisson error distribution with correction for underdispersion in the second model.

3.4.2. Egg size and mass

Mean egg volume of Blue Tits was not affected by food supplementation, regardless of its position in the laying sequence (Fig. 3.6, Table 3.3). There was significant inter-annual variation in the volume of all Blue Tit eggs and a significant positive effect of laying date on the volume of egg three (Table 3.3). Female age and clutch size did not affect the volume of Blue Tit eggs (Table 3.3). The explanatory power (R^2) of the Blue Tit egg size and mass models was low in most cases, however (Table 3.3).

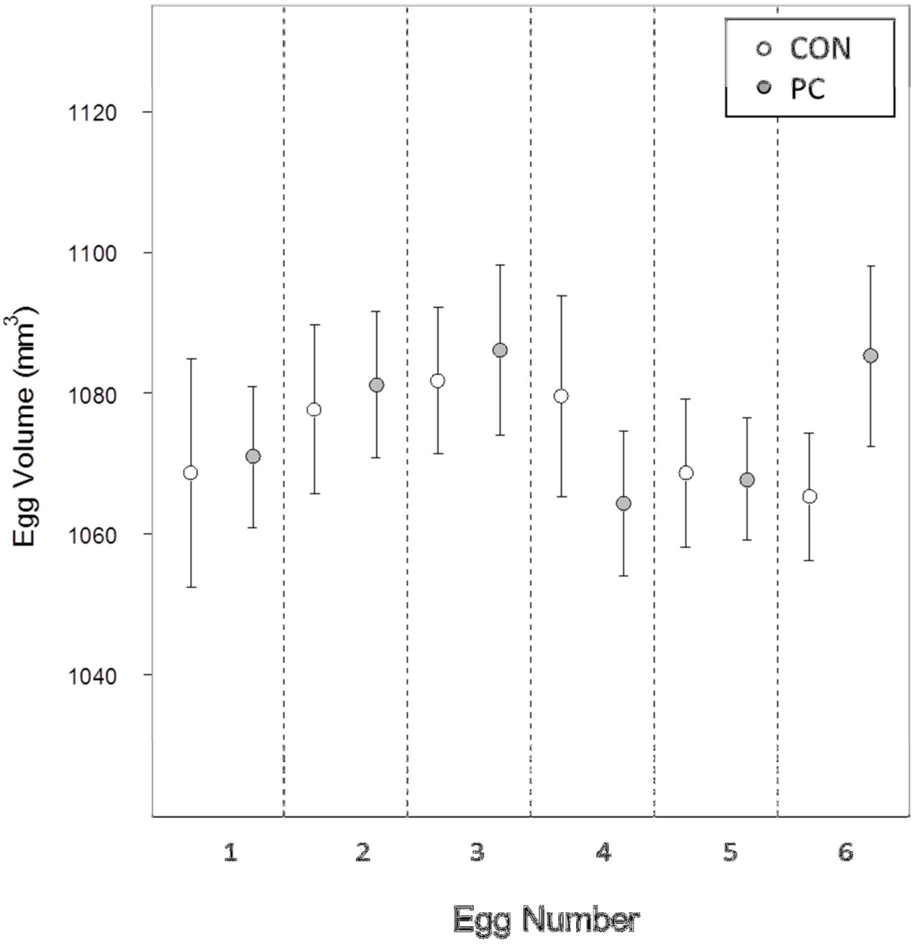

Figure 3.6. Egg volume (mean ± 1 SE from raw data) of the first six eggs of Blue Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK, from 2009 to 2011. Females breeding in the control area (CON) are represented by open circles and those breeding in the peanut cake-supplemented area (PC) by grey-filled circles. See Table 3.3 for statistical tests and text for details.

There was no significant effect of food supplementation on the mean mass of eggs 4 or 5 in Blue Tits (Fig. 3.7, Table 3.3). There was significant inter-annual variation in the mean mass of egg number 4 in Blue Tits but not in the mean mass of egg 5 (Table 3.3). No significant predictors of the variance in mass of egg 5 could be identified (Table 3.3).

Table 3.3. Results from simplified GLMM and GLM (last two models) regression analyses of egg volume and mass (Response) of individual eggs laid by female Blue Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2009 and 2011, regressed on significant explanatory variables (Fixed Effect). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 3.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	R^2
Egg 1 Volume	BT	Intercept	1115.551					0.09
2009 & 2011		Year: 2011	1042.717	0.018	13.9	**< 0.01**	2009 higher 2011 lower	
Egg 2 Volume	BT	Intercept	1133.505					0.15
2009 & 2011		Year: 2011	1052.232	0.016	22.1	**< 0.001**	2009 higher 2011 lower	
Egg 3 Volume	BT	Intercept	929.812					0.18
		Laying Date	5.006	0.002	9.0	**< 0.05**	Higher egg volume	
		Year: 2011	864.904	0.017	19.9	**< 0.01**	2009 higher 2011 lower	
2009 & 2011		Female Age	952.146	0.016	2.3	0.17		
Egg 4 Volume	BT	Intercept	1110.809					0.34
		Year: 2010	1010.894	0.040	11.9	**< 0.01**	2009 highest	
2009 - 2011		Year: 2011	1053.913	0.018			2010 lowest	
Egg 5 Volume	BT	Intercept	1099.536					0.63
		Year: 2010	1072.940	0.031	11.2	**< 0.01**	2009 highest	
2009 - 2011		Year: 2011	1050.173	0.014			2011 lowest	
Egg 6 Volume	BT	Intercept	1131.657					0.12
2009 & 2011		Year: 2010	1053.683	0.018	15.5	**< 0.01**	2009 higher 2011 lower	
Egg 4 Mass	BT	Intercept	0.947					0.15
		Year: 2010	0.856	0.045	0.092	**< 0.05**	2009 highest	
		Year: 2011	0.891	0.027			2010 lowest	
2009-2011		Female Age	0.987	0.026	0.031	0.12		
Egg 5 Mass	BT	Intercept	0.994					0.02
2009-2011		Female Age	1.018	0.010	0.017	0.23		

Random effect was nestbox in GLMM models. Tested fixed effects were dietary treatment (Intercept = control and PC = peanut cake), laying date, clutch size, year, egg removal and female age and only significant contributors to each model are displayed. All analyses used a Poisson error distribution with correction for overdispersion.

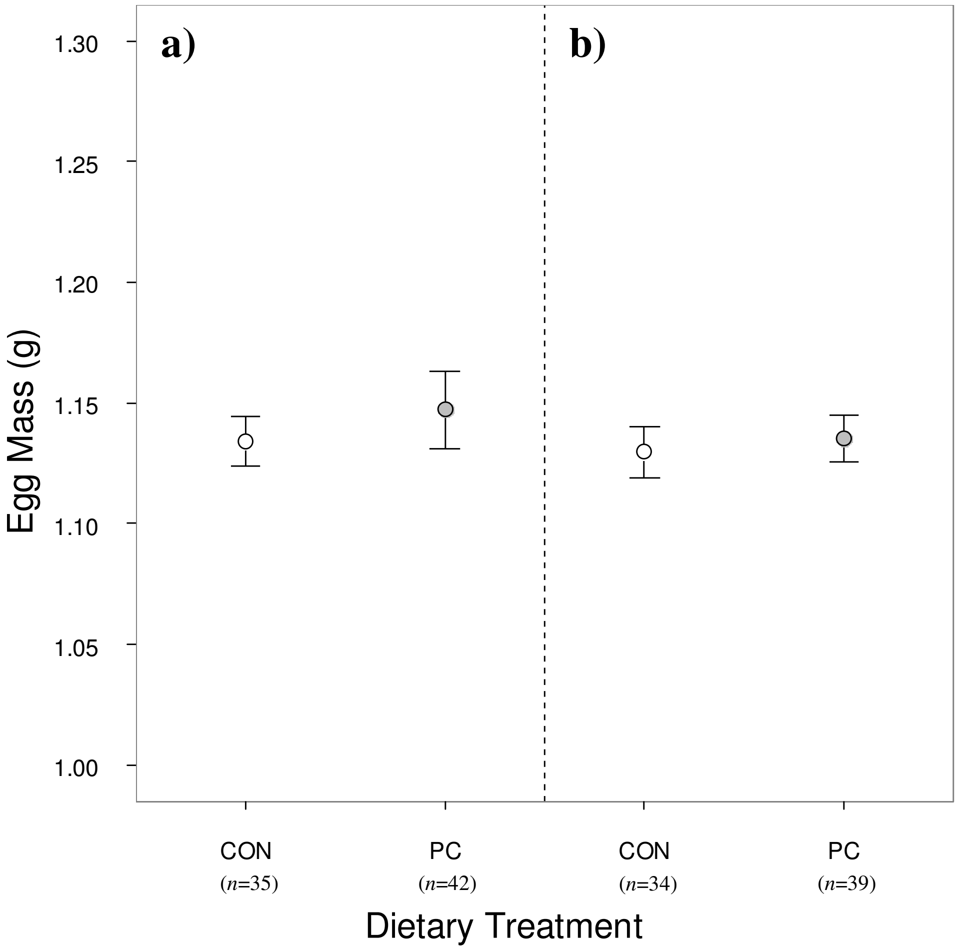

Figure 3.7. Mass (mean ± 1 SE from raw data) of (a) egg number 4 and (b) egg number 5 laid by female Blue Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK, from 2009 to 2011. Eggs from unsupplemented control (CON) females are represented by open circles and those from supplemented (PC) females are represented by grey-filled circles. Number of eggs is shown in parentheses below each axis label. See Table 3.3 for statistical tests and text for details.

There was a significant positive effect of food supplementation on mean egg volume in egg number 4 in the laying sequence of Great Tit clutches (Fig. 3.8, Table 3.4) but other eggs were unaffected. The fourth eggs of females were on average 577 mm^3 larger in supplemented first year breeders than those of first year breeders in the control area (Fig. 3.8, Table 3.4). There was a significant interaction between food supplementation

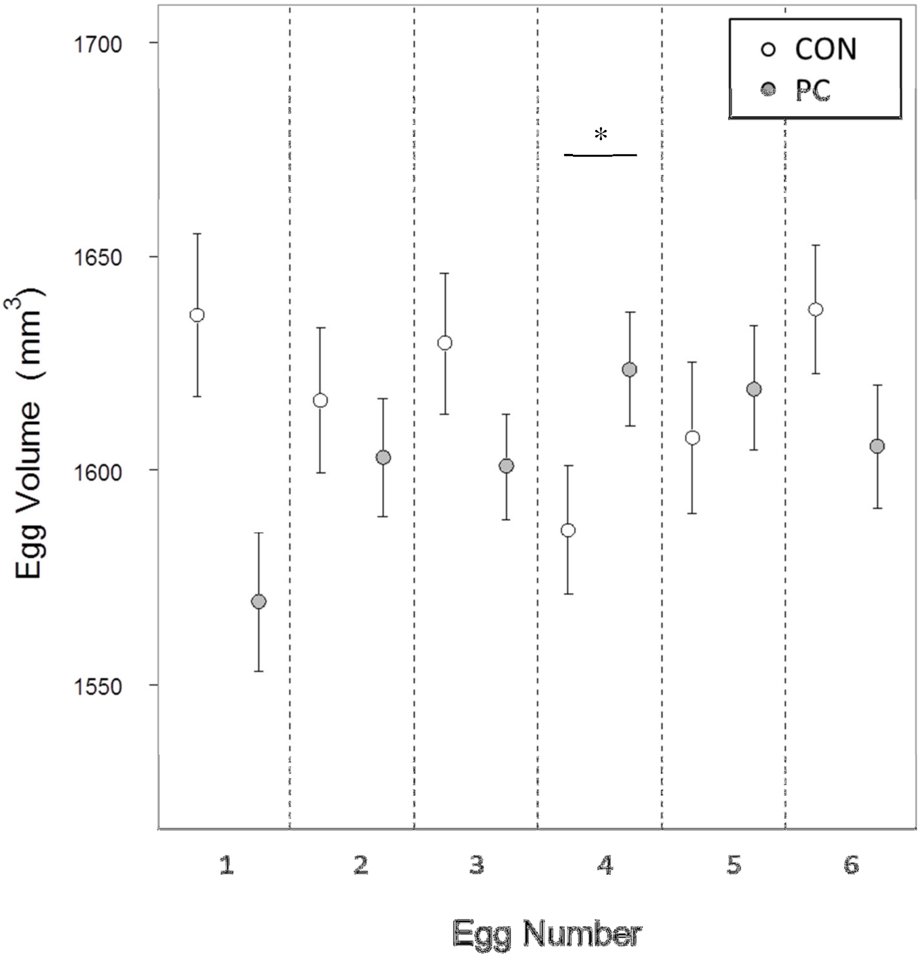

Figure 3.8. Egg volume (mean ± 1 SE from raw data) of the first six eggs of Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK, from 2009 to 2011. * = *P*<0.05. Females breeding in the control area (CON) are represented by open circles and those breeding in the peanut cake-supplemented area (PC) by grey-filled circles. See Table 3.4 for statistical tests and text for details.

treatment and female age, with the highest egg volumes found in first year breeders in the supplemented area, and lowest egg volume in the older females in the supplemented area (Table 3.4). There was a significant effect of food supplementation treatment on the mean mass of egg 5 but not egg 4 of Great Tits (Fig. 3.9, Table 3.4) and this was driven

Table 3.4. Results from simplified GLMM and GLM (last two models) regression analysis of egg volume and mass (Response) of individual eggs laid by female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2009 and 2011, regressed on significant explanatory variables (Fixed Effect). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented.. For details of statistical tests applied see section 3.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	R^2
Egg 1 Volume	GT	Intercept	384.728					0.09
		Clutch Size	75.747	0.078	5.6	**< 0.05**	Higher egg volume	
		Female Age	501.821	0.110	6.2	**0.05**	Higher egg volume	
2009 & 2011		Clutch Size × Female Age	-12.686	0.014	6.2	**< 0.05**	Lower egg volume with increasing clutch size and age	
Egg 2 Volume	GT	Intercept	1643.279					0.05
2009 & 2011		Year: 2011	1580.662	0.017	5.1	**< 0.05**	2009: higher 2011: lower	
Egg 3 Volume	GT	Intercept	1653.770					0.09
2009 & 2011		Year	1579.583	0.016	8.3	**< 0.05**	2009: higher 2011: lower	
Egg 4 Volume	GT	Intercept	1301.119					0.34
		Dietary Treatment: PC	1878.012	0.168	5.0	**< 0.05**	Higher egg volume	
		Year: 2010	1252.995	0.019	6.1	**< 0.05**		
		Year: 2011	1244.977	0.019				
		Female Age	1355.742	0.023	3.3	0.09		
2009-2011		Treatment × Female Age	1222.739	0.031	4.3	0.06		
Egg 5 Volume	GT	Intercept	1671.218					0.01
2009-2011		Clutch Size	-7.651	0.004	1.5	0.23		
Egg 6 Volume	GT	Intercept	1670.294					0.67
2009 & 2011		Year: 2011	1588.394	0.019	7.2	**< 0.05**	Lower egg volume	
Egg 4 Mass	GT	Intercept	1.831					0.19
		Dietary Treatment: PC	1.694	0.045	0.073	0.09		
		Laying Date	-0.009	0.002	0.010	**< 0.01**	Lower egg volume	
2009-2011		Dietary Treatment: PC × Laying Date	0.003	0.002	0.044	**< 0.01**	Higher egg volume in later PC clutches	

Egg 5 Mass	GT	Intercept	1.914					0.16
		Dietary Treatment: PC	1.704	0.059	0.062	**0.05**	Lower egg volume	
2009-2011		Laying Date	-0.014	0.002	0.043	**< 0.01**	Lower egg volume	

The random effect in GLMM models was nestbox. Tested fixed effects were dietary treatment (Intercept = control and PC = peanut cake), laying date, clutch size, year, egg removal and female age and only significant contributors to each model are displayed. All analyses used a Poisson error distribution with correction for overdispersion.

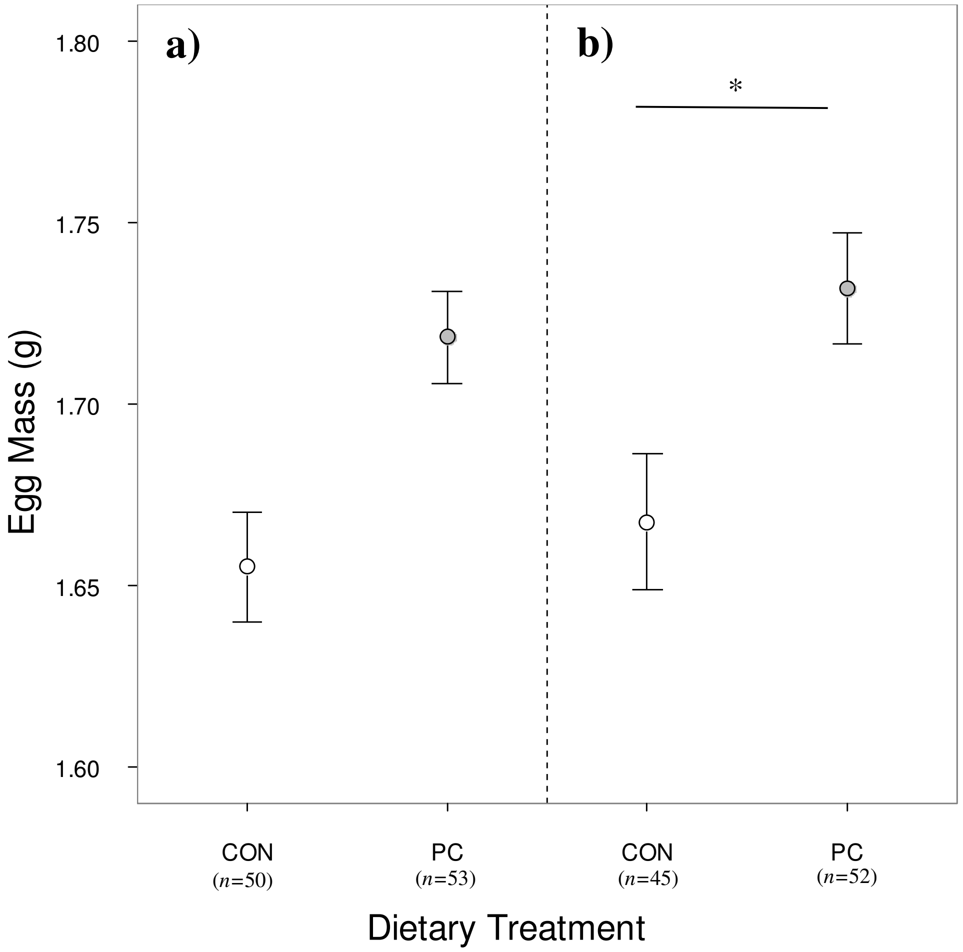

Figure 3.9. Mass (mean ± 1 SE from raw data) of (a) egg number 4 and (b) egg number 5 laid by female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK, from 2009 to 2011. Eggs from unsupplemented control (CON) females are represented by open circles and those from supplemented (PC) females are represented by grey-filled circles. * = $P < 0.05$. Number of eggs is shown in parentheses below each axis label. See Table 3.4 for statistical tests and text for details.

predominantly by an interaction between dietary treatment and laying date whereby the supplemented females breeding later in the season had heavier eggs 4 and 5 than the supplemented females breeding earlier (Figs 3.10 and 3.11, Table 3.4). The mean mass of egg 4 of supplemented females was 0.06 g heavier than control females although this

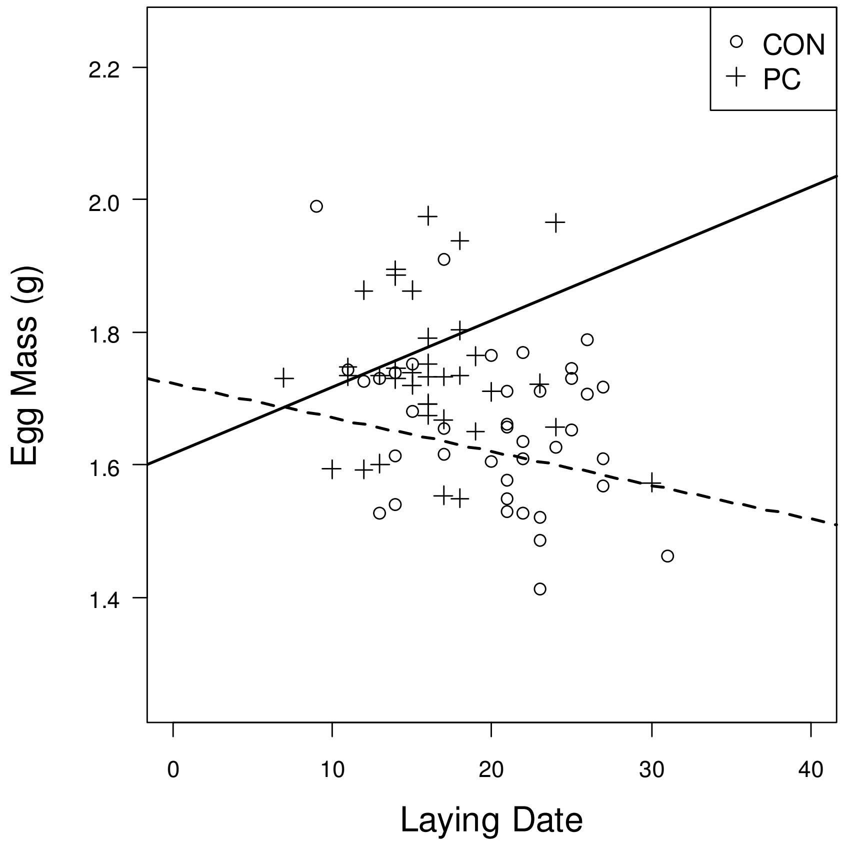

Figure 3.10. Mass of the fourth egg laid by female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2009 to 2011 in relation to laying date. Dates are in April Days, where 1=1st April. Unsupplemented control (CON) females are represented by open circles and the dashed regression line, and supplemented (PC) females by saltire crosses and the solid regression line. Regression lines are predicted from model estimates. See Table 3.4 for statistical tests and text for details.

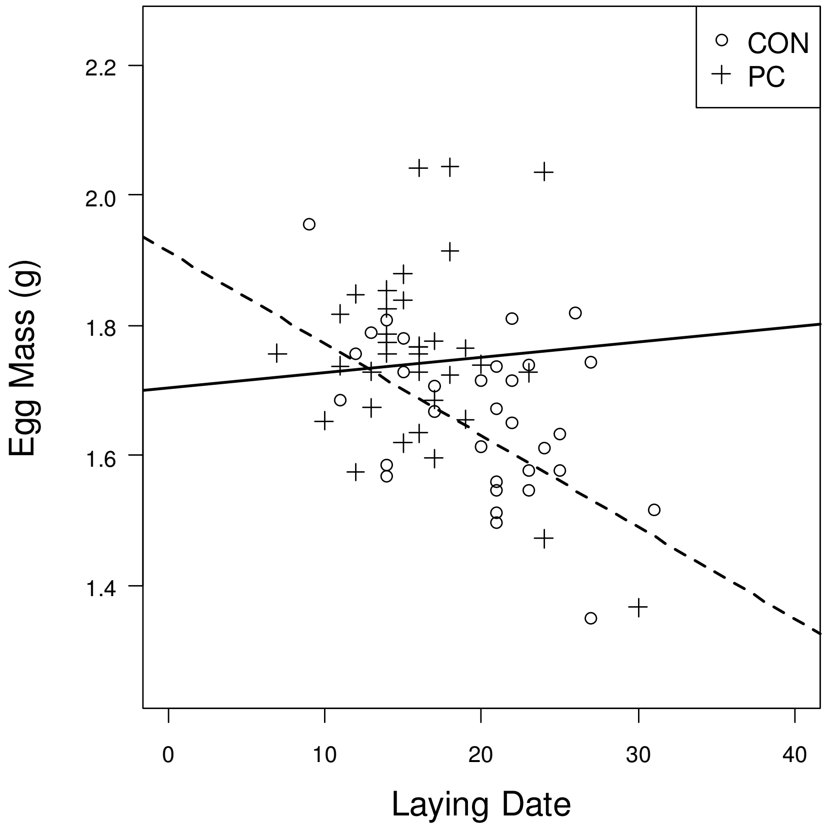

Figure 3.11. Mass of the fifth egg laid by female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2009 to 2011 in relation to laying date. Dates are in April Days, where 1=1st April. Unsupplemented control (CON) females are represented by open circles and the dashed regression line, and supplemented (PC) females by saltire crosses and the solid regression line. Regression lines are predicted from model estimates. See Table 3.4 for statistical tests and text for details.

relationship was not significant. The mean mass of egg 5 of supplemented females was also 0.06 g significantly heavier than control females representing a 4% increase in egg 5 mass from control to supplemented females. The explanatory power of Great Tit egg volume and egg mass models was fairly low in most cases (Table 3.4).

3.4.3. Female DEE during egg laying

Food supplementation significantly reduced female DEE during egg laying in both Blue and Great Tits (Fig. 3.12, Table 3.5). There was an average reduction of 15.58 kJ day^{-1} in

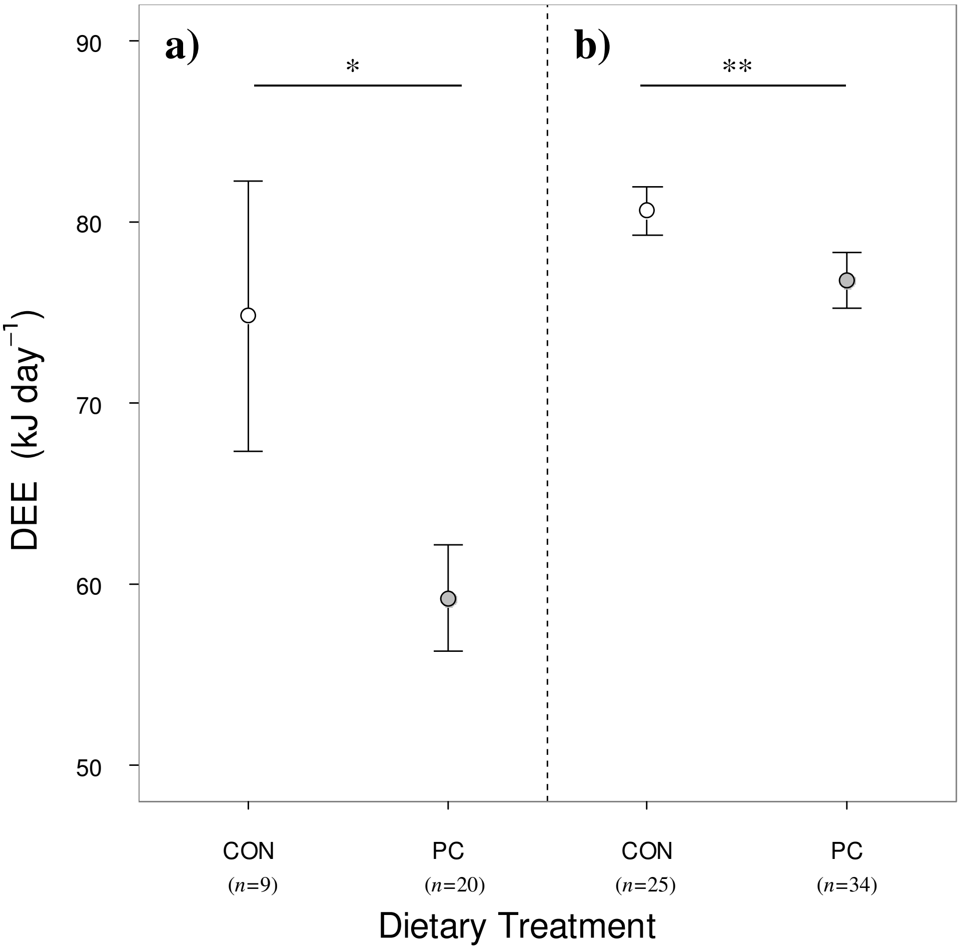

Figure 3.12. Daily Energy Expenditure (DEE) (mean ± 1 SE from raw data) of female (a) Blue and (b) Great Tits provisioning nestlings in Chaddesley Woods National Nature Reserve in Worcs., UK, in 2009 and 2010. ** = $P < 0.01$, * = $P < 0.05$. Number of females is shown in parentheses below each axis label. See Table 3.5 for statistical tests and text for details.

Blue Tits (control *versus* supplemented: 74.8 kJ day^{-1} *versus* 59.2 kJ day^{-1}), and a predicted mean reduction of 6.76 kJ day^{-1} in Great Tits when laying date was controlled for (control

Table 3.5. Results from simplified GLM regression analysis of the Daily Energy Expenditure (DEE) of female Blue (BT and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2009 and 2010, regressed on significant explanatory variables (Fixed Effect). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. For details of statistical tests applied see section 3.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	R^2
Female DEE	BT	Intercept	74.778	0.077				0.18
2009		Dietary Treatment: PC	59.200	0.097	22.875	**< 0.05**	Lower DEE in PC area	
Female DEE	GT	Intercept	89.701	0.054				0.27
		Dietary Treatment: PC	82.942	0.029	2.5886	**< 0.01**	Lower DEE in PC area	
		Laying Date	-0.463	0.002	2.0541	**< 0.05**	Lower DEE	
		Female Body Condition	13.958	0.046	3.7581	**< 0.01**	Higher DEE	
2009 & 2010		Laying Date × Female Body Condition	-0.561	0.002	4.5778	**< 0.05**	Lower DEE	

Tested fixed effects were dietary treatment (Intercept = control and PC = peanut cake), laying date, clutch size, year, egg removal, ambient temperature and female age and only significant contributors to each model are displayed. All analyses used a Poisson error distribution with correction for underdispersion.

versus supplemented: 89.70 kJ day^{-1} *versus* 82.94 kJ day^{-1}). In terms of multiples of BMR, the control Blue Tits expended energy at a mean of 3.7 × BMR (± 0.37 [1 SE], range: 2.65 – 6.08) while supplemented conspecifics expended energy at a mean of 2.9 × BMR (± 0.14 [1 SE], range:1.52 – 4.76). Equivalent figures for Great Tits were 2.7 × BMR for controls (± 0.04 [1 SE], range: 2.40 – 3.27), and 2.6 × BMR (± 0.05 [1 SE], range: 2.19 – 3.34) for supplemented birds.

There was a significant seasonal decrease in female DEE in Great Tits (Fig. 3.13, Table 3.5) but not in Blue Tits (Table 3.5). This seasonal decrease was driven partly by an interaction between laying date and female body condition (Fig. 3.14, Table 3.5). Female

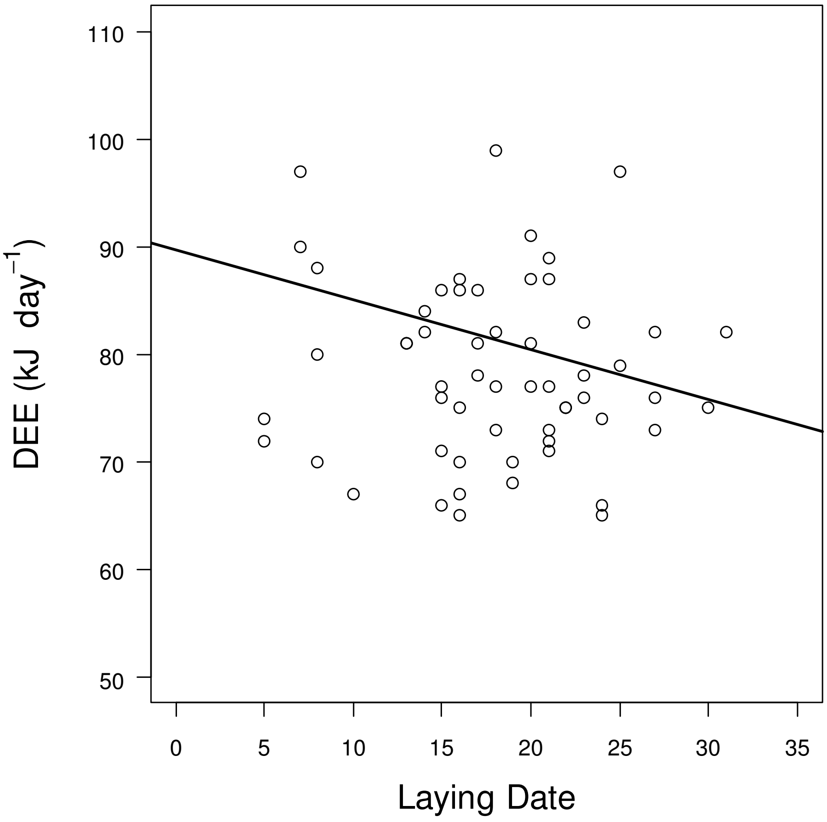

Figure 3.13. Daily Energy Expenditure (DEE) of egg laying female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2009 and 2010 in relation to laying date. Dates are in April Days, where 1=1st April. Regression line is predicted from model estimates. See Table 3.5 for statistical tests and text for details.

body condition had a significant positive effect on female DEE in Great, but not Blue, Tits (Table 3.5). Dietary treatment was the only significant predictor in Blue Tits (Table 3.5). The interaction between laying date and female body condition in Great Tits led to a decrease in female DEE as the season progressed and as female body condition increased (Figs 3.14 and 3.15, Table 3.5). Females in low body condition exhibited higher DEE when laying was initiated late in the season (Figs 3.14 and 3.15). There appeared to be a reduction in DEE by females in lower body condition (i.e. those whose body condition

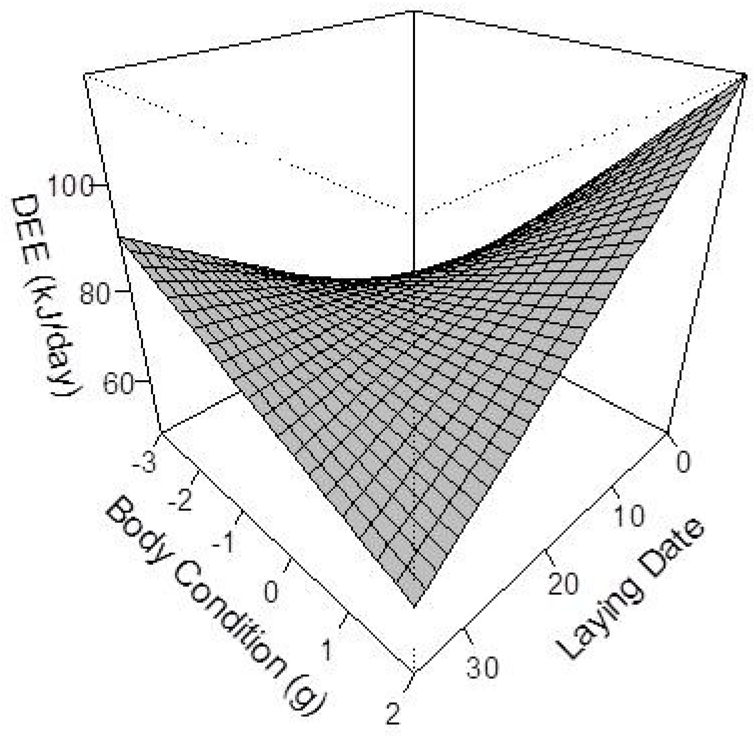

Figure 3.14. Daily Energy Expenditure (DEE) of egg-laying female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2009 and 2010 in relation to female body condition and laying date. Contour is predicted from model estimates controlling for dietary treatment. Dates are in April Days, where 1=1st April. See Table 3.5 for statistical tests and text for details.

value [i.e. residual] was negative) when they were laying early in the season (Fig. 3.15). The most energetically expensive relationship in this analysis was for females laying early in the breeding season that were in higher body condition (Figs 3.14 and 3.15). Females in higher body condition (i.e. those whose body condition had a positive residual value) reduced their DEE as the season progressed (Figs 3.14 and 3.15).

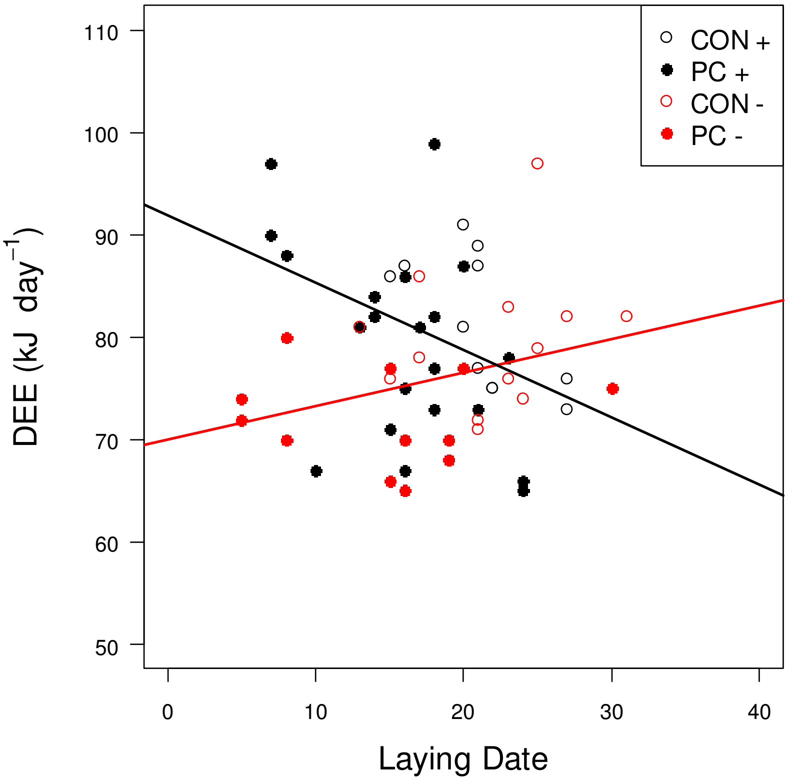

Figure 3.15. Daily Energy Expenditure (DEE) of egg-laying female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2009 and 2010 in relation to female body condition and laying date. Dates are in April Days, where 1=1st April. Females with positive body condition residuals are shown in black and those with negative body condition residuals are in red. Unsupplemented control (CON) females are shown with open circles and supplemented (PC) females with closed circles. Regression lines are from a simple linear model with females in higher body condition in black (F = 5.4, df = 30, P < 0.05) and females in lower body condition in red (F = 2.8, df = 23, P = 0.10). See Table 3.5 for statistical tests and text for details.

There was relatively low explanatory power (R^2) in the DEE models, in common with other studies where individual variance in DEE is high (e.g Stevenson & Bryant 2000).

3.5. Discussion

Food supplementation has been demonstrated to have significant negative effects on the clutch size of Blue and Great Tits breeding within this population (Harrison *et al.* 2010). When individual variation was included in clutch size analyses in my study to further the Harrison *et al.* (2010) study, Blue Tits were not affected by food supplementation, although Great Tit clutch size was significantly reduced (Fig. 3.3, Table 3.1). There was no intra-individual variation in clutch size in response to food supplementation in both species (Fig. 3.5, Table 3.2). The effects of food supplementation on the size and mass of eggs may be minimal (reviewed in Christians 2002) and there was very little effect of food supplementation on the size or mass of eggs laid by Blue Tits (Figs 3.6 and 3.7, Table 3.3). There were, however, some significant effects of food supplementation on the mass and size of eggs 4 and 5 of Great Tits (Figs 3.8 and 3.9, Table 3.4). Females of both species reduced DEE during egg laying in response to food supplementation (Fig. 3.12, Table 3.5), however, indicating that there may be energetic benefits of food supplementation due to easier foraging which are not converted into increased reproductive output. These life history responses to food supplementation during egg laying are discussed and a new theory of the role of food availability in the determination of clutch size is proposed.

3.5.1. Food supplementation and clutch size

The common lack of increase in clutch size in food supplementation studies seems to suggest that clutch size does not appear to be determined by food availability at the time of egg laying (reviewed in Nager 2006). Longer-term studies of natural food availability have found a correlation between resource availability and clutch size (reviewed in Martin

1987). Those studies of tit species that have found increases in clutch size in response to food supplementation have usually provided a high protein supplement (reviewed in Nager 2006, but see Nager *et al.* 1997) and availability of protein or amino acids is commonly cited as a potential factor limiting egg production (Murphy 1994, Reynolds *et al.* 2003a). Blue Tits in particular are limited by specific amino acids during egg laying (Ramsay & Houston 1998) if not by protein itself (Clamens & Isenmann 1989, Nilsson & Svensson 1993). This is consistent with a lack of response in clutch size to food supplementation with a protein-poor food such as peanut cake available to Blue Tits breeding in Chaddesley Woods. If energy is not a factor limiting egg production and if egg production is costly, no change in clutch size in food-supplemented females would be expected, as was the case for Blue Tits.

The consistent reduction in clutch size of food-supplemented Great Tits is puzzling, even if egg production is not constrained by food availability. Clutch size of Great Tits is density-dependent with smaller clutches being produced at higher breeding densities (Perrins & McCleery 1989), but there was no significant difference in occupancy of nestboxes between treatment blocks in my study (Appendix Three, Fig. A.3.1, Table A.3.1). Egg-laying females may be limited by other macro- (e.g. protein – Murphy 1994, Reynolds *et al.* 2003a) or micronutrients (e.g. calcium – Graveland & Drent 1997, carotenoids – Blount *et al.* 2002) in which peanut cake is deficient. In spite of the relatively poor nutritional composition of the peanut cake, food supplementation may be expected to reduce foraging time and increase time available to seek other nutrients, hence increasing clutch size. My results and those of other supplementation studies of small passerines (reviewed in Robb *et al.* 2008a) support the theoretical assertion that the maximum number of eggs produced is not primarily determined by the quantity of food available (Lack 1966) at the time of egg laying, even if the initiation of egg laying is related to resource availability (Chapter Two).

One possible reason for the lower clutch sizes in supplemented Great Tits is that if these females were cued to start laying eggs early, at a period of lower natural food availability they may be energetically constrained and unable to form a large clutch (Perrins 1970); under this scenario smaller clutch sizes but higher levels of energy expenditure would be expected. Supplemented females, however, demonstrated *lower* energy expenditure (Fig. 3.12), and thus confound this explanation. In these circumstances there should also be a curvilinear polynomial relationship between clutch size and laying date (Perrins & McCleery 1989), with smaller clutch sizes in earlier breeding attempts, but this was not the case and there was a linear seasonal decline in clutch size in both species (Fig. 3.4) in common with other studies (Klomp 1970).

Consistency of clutch size within individuals was high, and unrelated to any of the extrinsic factors which were tested in both species. The negative response to food supplementation in Great Tits appears to be at the mean population level and unaffected by individual variation. The intra-individual consistency in clutch size does not discount the possibility of a wholesale response to food supplementation, but indicates that responses to food supplementation are not consistent between individuals. Indeed, when the raw values of intra-individual clutch size were examined only 53% of female Great Tits reduced their clutch sizes in response to food supplementation. This variability in the response of individuals to food supplementation has been recorded before in terms of laying date and is related to territory quality (Svensson & Nilsson 1995) but not to clutch size; female Blue and Great Tits tend to be very faithful to nesting sites between years, so this individual variation may reflect territory quality.

3.5.2. Food supplementation and clutch size theory

According to both life history trade-off theory (Williams 1966, Stearns 1992) and the individual optimization hypothesis (Perrins & Moss 1975, Pettifor *et al.* 1988), short-lived species such as Blue and Great Tits would be expected to maximise their investment in the current reproductive attempt, particularly if provided with a predictable food resource throughout the breeding season. Although adherence to either theory cannot be accurately determined unless measures of offspring fitness and residual reproductive value are included (Pettifor *et al.* 1988, Vanderwerf 1992), a systematic reduction in clutch size in response to food supplementation in Great Tits would appear to be a detrimental life history strategy.

Larger clutches almost always produce more recruits (Perrins & McCleery 1989) and increased resource availability should theoretically raise the 'optimal' clutch size for maximising the number of offspring recruited into the breeding population (Boyce & Perrins 1987). Unpredictable food resources can also negatively affect the ability of female passerines to optimise their clutch size (Török *et al.* 2004), so Great Tits should have been better able to optimize their clutch size for maximal lifetime reproductive success in food-supplemented areas. This would not necessarily result in an increase in clutch size, but a reduction is not expected to be optimal. The downstream consequences of reduced clutch size will be examined in subsequent chapters to look for evidence of individual optimisation and smaller clutch sizes being equally as productive as larger ones.

There can also be life history costs specifically associated with producing more eggs in terms of reduced adult survival for future reproductive attempts (Nager *et al.* 2001, Visser & Lessells 2001) or reduced offspring-rearing capacity and lower fledgling mass in the current reproductive attempt (Sanz 1997, Monaghan *et al.* 1998). Great Tits may, therefore, have been trading off investment in their clutch size against increased investment in egg quality or in downstream parental care. The reason why the provision of a food supplement, representing a reduction in the cost of breeding, would elicit a life history trade-off in this manner is difficult to explain. Given that eggs were removed as part of the DLW protocol and did not appear to be replaced (Table 3.1), the downstream effects of egg removal (i.e. clutch reduction) should also be examined. This should be discussed in the context of food supplementation in order to determine whether there are trade-offs in the form of increased reproductive output from the current attempt or increased residual reproductive value. If there is evidence of improved offspring recruitment from reduced or smaller clutches, it would suggest that Great Tits breeding on unsupplemented territories are laying clutches larger than optimal for the maximised recruitment of offspring.

3.5.3. Theoretical framework - quantitative and qualitative cueing

This perplexing reproductive response to food supplementation seems suboptimal from a life history perspective. In light of this, and the fact that food-supplemented females do not appear to be energetically constrained during egg laying, a further hypothesis is required. I propose that the appearance of the food supplement acts as a cue to food availability later in the breeding season, but not in terms of the quantity of food that will be available, which has been proposed before (e.g. Ewald & Rohwer 1982, Perrins 1996, Nager *et al.* 1997). If the quantity of food is important for clutch size, an increase in clutch size would have been expected, or at the least no difference in clutch size if other selection pressures were acting against increased clutch size.

The experimental design was not intended to provide a sudden increase in nutrient resources: food supplementation started on 7th March, far in advance of egg laying. However, birds could be perceiving the appearance of feeders as a sudden increase in food resources, which has been widely hypothesised to be one of the potential fine-scale proximate triggers that allow birds to adjust their seasonal timing (Chapter Two, Martin 1987, Harrison *et al.* 2010). If this were the case the appearance of the food supplement might be acting as a *quantitative* signal to initiate behavioural and physiological changes required for egg laying (i.e. the cue hypothesis). It could even be construed as a signal that the season is advancing faster than birds had anticipated (Chapter 2), stimulating them to accelerate their breeding attempt. This could cause them to reduce the number of eggs laid in the clutch to enable earlier hatching (Cresswell & McCleery 2003).

This *quantitative* evaluation of food availability could occur in tandem with a *qualitative* evaluation of food value (also suggested by Harrison *et al.* 2010) where either protein content or the level of other micronutrients (summarised in Williams *et al.* 1993, Nager 2006) could act as a predictor of food quality later in the breeding season (Chapter 2). Indeed, Perrins (1991) suggested that the emergence of the first caterpillars coincides with egg number 5 laid by Blue and Great Tit in the Wytham Woods (Oxford, UK) population and that final clutch size could be affected at this point. Food supplementation in the form of peanut cake (i.e. a high fat, low protein supplement) in my study might represent an inhibitor of clutch size, and hence brood size (Harrison 2010) as the females would predict a poor quality of food supply later in the year and downscale their breeding attempt accordingly. There is a separation in feeding behaviours and locations between Blue and Great Tits during the nest-building phase (Gibb 1954), with Blue Tits foraging predominantly in the tips of branches and Great Tits foraging on the ground (Gibb 1954).

Blue and Great Tit females could, therefore, be using different cues not just to adjust their timing (Chapter Two), but also the number of offspring they can provision.

3.5.4. Food supplementation, and egg size and egg mass

Great Tit females laying smaller clutches could have traded off the quantity of eggs against their quality, laying larger eggs as a result (Föger & Pegoraro 1996, Williams 2001a). Larger eggs produce larger nestlings at hatching with improved early nestling phase growth (Schifferli 1973, Bolton 1991, Smith *et al.* 1993). Offspring from heavier eggs may then have improved survival prospects during the early nestling phase (Smith & Bruun 1998) and beyond (Tinbergen & Boerlijst 1990). In the Chaddesley population between 2009 to 2011, however, no effect of food supplementation on size or mass of individual Blue Tit eggs was found, in common with an earlier study in the same population (Harrison 2010). The interaction between treatment and clutch size, which enabled increased average egg size in larger supplemented clutches, found in a previous study of the same population (Harrison 2010) was not replicated at the individual egg level.

With no significant change in clutch size, a trade-off between egg size and number would not be detectable in Blue Tits. In Great Tits, however, females in the supplemented area laid a significantly larger egg 4 and a significantly heavier egg 5 than control birds. This increase in egg mass was driven by a seasonal increase in egg masses in the supplemented area in contrast to a seasonal decrease in mass of eggs laid by control birds. Seasonal increases in egg mass have been reported in Great Tits (Perrins 1970), as females increase egg sizes when temperatures rise and food increases. There may be a decrease in egg size in response to low food availability (Nager & Zandt 1994, Valkama *et al.* 2002) and a seasonal decrease is more common in egg size studies (reviewed in Christians 2002). The fact that the seasonal increase in egg size of supplemented Great Tits nesting in Chaddesley is not a very steep curve (Figs 3.10 and 3.11) supports the suggestion that a decrease may be related to food availability; the food supplement represents a stable and predictable food resource and, hence, a more consistent seasonal egg mass would be expected. Alternatively, the seasonal increase in egg size as a result of food supplementation could reflect females breeding at a suboptimal time (e.g. too early) and that the requisite resources are not available to produce large eggs. The steep seasonal decline in egg size in the control area could be associated with higher 'quality' females breeding earlier in the season (Verhulst *et al.* 1995), with females in higher body condition breeding earlier, or with younger females laying later, smaller eggs (Hipfner *et al.* 1997).

Egg size is very consistent within individuals and extrinsic factors such as ambient temperature and food availability have little effect (Christians 2002). This was supported in my study as unexplained variance in all egg size analyses was very high (Table 3.3 and 3.4), suggesting that individual variation accounted for almost all of the variation. The increase in egg mass between control and supplemented Great Tits in my population was only 4%. This is in conformity with a finding that of the 36% of studies that reported an effect of food supplementation on egg size (e.g. Reynolds *et al.* 2003a), most of these were substantially lower than a 13% shift in egg size (Christians 2002). The increase in egg mass of Great Tits has been demonstrated before across the clutch, and noted by Harrison (2010) as being unusual as supplemented birds that increase egg size are more associated with a high protein diet (Ramsay & Houston 1997, Reynolds *et al.* 2003a). The alteration in direction of the linear relationship between laying date and egg size in response to food supplementation develops this finding, however, and suggests that late-laying female Great

Tits are laying heavier eggs than they would otherwise be able to or that very early females are laying smaller eggs than would be expected.

3.5.5. Food supplementation and the energetic costs of egg production

There have been many theoretical and empirical studies on the cost of egg laying, based on the energy content of eggs (Ricklefs 1974, Walsberg 1983), life history trade-offs as a result of clutch manipulation (Verhulst & Tinbergen 1997, Visser & Lessells 2001), reducing thermal energetic costs (Yom-Tov & Wright 1993), or taking direct measures of metabolic rate or energy expenditure (Ward 1996, Stevenson & Bryant 2000, Nilsson & Råberg 2001). The key questions regarding what determines the quantity and quality of eggs laid by a breeding female remain unanswered, however (Williams 2005). The availability of food resources is widely acknowledged as being intrinsically related to the cost of egg laying, even if the mechanisms by which this constraint is exercised are still largely undefined (Nager & Zandt 1994, Monaghan & Nager 1997, Williams 2005). In light of this, my results are particularly exciting in finding a consistent association between food supplementation and the energy expenditure of egg-laying females. To my knowledge this is the first time that measures of energy expenditure were taken during egg laying in a food supplementation study and the first time that any measure of the energy expenditure during egg laying has been taken in Blue Tits.

Both supplemented Blue and Great Tit females reduced their DEE in response to food supplementation as predicted by my hypothesis. Compared to other studies of energy expenditure or metabolic rate during egg laying by Great Tits, the levels of DEE in both control and female Great Tits (around 2.5 × BMR) are of a similar magnitude (Stevenson & Bryant 2000, te Marvelde *et al.* 2011c). This level of energy expenditure is well below the theoretical metabolic ceiling of 4 × BMR which adults are thought to be unable or unwilling to exceed at any point in the annual cycle (Drent & Daan 1980) and even further below observed metabolic ceilings of up to 7 × BMR (Peterson *et al.* 1990). Blue Tits had a higher DEE relative to BMR (i.e. 3.1 × BMR), although the mean DEE of Blue Tits was lower than that of Great Tits. This is in concordance with inter-specific analyses where DEE or FMR scales to body mass (Nagy 1987, Bryant 1997). Given that several studies have reported relatively low DEE during egg laying in Great Tits in particular, it would appear that the energetic costs of egg laying for altricial species are possibly not as high as has been suggested (summarised in Perrins 1996, Nager 2006) or that breeding females reallocate energy between activities with high efficiency (Salvante *et al.* 2010).

The consistent reduction of Great Tit clutch size in this population indicated that these energetic savings were not channelled into increasing the number of offspring a female produced. Supplemented female Great Tits increased the mass of their eggs, however, suggesting that there is potentially increased investment in the quality of offspring. In contrast to other studies of energy expenditure or metabolic rate during egg laying, however, there was no correlation between the mass of the egg produced and DEE in either Blue or Great Tits (Stevenson & Bryant 2000, Nilsson & Råberg 2001, Vézina & Williams 2002). Despite there being no correlation between female DEE and the number or size of eggs she produced, I detected a seasonal decline in DEE, which is a novel finding. This decline indicated that early laying females were expending more energy than later laying birds (Fig. 3.13), but only existed when female body condition was accounted for. The earliest breeding birds that were in higher body condition decreased their DEE during the breeding season, whereas the females in lower body condition increased their DEE as the season progressed (Figs 3.14 and 3.15). This result is unprecedented, and may represent a condition-dependent, seasonal energetic strategy to enable earlier breeding; females in lower body condition were only able or willing to invest more energetically later in the season when feeding conditions were conducive to egg laying.

Females could be trading off the costs of earlier laying against egg size, explaining the change in the direction of the slope of egg mass in relation to date (Fig. 3.10). There were insufficient data to tease out an effect of female body condition and egg size in the DLW-sampled subset of the population, however, and measures of female body condition were not taken during egg laying in the remainder of the population. Egg size was found to increase with female body condition and mass in Great Tits (Nager & Zandt 1994), and also in European Pied Flycatchers and European Starlings (Smith *et al.* 1993), although both of these studies measured body condition during incubation rather than egg laying. Egg mass was unaffected by reduced female body condition in food-deprived European Starlings, although those females in low body condition significantly delayed their timing of breeding (Meijer & Langer 1995). There was no correlation between timing of breeding and egg size in relation to female body condition in any of these studies.

Females in low body condition are expected to delay breeding until environmental circumstances are more favourable. It is noteworthy, therefore, that within my population the earliest breeders were food-supplemented and that of the early supplemented females, those in lower body condition were exhibiting lower DEE (Fig. 3.15). This potentially identifies energy acquisition through food supplementation as the mechanism by which the females in lower body condition are able to advance laying and reflects the individual-level responses to food supplementation in clutch size. Food supplementation could encourage females in lower body condition to breed early through reducing the energetic cost of breeding at lower temperatures (te Marvelde *et al.* 2011c), possibly by decreasing foraging costs and enabling more resting (Ettinger & King 1980, Salvante *et al.* 2010). Alternatively, food supplementation could engender a maladaptive response, cueing females in low body condition to breed earlier than is optimal, resulting in lower reproductive output in terms of egg and clutch size. Although there were not smaller Great Tit clutches early in the season in the population as a whole (Fig. 3.4), there might be a more subtle individual response which is not detected at the population level. The exact nature of this timing/productivity trade-off could not be demonstrated unequivocally from my DEE data, possibly due to small sample sizes. It represents a promising avenue for further research, however.

One of the most fundamental issues surrounding the use of measures of energetic effort is whether extrinsic circumstances act as an enabling factor (wherein high DEE would represent increased investment whilst circumstances are good) or whether extrinsic circumstances act as a constraining factor (wherein high DEE would represent an increased cost associated with an activity). A reduction in DEE in response to food supplementation appears to support the latter, as females conserve energy when they can, as does the fact that females reduce their DEE in response to warmer temperatures in most studies (e.g. Stevenson & Bryant 2000, te Marvelde *et al.* 2011c). Within the latter study, however, females exhibiting higher DEE were thought to be using a nearby anthropogenic food resource to raise their DEE under less expensive foraging circumstances. These apparently conflicting results indicate that energetic trade-offs may be more complex and difficult to interpret than they may first seem. Energetic strategies may vary at the individual level (Vézina *et al.* 2006), so isolating inter-individual patterns in energetic trade-offs between individuals is problematic. This makes the fact that my study detected an inter-individual reduction in DEE in response to food supplementation even more striking.

Studies of energy expenditure are characterised by such high individual variability in measures such as BMR and DEE (Butler *et al.* 2004) and one of the potential issues could be the invasive nature of the technique. Female birds are particularly sensitive to disturbance during egg laying and incubation (Kania 1992), and handling of the females for the DLW injection could have impacted on their behaviour. In contrast to the use of the DLW technique during other times in the annual cycle, using egg albumen to measure DEE does not involve multiple captures of the females and the measurement period begins 24 hours after the injection. A number of studies have investigated whether the administration of DLW has a negative impact on animal behaviour or the DEE itself (reviewed in Speakman 1997) but the overriding conclusion is that it has a minimal impact. A comparable study of DEE of Great Tits during the egg laying period (te Marvelde *et al.* 2011a) concluded that desertion due to handling or injection of DLW was not significantly different from background desertion rates within the population. In my study there was an average desertion rate of 8% in Great Tits and 6% in Blue Tits. In addition 5% of Great Tits and 9% of Blue Tits changed nestbox without abandoning the breeding attempt altogether. There was no measureable impact of the nestbox switching in my analysis, however, due to the low number of females involved and more investigation would be required to ascertain whether relocating the nesting attempt has a noticeable effect on DEE. It has been noted already that the eggs removed from the clutch as part of the DLW protocol were not replaced in Great Tits, and that this needs to be taken into consideration during examination of downstream behaviour and breeding parameters.

The relative cost of egg laying within the annual cycle has been much debated, with measures of BMR indicating that there is very similar energy expenditure during egg laying as during brood rearing (Ward 1996, Vézina & Williams 2002), whereas theoretical estimates consider the latter to be more energetically demanding (Ricklefs 1974, Nager 2006). In subsequent chapters I will examine the relative costs of egg laying and of rearing nestlings.

3.5.6. Inter-specific differences in the response to food supplementation

There were many inter-specific differences in the response to food supplementation, with Blue Tits exhibiting far fewer changes in reproductive output than Great Tits (Chapter Two). Perhaps the most striking finding at the inter-specific level, however, is that Blue Tits were reducing their energy expenditure whilst egg laying but not translating this into changes in other reproductive parameters. Blue Tits might be using the savings afforded by food supplementation to advance their laying date without incurring energetic penalties. The possible difference between the two species in cueing mechanisms has already been discussed, but downstream reproductive effort, output and residual reproductive value will be examined in subsequent chapters to investigate whether such savings could translate into increased offspring or adult survival.

I have demonstrated that both Blue and Great Tits can make energetic savings during egg laying if a predictable food supplement is provided. In the next chapter I will examine how these savings affect the nestling phase, investigating the effects of food supplementation on nestling mortality, brood size, female energetic investment during brood rearing and parental work rates.

Chapter Four

The effects of food supplementation on parental investment during brood rearing

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4.1. Abstract

The cost of rearing offspring to avian parents is predicted to be inextricably linked to resource availability. Parental investment in the current breeding attempt must be balanced against the potential of future reproduction, and the investment of their partner. Parents of altricial nestlings may strategically reduce brood size in response to low food availability, as a means of increasing the allocation of resources to each nestling or improving their own survival chances. In order to clarify the role of food availability on parental investment during brood rearing, I measured the pair provisioning rates of Blue and Great Tits and the Daily Energy Expenditure (DEE) of female Great Tits between 2009 and 2011 within a food supplementation study. Food supplementation led to higher nestling mortality and smaller brood sizes in Great, but not Blue, Tits. Supplemented males of both species increased provisioning rates, whilst females reduced provisioning rates in response to natural food availability. Supplemented female Great Tits reduced DEE in spite of showing no change in provisioning rate, and the strategic implications of this are discussed. There was no effect of food supplementation on the body mass of Blue Tit nestlings, but body masses of Great Tit nestlings were lower in one year of the study. I conclude that food supplementation may compromise parental investment in offspring but that supplements appropriate for nestling-feeding may mitigate for this.

4.2. Introduction

Although the theoretical relationship between resource availability and the cost of reproduction to avian parents has been long established (Lack 1947, Williams 1966, van Noordwijk & de Jong 1986), the mechanisms through which life history trade-offs are enacted in response to food availability remain contentious (reviewed in Zera & Harshman 2001, Williams 2005). Individuals of species which exhibit bi-parental care must balance their reproductive investment in the face of the demands of their offspring against the investment of their partner (e.g. Hinde 2006) to maximise the number of offspring being produced without jeopardising their future reproductive potential (as proposed by life history theorists e.g. Stearns 1992, Mock & Forbes 1994). Increased food availability has the potential to reduce foraging and provisioning costs significantly (e.g. in Great Tits – Tinbergen & Dietz 1994, and in Blue Tits – Thomas *et al.* 2001) and so may lessen the cost of investment in the current breeding attempt. This is theorised to benefit residual reproductive value for adults (Lessells 1991) and may improve the share of parental care for offspring in practice if provision of food to nestlings is increased (e.g. Simons & Martin 1990). The previously reported smaller clutches in a population of food-supplemented Blue and Great Tits (Chapter Three, Harrison *et al.* 2010) may further improve the prospects for individual nestlings in terms of a greater share of the parental brood investment.

4.2.1. Food availability and parent-offspring conflict

The relationship between the number of offspring produced within reproductive attempts and potential life history trade-offs has been much investigated (reviewed in Martin 1987, Dijkstra *et al.* 1990, Godfray 1991). Species such as Blue and Great Tits time the period of maximum nestling demand to an ephemeral peak in abundance of their caterpillar prey (Perrins 1991). The timing of reproduction in such seasonally breeding species is one of the key determinants of offspring survival (van Noordwijk *et al.* 1995) and the cost to adults of raising those offspring (Nilsson 1994, te Marvelde *et al.* 2011b). An increase in phenological mismatching as a result of food supplementation (Chapter Two) could, therefore, have significant implications for the cost of a reproductive attempt to adults and the survival prospects of offspring. The decision of when to initiate breeding and how many offspring to produce is made far in advance of the peak in invertebrate availability (Perrins 1970). Females are able to fine-tune both the timing of hatching and the number of nestlings that survive, however, through the use of asynchronous hatching (Clark & Wilson 1981, Haftorn 1981). Through this mechanism a size hierarchy is created amongst offspring, and if food availability is low the smallest nestlings in whom the parents have invested least are predicted to perish (Lack 1954, Clark & Wilson 1981). Thus, parents may be able to adjust brood size to the prevailing conditions and recover some of the costs of mistimed breeding. There is, however, little evidence to demonstrate whether this is the case from long-term studies of trophic interactions or food supplementation.

There has been much debate as to whether such brood reduction is an adaptive strategy to maximise the number of offspring that survive (as demonstrated experimentally by Husby 1986, Amundsen & Slagsvold 1998) or whether it provides evidence of parent-offspring conflict, where the energetic demands on parents of brood rearing directly affect adult future survival and fecundity (Nur 1984b, Hõrak 1995, Nilsson 1995). Brood reduction is commonly considered in theoretical studies to be a reaction to unreliable food resources (Lack 1947, Stenning 1996). Parents appear to maximise the fitness value of their current reproductive attempt by scaling the number of offspring produced to resource availability (Lack 1954), although the efficacy of such an adaptation for short-lived species has been called into question (e.g. Clark & Wilson 1981, Slagsvold 1985). With high levels of nestling mortality the brood may appear devalued to parents, however, and they may reduce risk taking to increase their own survival prospects (Tilgar & Kikas 2009), improving residual reproductive value to the detriment of current reproductive value (Daan *et al.* 1990).

Food supplementation may be able to decrease nestling mortality through reducing the costs of foraging for parents provisioning nestlings (e.g. Simons & Martin 1990, Jodice *et al.* 2002). By way of example, increased mortality of Florida Scrub-Jay nestlings has been recorded in poorer habitats (which have been fragmented by recent urban developments) with lower arthropod abundance (Shawkey *et al.* 2004). Reduced nestling mortality has been found in food-supplemented European Pied Flycatchers (Moreno *et al.* 1999) and Eurasian Kestrels (Wiehn & Korpimäki 1997). A predictable food resource may, therefore, reduce the need for adults to 'trim' the number of offspring strategically in order to maintain their own residual reproductive value. Alternatively, there may be higher nestling mortality in response to food supplementation if phenological mismatch has occurred and adults strategically 'trim' brood sizes to maximise reproductive investment or to assure their own survival. Such a strategy would be revealed either by increased nestling body mass or improved adult survival rates although few studies have provided food supplements through successive generations to examine these interactions.

4.2.2. Food availability and parental provisioning rates

The provisioning rate of adults feeding altricial nestlings has long been used as a measure of parental investment in offspring (Trivers 1972), with early ecological studies (e.g. Kluijver 1950) leading to intricate models of parent-offspring conflict (e.g. Trivers 1974) and parental division of labour in a game theoretic framework (e.g. McNamara *et al.* 2000). New technology such as PIT tags using Radio-frequency Identification (RFID) allows each partner in a pair to be identified, enabling provisioning effort to be modelled automatically at the individual level (Bonter & Bridge 2011). Adult feeding rates of nestlings have been related to faster nestling growth and higher nestling survival (e.g. Naef-Daenzer & Keller 1999) so there are fitness advantages to increased provisioning effort. Parents are expected to balance their investment against that of their partner (Chase 1980), however, and the energetic cost of this parental investment may be closely related to food availability (Tinbergen & Dietz 1994, te Marvelde *et al.* 2011b).

Females and males of monogamous pairs should provision at similar rates during the period of maximum nestling demand (e.g. Great Tits - Royama 1966, Wilkin *et al.* 2009a). Empirical evidence has demonstrated significant proportional relationships in within-pair provisioning rates in some species such as the Dunnock (*Prunella modularis*) (Hatchwell & Davies 1990), but non-significant relationships in European Pied Flycatchers (Moreno *et al.* 1999) and House Sparrows (*Passer domesticus*) (Nakagawa *et al.* 2007). In one study the brood provisioning rates of male and female Great Tits were not correlated at the brood level (Michler *et al.* 2010) and in another study pair provisioning rates to broods were positively correlated (Hinde & Kilner 2007). In the former study all broods had the sex ratio of nestlings manipulated with no data from unmanipulated nests reported; although no correlation between parents in provisioning rate was detected at the (manipulated) brood level, there was a positive correlation at the individual nestling level. Results where parental rates are not correlated indicate that there may be evidence of sex-specific investment strategies and a full exploration of the ecological circumstances under which these strategies are being employed would be highly informative.

4.2.3. Food supplementation and DEE during nestling provisioning

The reproductive season is one of the most energetically demanding periods of the avian annual cycle (e.g. Green *et al.* 2009) with the late nestling phase being particularly so for parents feeding altricial nestlings (e.g. Nilsson & Råberg 2001). Brood size represents a composite of all the reproductive effort invested to that point (Martin 1987), and the energy requirements of brood-provisioning adults have been used by many studies (e.g. Bryant & Westerterp 1983a, Williams 1985, Tinbergen & Dietz 1994, Moreno *et al.* 1995, Sanz *et al.* 2000b) as a proxy for adult energetic investment in the reproductive attempt as a whole. Although there is seemingly an inextricable link between food or nutrient availability and allocation of energetic resources between different reproductive activities (King 1973), it is surprising that only two studies (i.e. Moreno *et al.* 1999, Jodice *et al.* 2002) have experimentally manipulated food availability during brood rearing and then measured energy expenditure. In the former there was no change in female DEE in response to food supplementation but in the latter supplemented parents reduced DEE. Both studies provided food only during nestling provisioning but neither measured natural food availability. Therefore, seasonal timing of reproduction to prey availability and the interaction between brood reduction and parental effort could not be accounted for. There still remain many unanswered questions regarding how food availability affects parental energetic investment in the brood.

Many studies (reviewed in Bryant & Tatner 1991) have endeavoured to quantify natural food availability without dietary manipulation and relate this to energetic measures, generating some key theories concerning adult reproductive investment. The ‘prudent parent’ hypothesis of Drent and Daan (1980), itself a refinement of the ‘Optimal Working Capacity’ hypothesis of Royama (1966), proposed that parents are likely to work only to a maximum theoretical level (i.e. 4 × BMR). This is because increased work rate could theoretically result in a loss of adult body condition and ultimately reduce adult survival. Adults are predicted to be able to metabolise and expend only a certain amount of energy (Peterson *et al.* 1990), so reproductive investment may have an upper energetic limit. Some of the subsequent studies on Great Tits supported this hypothesis, with a fixed energetic ceiling evident during feeding of enlarged Great Tit broods (e.g. Tinbergen & Verhulst 2000), whereas others (e.g. Sanz & Tinbergen 1999) suggested that adults are able to raise their energy expenditure without evidence of levelling off as manipulated brood size increased.

4.2.4. Food supplementation, parental effort and nestling body mass

One of the ultimate consequences of increased parental effort should be increased nestling body mass, which may then enhance post-fledging survival (Tinbergen & Boerlijst 1990) and offspring recruitment prospects (Naef-Daenzer *et al.* 2001). Studies have related increased parental effort in the form of provisioning rate (Naef-Daenzer & Keller 1999) and DEE (Moreno *et al.* 1997) to nestling body mass. An increase in nestling body mass is also characteristic of improved food availability, whether this is natural prey abundance (Wilkin *et al.* 2009a) or supplementary food (von Brömssen & Jansson 1980).

4.2.5. Food supplementation and parental investment – the current study

In this integrative study I aimed to link both natural and supplementary food availability to nestling mortality, brood size and parental investment in Blue and Great Tits using the metrics of adult provisioning rate and DEE (female Great Tits only). The consequences of any changes in parental investment for offspring were examined by 1) measuring nestling body mass and nestling mortality and 2) analysing these in relation to parental investment. This approach was used to investigate whether any brood reduction appeared to be adaptive (represented by increased nestling body mass) or a proponent of parent-offspring conflict (represented by reduced adult investment). I predicted that food supplementation should reduce nestling mortality (e.g. Simons & Martin 1990) as parents should have reduced foraging costs and could feed nestlings mealworms. Similarly, I anticipated that parents should exhibit higher provisioning rates in the food supplemented areas (e.g. Cucco & Malacarne 1997) as easier foraging should mean parents could bring prey more often, due to less time required for self-feeding. Females and males have exhibited different responses to food supplementation (e.g. Wiehn & Korpimäki 1997), however, so with the PIT tag technology I examined sex-specific differences in provisioning rate to further previous work completed on pair provisioning rates in the population (Smith 2011). I predicted, however, that there would be no difference between levels of energy expenditure in supplemented and unsupplemented (control) female Great Tits as the extra effort required to provision more frequently was offset by reduced foraging costs as a result of the food supplement. With a reduction in foraging costs for somatic maintenance, but no difference in DEE, I also predicted that females would reallocate energy into providing better quality food for their offspring which would be evident in increased nestling body mass in supplemented areas.

4.3. Methods

4.3.1. Nestbox study

Details of the study site and routine monitoring of nestboxes between 2007 and 2011 are provided in Chapter Two. Breeding female and male Blue and Great Tits were captured in all years between nestling days 9 and 14 (where hatching day = 0) using a metal spring trap placed inside the nestbox (Harrison 2010, Smith 2011). Adults were then identified with a pre-existing, uniquely numbered BTO metal ring, or ringed upon capture if unringed, measured and weighed. A split plastic colour ring was also placed on the same leg as the BTO ring of birds caught during egg laying or roost catches (Chapter Two, Chapter Three) in 2010 and 2011 to denote which food treatment area they were breeding in to enable monitoring of feeders (Chapter Two). All adults were aged according to Svensson (1992), using moult limits, as either first year breeders or older. Adult wing length (maximum chord) and tarsus length were measured (Svensson 1992), and they were weighed to the nearest 0.1 g using a SATRUE-500 electronic balance. In 2010 and 2011 the birds were given a unique combination of two plastic colour rings on the leg not carrying a BTO ring and area marker upon completion of the provisioning rate and energetics sampling protocols. This was for individual identification (at a distance using binoculars) at a later date. In all years nestlings were ringed with uniquely numbered BTO metal rings between 10 and 14 days of age and weighed to the nearest 0.1 g on a SATRUE-500 balance on nestling day 11 in 2010 and 2011. Nestling day 11 was selected as mass at this point in the nestling phase has been found to be a good predictor of fledgling survival (Schwagmeyer & Mock 2008). Individual nestling body masses were then divided by brood size to derive an average nestling mass for each brood to avoid pseudoreplication of data points from each brood.

4.3.2. Food supplementation

Details of the food supplementation regime are provided in Chapter Two. Between 2007 and 2009 inclusive, woodland areas were unsupplemented (Control, CON), supplemented with peanut cake (PC) or supplemented with peanut cake and mealworms (PCM). In 2010 and 2011, woodland areas were either unsupplemented (CON) or supplemented with peanut cake (PC).

4.3.3. Caterpillar biomass

Details of the methodology used to measure caterpillar biomass and determine timing of the seasonal peak in their availability are provided in Chapter Two.

4.3.4. Measurement of female and male provisioning rates

The rate at which both male and female Blue and Great Tit parents were provisioning nestlings was measured using RFID technology and Trovan Unique™ ID 100A PIT tags. PIT tags are small microchips that emit a unique hexadecimal code when they pass through the radio waves generated by the antenna of the Trovan Unique™ LID665 reader. The antenna of the reader was attached to the nestbox and each time the bird carrying the PIT tag passed through the antenna loop, the date, time and PIT tag number were recorded. The PIT tags were glued onto two coloured plastic rings and then attached to the leg of the male and female in each pair when they were caught before weighing and measuring (Fig. 4.1a).

Figure 4.1 (a) Male Great Tit wearing Passive Integrated Transponder tag on left leg (Photo: P. Beasley) and (b) PIT tag antenna in position on nestbox (Photo: T. Millar).

The antenna was attached to the outside of the nestbox using heavy duty Velcro™ or black gaffer tape (Fig. 4.1b) at the end of the initial capture of the breeding pair between nestling days 9 and 14 (in 2009) or on nestling day 10 (in 2010 and 2011). The LID665 datalogger was hidden behind the tree and left in place for at least 24 hours. Both males and females were recaptured where possible on nestling day 11 at the end of the sampling period in 2010 and 2011, when the PIT tags were removed and replaced with the unique colour ring combination described in section 4.3.1.

Calculating provisioning rates from PIT tag data

Once the reader was removed from the nestbox, the data were downloaded using Trovan Dorset ID software, version V710. Due to erroneous readings caused by the parents sitting in the nestbox entrance, the number of entries per day could not be used as a reliable measure of nestbox visit rate (te Marvelde *et al.* 2011b). Therefore, the number of mins per hour that had a registered entry for each of the PIT tags was counted (Wilkin *et al.* 2009a), based on the assumption that Blue and Great Tits are unlikely to provision more than twice in 1 min (Wilkin *et al.* 2009a). This assumption was verified by visual observations and video recordings of nestboxes with PIT tag readers using a Sony Handycam DCR-SR90E positioned approximately 10 m from the nestbox. The counts from the PIT Tag readers were then averaged across the sample period to provide a mean provisioning rate per hour. Recordings at the beginning or end of a sampling period or day (partial samples) for which there was not an entry in the first 10 mins or the last 10 mins of an hour were not used. The provisioning rate per hour was divided by the brood size to give an hourly provisioning rate per nestling.

4.3.5. Measurement of female DEE

The DLW method was used in 2010 and 2011 to gain measures of DEE for female Great Tits during the brood-rearing period; details of the theoretical background behind the technique are provided in Chapter One and Chapter Three.

Upon capture on nestling day 10, female Great Tits were injected intra-peritoneally with 100 µl of DLW (2010: 13.2162 g 98.44% ^{18}O, 6.8111 g 99.9% ^{2}H, mixed 2:1; 2011: 4.63184 g 98.44 % ^{18}O, 2.40526 g 99.9 % ^{2}H) using a BD Micro-Fine™ 0.3 ml 30G insulin syringe with an 8 mm needle (Fig. 3.2). All procedures were carried out under Home Office Licence (PPL 40/2926). The syringes had been filled and weighed to the nearest 0.0001 g prior to injection to determine the exact dose administered (Speakman 1997). The birds were then kept in a thermo-neutral cotton bag for a minimum of 30 mins (mean ± SE = 44 ± 1 mins) to allow the DLW to reach equilibration with the female's body water pool (Tatner & Bryant 1987, Speakman 1997). Although one hour is usually allowed for equilibration of DLW in Great Tits (Tinbergen & Dietz 1994), I wished to minimise the risk of desertion of the brood by reducing female detention while achieving equilibration (*sensu* Hinsley *et al.* 2011). Equilibration times have been calculated for a wide range of species based on body mass and then tested (reviewed in Speakman 1997). A 30 µl blood sample (Fig. 4.2) was taken from the female by puncturing the brachial vein on the left wing using a BD Microlance™ 3 27G 19 mm needle (Fig. 4.2). The blood was then stored in 2 × 25 µl Vitrex non-heparinized pre-calibrated microcapillary tubes (Fig. 4.2) which were flame-sealed within 5 mins using a butane blowtorch and stored in labelled plastic test tubes. After approximately 24 hours (mean ± SE: 24 hrs 55 mins ± 8 mins) the females were recaptured for a second blood sample of 45 µl, weighed and released. In 2010 the desertion rate was 3 females out of 62 females injected with DLW (2%) based on missing PIT tag data on the reader, of which only one brood was completely abandoned by both parents. In 2011 the desertion rate was 1 female out of 25

Figure 4.2. Venipuncture of a female Great Tit 30 mins after DLW injection using a non-heparinized pre-calibrated microcapillary tube (Photo: S. Webber)

females injected with DLW (4%). A single additional blood sample was taken each year from a female that had not been injected with DLW to provide background isotope levels. Mass spectrometry analysis of blood samples was carried out at the University of Groningen Centre for Isotope Research in The Netherlands. Calculations of DEE analyses and conversion to multiples of BMR were conducted as detailed in Chapter Three.

4.3.6. Measuring ambient temperature

Details of ambient temperature measurements using Thermochron® DS1921G_iButtons®, are provided in Chapter Three.

4.3.7. Statistical analyses and data filtering

Only first breeding attempts were included and known or suspected second broods were excluded from analyses based on criteria specified in Chapter Two. Nestling mortality and brood size analyses only used data from breeding attempts where female identity and the timing of breeding in relation to peak caterpillar availability were known. The mean caterpillar biomass within the woodland block in which the breeding attempt occurred was used in subsequent analyses to allow hatch date to be controlled for. Provisioning rates and female DEE were only examined if provisioning rate data for both parents were available. Nestling mortality was defined as the number of nestlings dying before ringing age (at days 10 to 14) and was calculated by subtracting the number of surviving young from clutch size, minus any un-hatched eggs. Brood size was defined as the number of nestlings alive at ringing age minus any dead nestlings found in the nest material post-breeding. Nestling mortality is likely to be higher in the early nestling period (i.e. before day 10), as nestlings are unable to regulate their own body temperature and so more at risk of fluctuations in ambient temperature (Kluijver 1951). Although adult Blue and Great Tits remove dead nestlings when they are young, they do not remove larger nestlings (e.g. Smith *et al.* 1989), so the number of nestlings at day 10, minus any dead nestlings found post-breeding, provides a good proxy for the number of young that fledge successfully from each brood. Disturbance later in the nestling phase (i.e. after day 15) may cause premature fledging of nestlings (Gosler 1993), which could affect downstream survival of the brood. Female and male body condition was calculated by using the residuals of the regression of body mass on tarsus length. Reduction in clutch size due to egg removal as part of earlier protocols (Chapter Three) was tested in all models and included if significant. The day of capture of adults varied only in 2009 and nestling age was tested in the provisioning rate models for this year. Two outlying DEE data points (possibly due to processing error from low blood sample volumes) (Speakman 1997) were excluded from analyses.

All statistical analyses were conducted using Generalized Linear Models (GLMs – glm function) or Generalized Linear Mixed Models (GLMMs – glmmadmb function in glmmADMB package or glmmPQL in MASS package) in R version 2.13.1 (R Development Core Team 2011). All response variables were tested for normality using the Shapiro-Wilk test for normality (Crawley 2007) and then checked against a negative binomial or Poisson error structure using a diagnostic distribution plot. All response variables were then checked for under- and overdispersion and corrected where necessary using a quasi-likelihood error structure (Crawley 2005, Zuur *et al.* 2009a). Collinearity was checked in all models using Variance Inflation Factors (VIF). Nestling mortality analyses included a high proportion of zeros and GLMM model analyses were conducted correcting for zero-inflation. All interactions between explanatory variables were tested using backward stepwise regression to find the minimal adequate model, and quadratic terms tested where appropriate. This model simplification was conducted using likelihood ratio tests, Wald tests or comparisons of residual deviance to compare models with and without terms and interactions (Crawley 2007). Pseudo R^2 values were calculated where possible for GLMMs, to illustrate model fit, using the R^2 value from a linear model of the correlation between fitted and observed values. Pseudo R^2 values for GLMs were calculated from the proportion of residual deviance in the model.

4.4. Results

A total of 240 Blue Tit and 210 Great Tit broods from 2007 to 2011 were analysed for variation in partial brood mortality and brood size in response to breeding parameters including caterpillar biomass and food supplementation treatment. Within-individual analyses of nestling mortality and brood size were conducted for 55 Blue and 58 Great Tit females which bred in more than one year. Measures of provisioning rates were examined for 81 Blue Tit broods, and 156 Great Tit broods between 2009 and 2011 and estimates of DEE were gained for 66 females provisioning nestlings in 2010 and 2011. Nestling body masses were obtained for 131 Blue Tit and 126 Great Tit broods in 2010 and 2011.

4.4.1. Food supplementation and nestling mortality

There was no significant effect of food supplementation treatment on nestling mortality before nestling day 11 in Blue Tits at either the population (Fig. 4.3, Table 4.1) or the

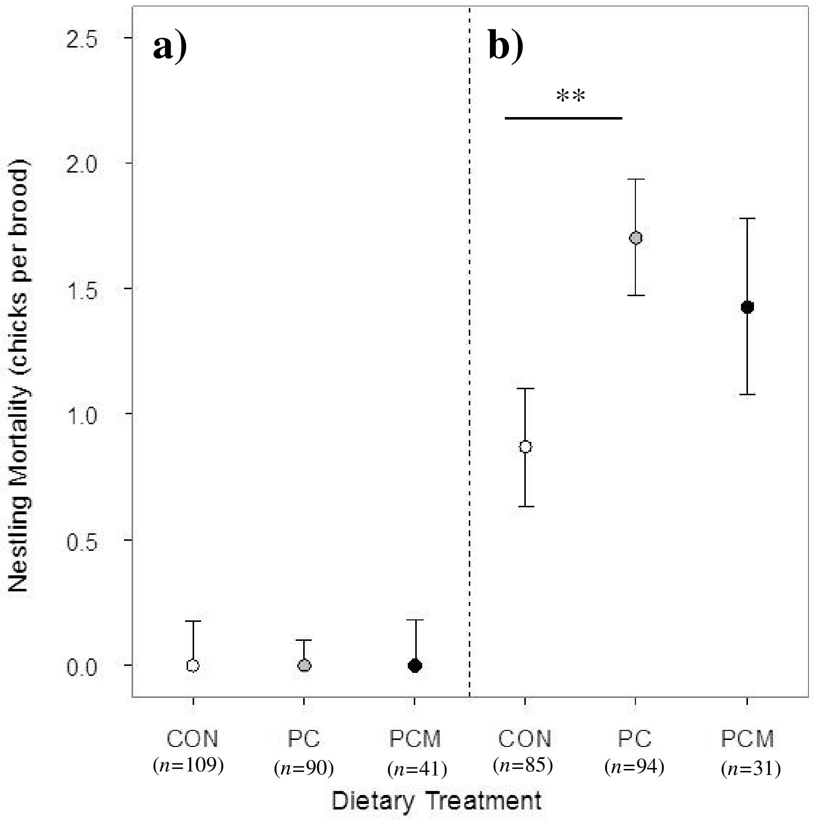

Figure 4.3. Nestling mortality per brood (mean + 1 SE or mean ± 1 SE) from model estimates) of (a) Blue and (b) Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011 (excluding 2009). Broods in the control treatment area (CON) are represented by open circles, those in the peanut cake treatment area (PC) by grey-filled circles and those in the peanut cake and mealworms treatment area (PCM) by black-filled circles. ** = P < 0.01. Number of broods is shown in parentheses below axis labels. See Table 4.1 for statistical tests and text for details.

individual (Fig. 4.4, Table 4.2) level. In Great Tits, however, there was significantly higher nestling mortality in broods supplemented with peanut cake than in unsupplemented broods but no difference from those supplemented with peanut cake and mealworms at the

Table 4.1. Results from simplified GLMM analysis of nestling mortality per brood (Response) of female Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011 regressed on significant explanatory variables (Fixed Effect). Chi-squared results from likelihood ratio (Chisq) detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 4.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	R^2
Nestling Mortality	BT	Intercept	0.004						0.27
		Year			3,330	8.87	**< 0.05**	2007: lowest 2010: highest	
		Number Hatched	0.0007	0.057	1,330	12.77	**< 0.001**	Higher mortality	
2007, 2008, 2010, 2011		Hatch Date	0.0003	0.022	1,330	12.22	**< 0.001**	Higher mortality	
Nestling Mortality	GT	Intercept	0.871						0.81
		Dietary Treatment: PC	1.704	0.233	1,284	8.29	**< 0.01**	Higher mortality	
		Dietary Treatment: PCM	1.428	0.351	1,284		0.16		
		Year			3,284	34.76	**< 0.001**	2007: lowest 2010: highest	
		Number Hatched	1.060	0.043	1,284	18.74	**< 0.001**	Higher mortality	
		Hatch Date	-0.006	0.022	1,284	8.93	**< 0.01**	Lower mortality	
2007, 2008, 2010, 2011		Egg Removal	-0.360	0.187	1,284	8.75	**< 0.01**	Lower mortality	

Random effects were nestbox and female identity in all models. Tested fixed effects were dietary treatment (Intercept = control, ‘PC’ = peanut cake, ‘PCM’ = peanut cake and mealworms), year, number of hatched young, mismatch from the peak in caterpillar biomass and female age. Only significant contributors to each model are displayed. Analyses are corrected for zero-inflation or underdispersion where required and used a negative binomial or Poisson error distribution.

population level (Fig. 4.3, Table 4.1) and significantly higher nestling mortality in the broods supplemented with peanut cake than both of the other treatment groups at the individual level (Fig. 4.4, Table 4.2). There was a significant increase in early nestling mortality in relation to larger original brood size in both Blue and Great Tits (Table 4.1). There was also a significant increase in nestling mortality associated with advancing hatch date in Blue Tits, but a decrease in nestling mortality in relation to advancing hatch date in the Great Tits (Table 4.1). The removal of eggs during egg laying had a significant

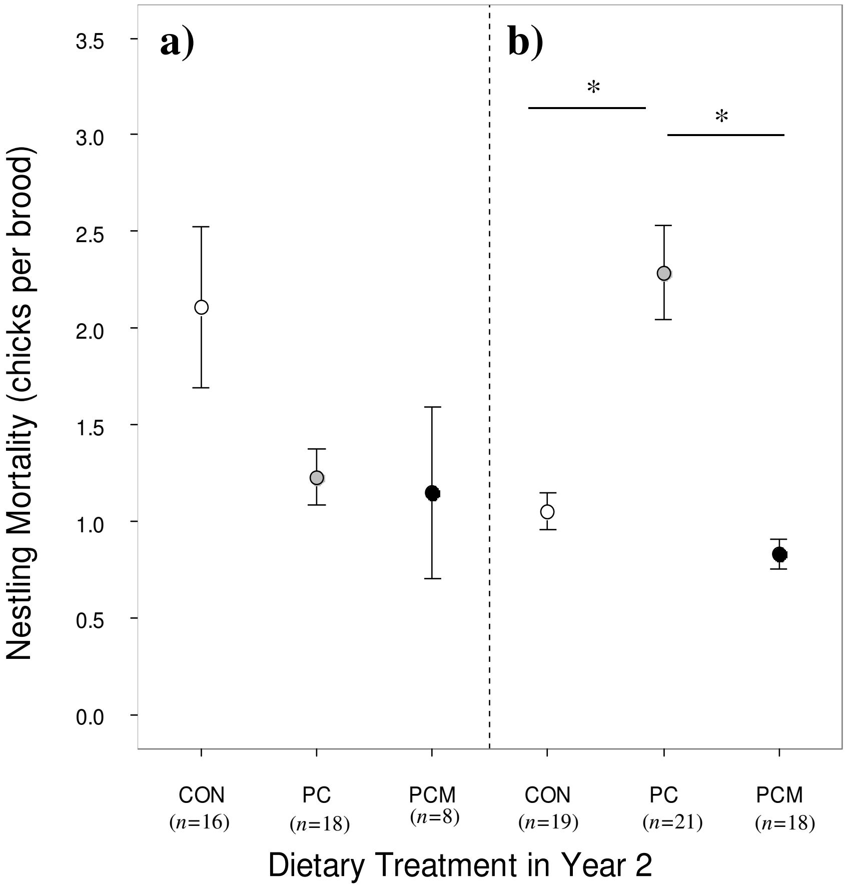

Figure 4.4. Nestling mortality per brood (mean ± 1 SE from model estimates) in the second year of (a) Blue and (b) Great Tit females breeding for two consecutive years in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011. Broods in the control treatment area (CON) are represented by open circles, those in the peanut cake treatment area (PC) by grey-filled circles and those in the peanut cake and mealworms treatment area (PCM) by black-filled circles. * = $P < 0.05$. Number of broods is shown in parentheses below axis labels. See Table 4.2 for statistical tests and text for details.

negative effect on nestling mortality in Great but not Blue Tits after controlling for the reduced clutch size (i.e. Number Hatched, Table 4.1). There was a seasonal increase in nestling mortality associated with advancing hatch date in Blue Tits at the individual level (Table 4.2).

Table 4.2. Results from simplified GLMM analysis of nestling mortality per brood (Response) of female Blue (BT) and Great (GT) Tits breeding in the second of two consecutive years in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011 regressed on significant explanatory variables (Fixed Effect). Only significant contributors to the model are displayed. Chi-squared results from Wald tests (Chisq) detail the relative contribution of each fixed effect. A pseudo-R^2 value is presented. For details of statistical tests applied see section 4.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	R^2
Nestling Mortality Year Two (within female) 2007-2011	BT	Intercept	0.003						0.09
		Hatch Date Year 2	0.001	0.126	1,41	4.9	**< 0.05**	Higher mortality	
		Number Hatched Year 2	0.001	0.032	1,41	6.8	**< 0.05**	Higher mortality	
Nestling Mortality Year Two (within female) 2007-2011	GT	Intercept	0.171						0.29
		Dietary Treatment Year 2: PC	0.359	0.362	1,57	4.5	**< 0.05**	Higher mortality	
		Dietary Treatment Year 2: PCM	0.132	0.464	1,57	0.33	0.58		
		Number Hatched Year 2	0.042	0.077	1,57	8.6	**< 0.01**	Higher mortality	

Random effect was year in all models. Tested fixed effects were mortality in year 1, dietary treatment in year 2 (Intercept = control, ‘PC’ = peanut cake, ‘PCM’ = peanut cake and mealworms), number of hatched young, hatch date in year 2 and female age in year 1. Analyses were corrected for underdispersion where required and a Poisson error distribution was used.

4.4.2. Food supplementation and brood size

There was no significant difference in brood size between unsupplemented and supplemented Blue Tits (Fig. 4.5, Table 4.3), but there were significantly smaller brood sizes in Great Tits supplemented with peanut cake than in unsupplemented Great Tits (Fig. 4.5, Table 4.3). There was significant inter-annual variation in brood size in Great but not Blue Tits (Table 4.3). There was a significant effect of hatch date on brood size in both species although this relationship was negative in Blue Tits and positive in Great Tits.

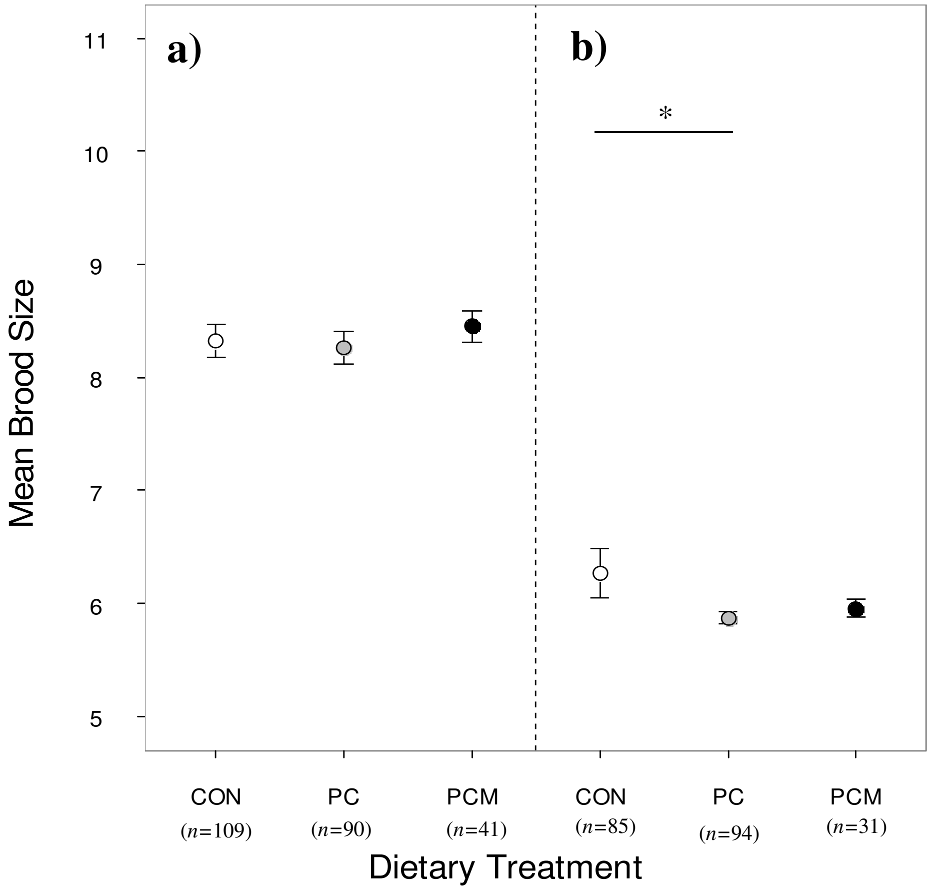

Figure 4.5. Brood size (mean ± 1 SE from model estimates) of (a) Blue and (b) Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2011 (excluding 2009). Estimates control for seasonal changes in brood size and use day 30 as a baseline. Broods in the control treatment area (CON) are represented by open circles, those in the peanut cake treatment area (PC) by grey-filled circles and those in the peanut cake and mealworms treatment area (PCM) by black-filled circles. * = $P < 0.05$. Number of broods is shown in parentheses below each axis label. See Table 4.3 for statistical tests and text for details.

Caterpillar biomass was significantly positively related to brood size in Blue Tits but not Great Tits. Great Tit broods in the peanut cake-supplemented area were on average 1.4 chicks smaller than broods in the unsupplemented area and 1.3 chicks smaller than broods in the peanut cake and mealworms area. There was high unexplained variance in the Blue Tit brood size model, however (Table 4.3).

Table 4.3. Results from simplified GLMM analysis of brood size (Response) of female Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011 regressed on significant explanatory variables (Fixed Effect). Chi-squared results from Wald tests (Chisq) detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo-R^2 value is presented. For details of statistical tests applied see section 4.3.7.

Response	Sp.	Fixed Effect	Estimate	SE	df	*Chisq*	*P*	Direction	R^2
Brood Size	BT	Intercept	12.701						0.09
		Hatch Date	-0.146	0.003	1,322	8.4	**0.001**	Lower brood size	
		Caterpillar Biomass	68.382	0.579	1,322	2.8	**< 0.01**	Higher brood size	
2007,2008 2010,2011									
Brood Size	GT	Intercept	3.750						0.33
		Dietary Treatment: PC	3.354	0.056	1,276	4.1	**0.05**	Lower brood size	
		Dietary Treatment: PCM	3.438	0.077	1,276	1.3	0.26		
		Year			3,276	48.6	**< 0.001**	2007: highest 2010: lowest	
2007,2008 2010,2011		Hatch Date	0.084	0.005	1,276	18.3	**< 0.001**	Higher brood size	

Nestbox was included as a random effect and tested fixed effects were dietary treatment (Intercept = control, 'PC' = peanut cake, 'PCM' = peanut cake and mealworms), year, caterpillar biomass, the quadratic term of mismatch and female age. Analyses were corrected for over- or underdispersion and a Poisson error distribution was used.

When considering the within-individual relationship between food supplementation treatment and brood size, there was no effect in Blue Tit females (Table 4.4) and only the age of the female in the first of the two consecutive years was found to affect the brood size in the second year (Table 4.4). Females that were older in the first year of capture had significantly smaller broods than those that were in their first breeding year in the first year of capture. In Great Tits there was a significant negative effect of supplementation on brood size when peanut cake was provided but not when both peanut cake and mealworms

Table 4.4. Results from simplified GLMM analysis of specified breeding parameters (Response) of female Blue (BT) and Great (GT) Tits breeding in the second of two consecutive years in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2011 regressed on significant explanatory variables (Fixed Effect). Chi-squared results from Wald tests (Chisq) detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo-R^2 value is presented. For details of statistical tests applied see section 4.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	R^2
Brood Size Year Two (within female) 2007-2011	BT	Intercept	9.593						0.14
		Female Age	7.818	0.089	1,41	5.6	**< 0.05**	Lower brood size in older females	
Brood Size Year Two (within female) 2007-2011	GT	Intercept	5.133						0.24
		Dietary Treatment Year 2: PC	3.965	0.106	1,57	6.4	**< 0.05**	Lower brood size	
		Dietary Treatment Year 2: PCM	5.012	0.107	1,57	0.05	0.55		
		Brood Size 1	0.270	0.022	1,57	5.9	**< 0.05**	Higher brood size	

Year was included as a random effect and tested fixed effects were dietary treatment in year 2 (Intercept = control, 'PC' = peanut cake, 'PCM' = peanut cake and mealworms), hatch date in year 2, brood size in year 1 and female age in year 1. Analyses were corrected for over- or underdispersion and a Poisson error distribution was used.

were provided. There was a significant positive relationship between brood size in the first and second years (Table 4.4).

4.4.3. Food supplementation and provisioning rates

Female and male Blue Tit provisioning rates per nestling were significantly positively correlated with the provisioning rates of their partners (Table 4.5), but they did not proportionately increase their provisioning rate per nestling with brood size. Therefore, larger Blue Tit broods received fewer feeding visits per nestling (Table 4.5). Female Blue and Great Tits significantly reduced their provisioning rates in conditions of higher

Table 4.5. Results from simplified GLM analysis of specified breeding parameters (Response) of pairs of Blue (BT) and Great (GT) Tits provisioning nestlings in Chaddesley Woods National Nature Reserve in Worcs., UK between 2009 and 2011 regressed on significant explanatory variables (Fixed Effect). Chi-squared results from Wald tests (Chisq) detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo-R^2 value is presented. For details of statistical tests see section 4.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	R^2
Female Provisioning Rate per Nestling 2009 & 2011	BT	Intercept	3.104						0.54
		Brood Size	-0.187	0.019	1,63	15.07	**< 0.01**	Lower fem prov rate	
		Male Prov Rate per Nestling	0.388	0.029	1,63	4.07	**< 0.001**	Higher fem prov rate	
		Year			1,63	0.96	0.07		
Female Provisioning Rate per Nestling (including caterpillar biomass) 2011	BT	Intercept	1.879						0.68
		Caterpillar Biomass	-1.879	4.805	1,21	11.71	**< 0.01**	Lower fem prov rate	
		Male Prov Rate per Nestling	0.476	0.047	1,21	5.06	**< 0.001**	Higher fem prov rate	
		Male Body Condition	0.772	0.182	1,21	4.16	0.07		
Male Provisioning Rate per Nestling 2009 & 2011	BT	Intercept	11.014						0.59
		Brood Size	-0.618	0.017	1,63	8.22	**< 0.01**	Lower male prov rate	
		Hatch Date	-0.276	0.006	1,63	6.02	**< 0.001**	Lower male prov rate	
		Female Prov Rate per Nestling	0.818	0.025	1,63	1.50	**< 0.01**	Higher male prov rate	
Male Provisioning Rate per Nestling (including caterpillar biomass) 2011	BT	Intercept	5.198						0.60
		Dietary Treatment: PC	6.674	0.090	1,21	0.95	**< 0.05**	Higher male prov rate	
		Brood Size	-0.402	0.016	1,21	3.49	**< 0.001**	Lower male prov rate	
		Caterpillar Biomass	346.523	3.403	1,21	0.23	0.23		
Female Provisioning Rate per Nestling 2009-2011	GT	Intercept	10.235						0.23
		Brood Size	-0.861	0.021	1,94	6.35	**< 0.001**	Lower female prov rate	
		Hatch Date	-0.113	0.005	1,94	0.92	**< 0.05**	Lower female prov rate	
		Male Prov Rate per Nestling	-0.535	0.034	1,94	0.63	0.12		
		Female Body Condition	-3.084	0.205	1,94	1.10	0.08		

Female Provisioning Rate per Nestling (including caterpillar biomass) 2010 & 2011	GT	Intercept	5.600						0.24
		Brood Size	-0.311	1.811	1,61	11.62	**< 0.05**	Lower female prov rate	
		Caterpillar Biomass	-5.549	0.024	1,61	3.58	**< 0.05**	Lower female prov rate	
Male Provisioning Rate per Nestling 2009-2011	GT	Intercept	11.525						0.42
		Hatch Date	-0.128	0.004	1,94	4.02	**< 0.05**	Lower male prov rate	
		Brood Size	-1.185	0.014	1,94	20.21	**< 0.001**	Lower male prov rate	
Male Provisioning Rate per Nestling (including caterpillar biomass) 2010 & 2011	GT	Intercept	6.690						0.53
		Dietary Treatment: PC	8.131	0.079	1,61	7.37	**< 0.05**	Higher male prov rate	
		Brood Size	-2.133	0.017	1,61	12.09	**< 0.001**	Lower male prov rate	
		Caterpillar Biomass	14.99	1.405	1,61	0.183	0.41		

Tested fixed effects were dietary treatment (Intercept = control, ‘PC’ = peanut cake), nestling mortality, hatch date, brood size, female age and male age, female and male body condition, caterpillar biomass, nestling age (2009 only) and female and male provisioning rates. Analyses were corrected for over- or underdispersion and a Poisson error distribution was used.

caterpillar biomass (Fig. 4.6, Table 4.5). The provisioning rates of males of both species were unaffected by caterpillar biomass. Both Blue and Great Tit males exhibited a seasonal reduction in provisioning rates per nestling, however, with later broods having fewer feeding visits per nestling from males (Table 4.5). This tendency was exhibited by female Great Tits when caterpillar biomass was not included in the model (Table 4.5), but not in Blue Tit females. In both Blue and Great Tit males there was a significant positive effect of food supplementation with peanut cake on the provisioning rates per nestling when caterpillar biomass was controlled for (Figure 4.7, Table 4.5). There was no effect of food supplementation on the provisioning rates of either female Blue or Great Tits (Table 4.5).

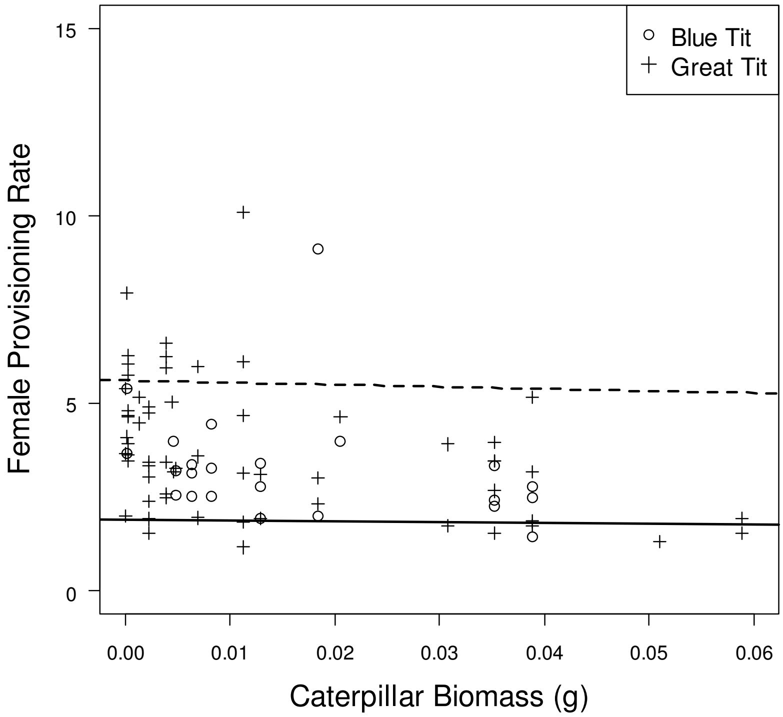

Figure 4.6. Mean hourly provisioning rate per nestling of female Blue (circles) and Great (saltire crosses) Tits breeding in the Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 (Great Tits only) and 2011 in relation to caterpillar biomass. Regression lines are from GLM models, with Blue Tit females represented by a solid line and Great Tit females by a dashed line. See Table 4.5 for statistical tests and text for details.

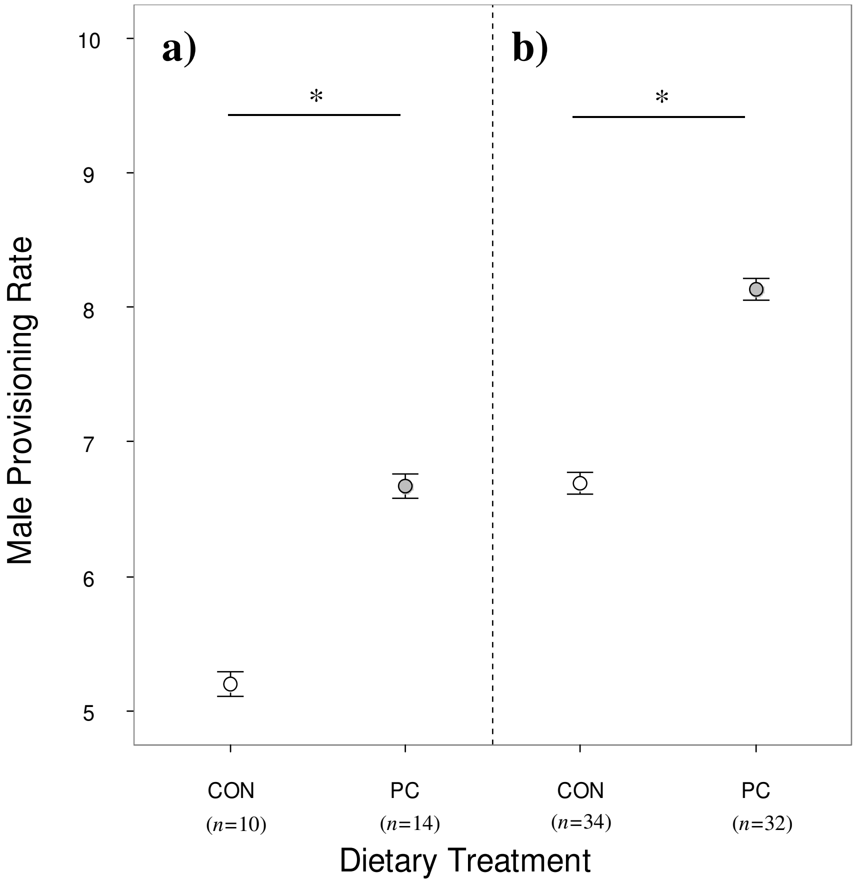

Figure 4.7. Hourly provisioning rates per nestling (mean ± 1 SE from model estimates) of (a) Blue and (b) Great Tit males breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 (Great Tits only) and 2011. Broods in the control treatment area (CON) are represented by open circles and those in the peanut cake treatment area (PC) by grey-filled circles. * = $P < 0.05$. Number of broods is shown in parentheses below axis labels. See Table 4.5 for statistical tests and text for details.

4.4.4. Food supplementation and female DEE during brood rearing

There was a significant negative effect of food supplementation with peanut cake on the DEE of female Great Tits when they were provisioning nestlings (Fig. 4.8, Table 4.6). Supplemented females expended on average 7.5 kJ less per day than unsupplemented females (Table 4.6) which represents 6% of mean DEE. There was a significant inter-annual difference in female DEE, with higher energy expenditure in 2011 than 2010

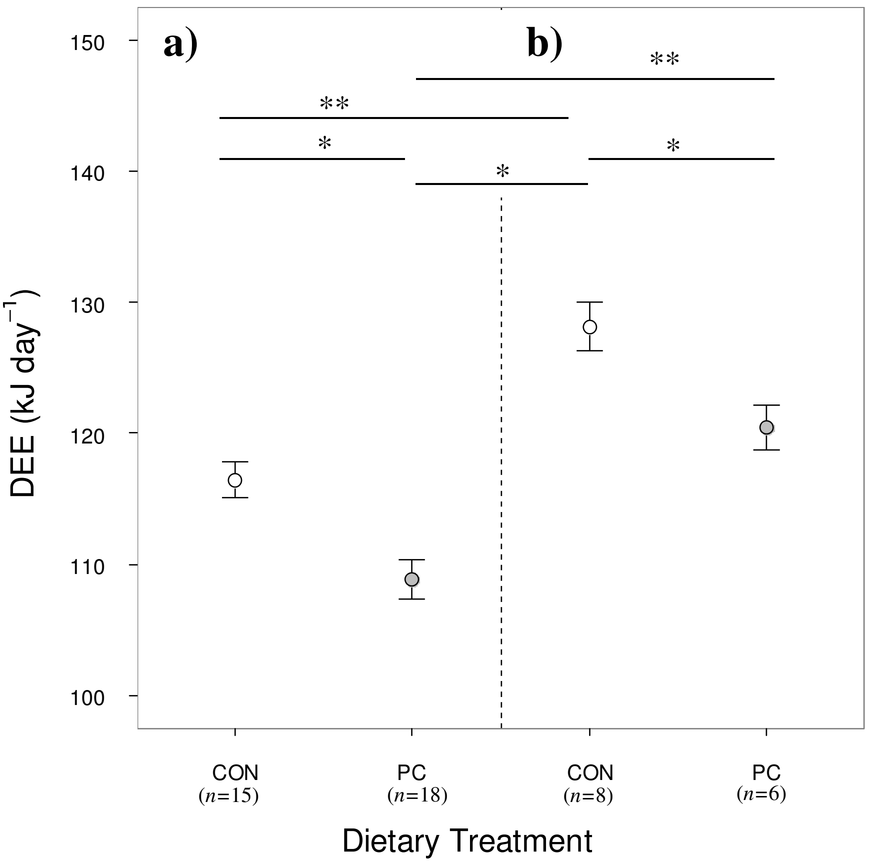

Figure 4.8. Daily Energy Expenditure (DEE) (mean ± 1 SE from model estimates) of female Great Tits provisioning nestlings in Chaddesley Woods National Nature Reserve in Worcs., UK in (a) 2010 and (b) 2011. Females breeding in the control treatment area (CON) are represented by open circles and those breeding in the peanut cake treatment area (PC) by grey-filled circles. * = *P* < 0.05, ** = *P* < 0.01. Number of broods is shown in parentheses below axis labels. See Table 4.6 for statistical tests and text for details.

(Fig. 4.9, Table 4.6). Females provisioning in pairs exhibited lower DEE with increasing provisioning effort from their partner, saving 2.9 kJ per day (or 3% of their total DEE) for every extra visit per nestling provided by the male (Fig. 4.9, Table 4.6). Female DEE was unrelated to brood size and ambient temperature when broods with no males were

Table 4.6. Results from simplified GLMM analysis of female Daily Energy Expenditure (DEE) of female Great Tits (GT) breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 and 2011 regressed on significant explanatory variables (Fixed Effect). Chi-squared results from Wald tests (Chisq) detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo-R^2 value is presented. For details of statistical tests applied see section 4.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	R^2
Female DEE	GT	Intercept	116.944						0.33
		Dietary Treatment: PC	109.922	0.031	1,66	4.3	**0.05**	Lower DEE	
		Year: 2011	126.710	0.032	1,66	6.8	**< 0.05**	Higher DEE in 2011	
		Brood Size	1.968	0.008	1,66	5.2	**< 0.05**	Higher DEE	
		Temperature	-1.438	0.006	1,66	5.2	**< 0.05**	Lower DEE	
2010 & 2011									
Female DEE (excluding broods with no male)	GT	Intercept	116.425						0.51
		Dietary Treatment: PC	108.884	0.029	1,47	6.0	**< 0.05**	Lower DEE	
		Year: 2011	128.130	0.029	1,47	12.5	**< 0.01**	Higher DEE in 2011	
		Male Provisioning Rate per Nestling	-2.9345	0.011	1,47	5.9	**< 0.05**	Lower DEE	
		Female Body Condition	7.006	0.019	1,47	10.3	**< 0.01**	Higher DEE	
2010 & 2011									

Random effect was woodland area and tested fixed effects were dietary treatment (Intercept = control, 'PC' = peanut cake), nestling mortality, hatch date, brood size, caterpillar biomass, year, ambient temperature, female and male age, female and male body condition, and female and male provisioning rates. Analyses were corrected for over- or underdispersion and a Poisson error distribution was used.

excluded (Table 4.6), but was significantly positively correlated with female body condition, with females in higher body condition exhibiting higher DEE (Table 4.6).

In terms of multiples of BMR, the DEE of females provisioning in pairs represented a mean of 3.9 × BMR (± 0.07 [1 SE], range: 3.02 – 4.27).

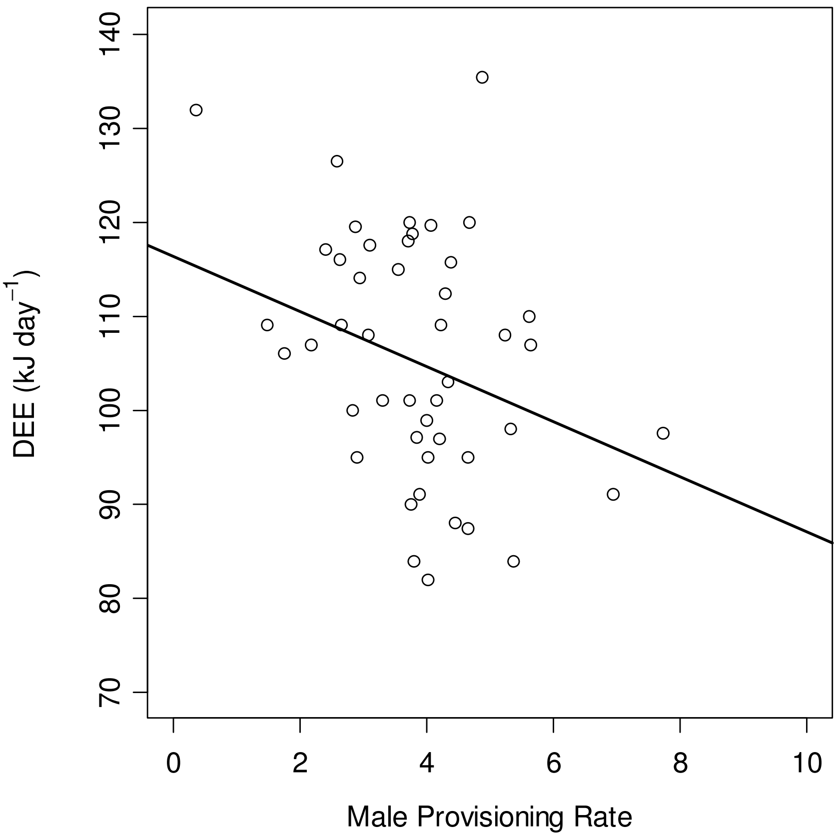

Figure 4.9. Daily Energy Expenditure (DEE) of female Great Tits breeding in the Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 and 2011 in relation to hourly male provisioning rates per nestling. Regression line is from a GLMM model, correcting for food treatment group, female body condition and year. See Table 4.6 for statistical tests and text for details.

4.4.5. Food supplementation and nestling body mass

There was no significant effect of food supplementation on the average nestling body mass at day 11 of Blue Tit broods (Fig. 4.10, Table 4.7). There was, however, a change in the direction of the relationship between female body condition and average nestling body mass, with supplemented females in higher body condition associated with lower nestling body mass than unsupplemented females (Table 4.7). There was a significant increase in

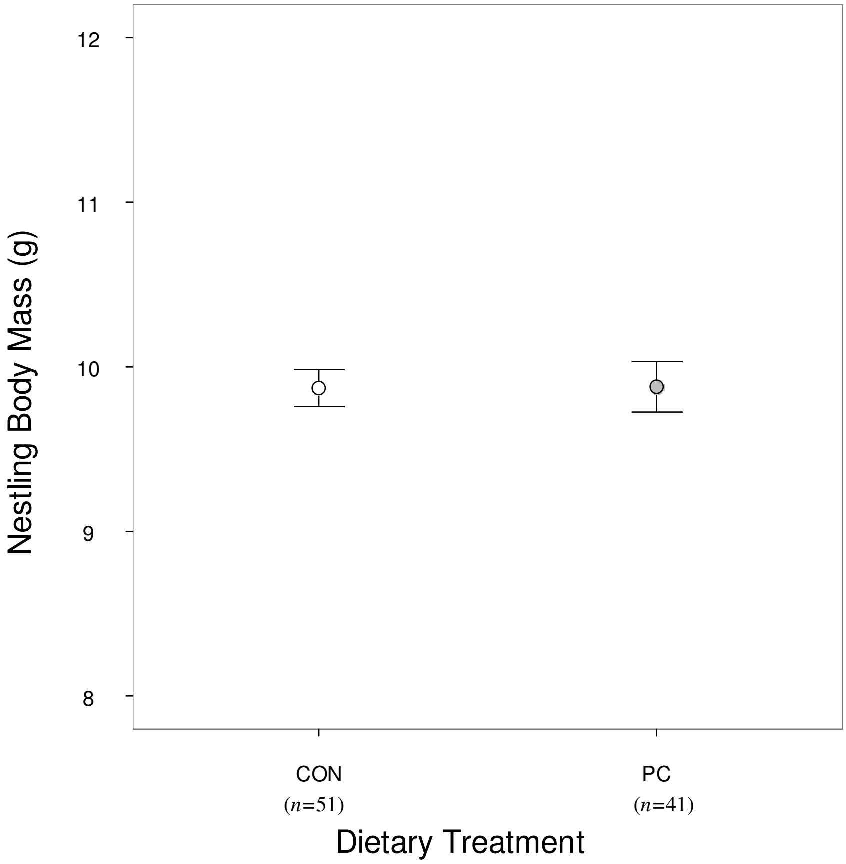

Figure 4.10. Nestling body mass (mean ± 1 SE from model estimates) of Blue Tit broods in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 and 2011 with hatch date set to day 30. Broods from the control treatment area (CON) are represented by open circles and those from the peanut cake treatment area (PC) by grey-filled circles. Number of broods is shown in parentheses below axis labels. See Table 4.7 for statistical tests and text for details.

nestling body mass under conditions of higher caterpillar availability and a seasonal decline in nestling body mass in Blue Tit broods (Table 4.7). Improved body condition of male Blue Tits was also associated with higher nestling body mass (Table 4.7).

In Great Tit broods there was a significant negative effect of food supplementation on nestling body mass, with lower body mass in the peanut cake-supplemented area in 2010, but higher nestling body mass in 2011 when hatch date, male body condition and year were controlled for (Figure 4.11, Table 4.7). In common with Blue Tits, there

Table 4.7. Results from simplified GLMM analysis of average nestling body mass (Response) of female Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 and 2011 regressed on significant explanatory variables (Fixed Effect). Chi-squared results from Wald tests (Chisq) detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo-R^2 value is presented. For details of statistical tests applied see section 4.3.7.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	R^2
Average Nestling Body Mass	BT	Intercept	11.247						0.35
		Dietary Treatment: PC	11.259	0.014	1,89	2.3	0.94		
		Caterpillar Biomass	22.471	0.276	1,89	0.18	**< 0.001**		
		Hatch Date	-0.046	0.002	1,89	5.5	**< 0.05**	Lower body mass	
		Year: 2011	11.407	0.025	1,89	0.02	0.57		
		Male Body Condition	0.514	0.018	1,89	1.5	**0.01**	Higher body mass with better male condition	
		Female Body Condition	0.821	0.107	1,89	0.001	0.51		
		Dietary Treatment × Female Body Condition	-3.1391	0.101	1,89	7.3	**< 0.05**	Lower body mass with better female body condition in PC area	
2010 & 2011		Year × Female Body Condition	2.385	0.110	1,89	12.1	**< 0.05**	Higher body mass with better female body condition in 2011	
Average Nestling Body Mass	GT	Intercept	20.858						0.24
		Dietary Treatment: PC	18.919	0.026	1,110	14.9	**< 0.001**	Lower body mass	
		Hatch Date	-0.106	0.002	1,110	5.8	**< 0.05**	Lower body mass	
		Year: 2011	18.900	0.040	1,110	6.3	**< 0.05**	Lower body mass in 2011	
		Male Body Condition	3.216	0.036	1,110	16.7	**< 0.001**	Higher body mass with better male body condition	
2010 & 2011		Dietary Treatment PC × Year 2011	23.973	0.046	1,110	9.9	**0.01**	Higher body mass in PC area in 2011	

Tested fixed effects were dietary treatment (Intercept = control, 'PC' = peanut cake), nestling mortality, hatch date, brood size, caterpillar biomass, year, female and male feeding rates, female and male age and female and male body condition. Analyses were corrected for over- or underdispersion and a Poisson error distribution was used.

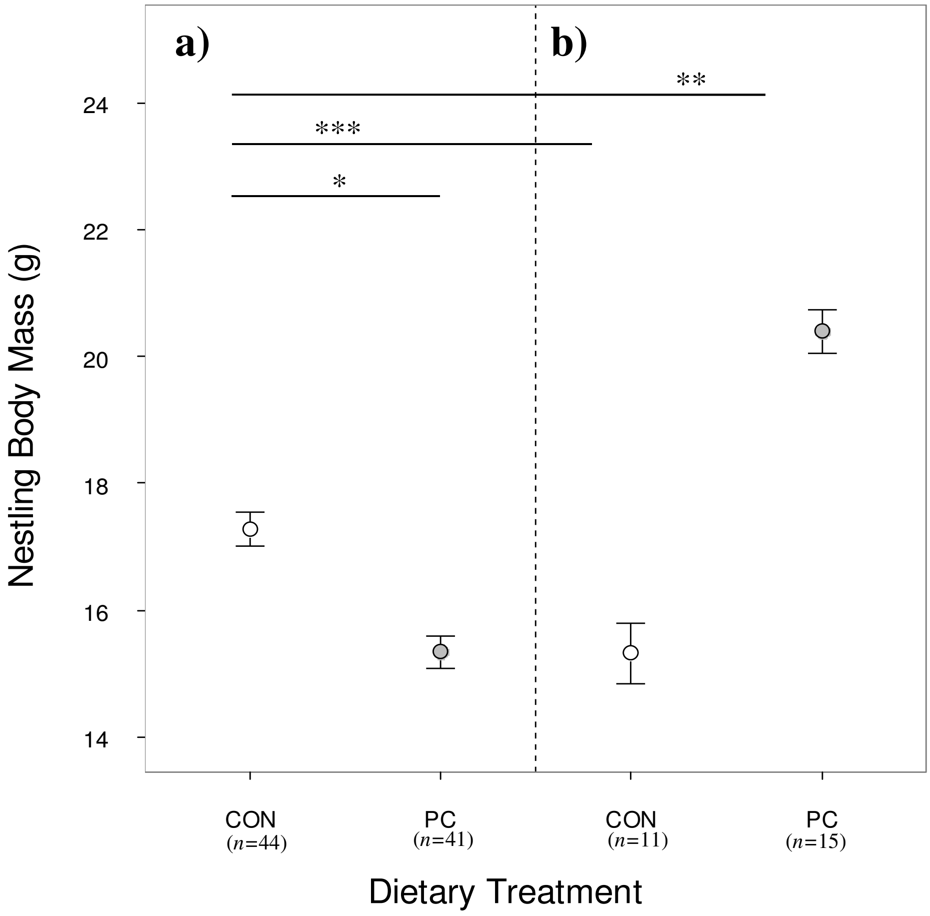

Figure 4.11. Nestling body mass (mean ± 1 SE from model estimates) of broods of Great Tits in Chaddesley Woods National Nature Reserve in Worcs., UK in (a) 2010 and (b) 2011 in relation to food supplementation, with hatch date set to day 30. Broods from the control treatment area (CON) are represented by open circles and from the peanut cake treatment area (PC) by grey-filled circles. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Number of broods is shown in parentheses below each data axis label. See Table 4.7 for statistical tests and text for details.

was a significant positive association between male body condition and average nestling body mass. There was no significant effect of increasing brood size on nestling mass in either species (Table 4.7), and although there was a seasonal decline in nestling body mass with advancing hatch date in Great Tits, there was no significant effect of caterpillar biomass (Table 4.7).

4.5. Discussion

In common with the tendency identified in previous chapters and in earlier results from this population (Harrison 2010, Smith 2011), there was a significant impact of food supplementation on breeding parameters of Great, but not Blue, Tits during the nestling phase. There were higher levels of nestling mortality and lower brood sizes in Great Tits supplemented with peanut cake at both the population- (Figs 4.3 and 4.5) and intra-individual (Fig. 4.4, Table 4.2) level, but no difference between control broods and those supplemented with peanut cake and mealworms (Figs 4.3, 4.4 and 4.5).

Male partners in both Blue and Great Tit broods increased their hourly provisioning rate in response to food supplementation with peanut cake (Fig. 4.7), but females of both species did not significantly change their hourly provisioning rates when supplemented with either peanut cake or peanut cake and mealworms (Table 4.5). Female Blue and Great Tits reduced their hourly provisioning rates in response to increased natural food availability in the form of caterpillars (Fig. 4.6). Female Great Tits reduced their DEE in response to supplementation with peanut cake (Fig. 4.8) but there was no effect of caterpillar availability on DEE (Table 4.6). Female DEE was also lower when the provisioning rate of their partner was higher (Fig. 4.9).

There was a seasonal decline in nestling body mass in both species (Table 4.7), but only in Blue Tits was the relationship between caterpillar availability and nestling body mass significant (Table 4.7). Great Tit nestlings in the peanut cake supplemented area were significantly lighter than control nestlings in 2010 but significantly heavier than nestlings in the control area in 2011 (Fig. 4.11).

4.5.1. Food supplementation, nestling mortality and brood size

There is a precedent in other studies for nestling mortality either to decrease or remain unchanged in response to food supplementation (Wiehn & Korpimäki 1997, Moreno *et al.* 1999), as was demonstrated in the Blue Tits in my population. Interpreting an increase in nestling mortality in conjunction with a reduction in clutch size (Chapter Three) of Great Tits is more problematic. It is particularly surprising that the Great Tit broods in the peanut cake supplemented area experienced higher nestling mortality as the females had already reduced clutch size in response to food supplementation (Chapter Three). In addition, reduced clutch size as a consequence of egg removal through the DLW protocol led to a reduction in nestling mortality. It could be that supplemented female Great Tits were breeding too early in relation to the peak in natural food availability (Nilsson 1994, Mock *et al.* 2009), and that the mealworms mitigated for this mismatch in being a supplement that could be fed to nestlings instead of caterpillars (e.g. Moreno *et al.* 1999). Brood reduction has been much investigated as a life history strategy to maximise the chances of offspring survival (Pijanowski 1992) or to increase adult survival (Hõrak 1995), particularly under conditions of lower food availability (Stenning 1996). Brood reduction is predicted to be favoured by natural selection under conditions of low food availability if it lowers parental provisioning effort per nestling (Mock & Forbes 1994). Therefore, higher nestling mortality could have been a strategic response to low natural food availability.

The suggestion that nestling mortality is connected to a seasonal paucity of natural food is corroborated by a seasonal decrease in mortality associated with hatch date in Great Tits; this is unexpected as delayed or late breeders usually exhibit higher nestling mortality (Ewald & Rohwer 1982, Barba *et al.* 1995), as in Blue Tits. The earliest Great Tit broods experienced higher levels of nestling mortality, reversing this relationship, and although it could be theorised that this is due to being mismatched from peak caterpillar availability, there was no relationship between caterpillar availability and Great Tit nestling mortality. It was also not possible to resolve whether the reversal in the relationship between hatch date and nestling mortality (and hence the high nestling mortality early in the season) occurred as a result of food supplementation. This is because an interaction between dietary treatment and hatch date in Great Tits could not be included due to high collinearity between the two covariates.

The cumulative effect of reduced clutch size and increased nestling mortality in Great Tits supplemented with peanut cake resulted in broods that were on average 1.4 chicks smaller than those in the unsupplemented area and 1.3 chicks smaller than those in the peanut cake and mealworms area. There was no significant difference in brood size between dietary treatments in Blue Tits, consistent with no difference being found between treatment groups in clutch size (Chapter Three) or nestling mortality. Blue Tit brood size, and in subsequent analyses nestling body mass, were correlated with caterpillar availability. This suggests that Blue Tit breeding success may be more closely linked to the availability of specific prey rather than a general seasonal increase in invertebrates and that they are able to cue more accurately to the availability of these invertebrates than Great Tits (see discussion of this point in Chapter Two). Indeed, although there was no difference between Blue Tit treatment groups in terms of partial brood mortality, previous analyses from this study population have found a higher complete brood mortality in supplemented Blue Tits (Harrison 2010). The penalty of mistiming could, therefore, be more dramatic for Blue Tits and further analyses of aborted nesting attempts could provide additional insights.

Evidence suggests that nestling mortality is an example of parent-offspring conflict rather than an adaptive response where parents and surviving offspring cooperate to improve individual offspring prospects (reviewed in Nilsson 1995). Higher nestling mortality may be beneficial to Great Tit offspring if provisioning rates per nestling were increased, or if nestling body mass was higher in broods where mortality had occurred. If brood reduction favours adult survival through reduced reproductive effort, and it is an exponent of parent-offspring conflict, I would expect parental effort to be reduced in broods suffering higher mortality. In the latter scenario parents may reduce provisioning effort in order to conserve resources for future reproduction (e.g. Hõrak 1995).

4.5.2. Food supplementation and parental provisioning rates

There was a significant negative relationship between male and female provisioning rate per nestling and brood size in both species. The result of this was that nestlings in larger broods each received fewer feeding visits from parents than those in smaller broods. This reduction of visits per nestling with increasing brood size has been reported in some studies of Great Tits (e.g. Gibb 1950), but not in others (e.g. Kluijver 1950). The smaller broods that had experienced nestling mortality could, therefore, have benefitted from higher provisioning rates per nestling, although mortality was not a significant factor in any of the analyses, possibly due to the high number of broods experiencing no mortality.

There was a differential response in males and females of both species to both natural and supplementary food in terms of the nature of the food type and the direction of the response. Males increased their provisioning rate in response to conditions of higher food availability (i.e. peanut cake), whereas females reduced their provisioning rate in response to higher food availability (i.e. caterpillars). The validity of using provisioning rate to measure parental effort or to make inferences about the nutritional value of food being delivered to the nestlings has been questioned many times (e.g. Royama 1966, Bryant 1988). Provisioning rate is commonly positively correlated with DEE in many species (Bryant & Tatner 1991), however. Therefore, it may represent parental effort to some extent (but see Moreno *et al.* 1995, Verhulst & Tinbergen 1997), even if an increase in DEE does not reflect an increase in the quality of food being delivered to the nestlings. A relationship between prey size and delivery rate is commonly reported, however, but it tends to be negative (e.g. van Balen 1973, Schwagmeyer & Mock 2008).

A reduction in provisioning rate under conditions of higher caterpillar availability may not indicate that a female is investing less effort as she may be bringing fewer items of higher quality (Grieco 2002a) or providing larger caterpillars (Kluijver 1950), indicating more selective foraging potentially with associated energetic costs. Indeed, studies of Great Tits have found that adults will provide food other than caterpillars until the larvae reach a threshold size (e.g. Naef-Daenzer *et al.* 2000). In addition, higher provisioning rates have been recorded in circumstances of lower available caterpillar biomass (van Balen 1973, Naef-Daenzer *et al.* 2000) and lower provisioning rates are associated with a higher proportion of caterpillars being delivered (Wilkin *et al.* 2009a). Increasing caterpillar availability has been associated with a reduction in foraging time (Naef-Daenzer & Keller 1999), however, which could lead to an increase in provisioning rate.

Males provisioning at higher rates in response to food supplementation with peanut cake may, therefore, have been working harder to compensate for low food availability or using the predictable calorie supply in the peanut cake to boost their provisioning performance. Without details of energy expenditure or of prey items delivered, it is difficult to differentiate between such scenarios. Hatch date was not controlled for in the male provisioning models which had a significant positive effect of food supplementation, so although a significant factor, food supplementation might be acting through covariance with hatch date and earlier, smaller broods. It seems unlikely, based on evidence from previous studies (e.g. Royama 1966, van Balen 1973), that the increase in provisioning rate is indicative of better quality provisioning to nestlings, even if parents are working harder. The fact that male provisioning rates were unaffected by caterpillar availability could be a sex-specific response in that males are less likely to reduce their provisioning rate when food availability is higher. Both partners of a pair tend to reduce their provisioning rates when individual costs are experimentally increased, through tail weighting for example (Wright & Cuthill 1989). Fewer examples exist, however, of incidences where foraging costs are reduced to one or both of the provisioning partners.

Previous studies of provisioning rate and food supplementation (e.g. Wiehn & Korpimäki 1997, Dawson & Bortolotti 2002, Eldegard & Sonerud) have found that females, rather than males, were more strongly affected by food supplementation. Studies of longer-lived species reported a decrease in female provisioning rate with supplementation of nestlings in Eurasian Kestrels (*Falco tinnunculus*) and American Kestrels, and Tengmalm's Owls (*Aegolius funereus*), for example. The inverse has been found in food-supplemented shorter-lived species such as the Black Redstart (Cucco & Malacarne 1997) and Stitchbird (*Notiomystis cincta*) (Low *et al.* 2011); females increased their provisioning rate in response to food supplementation, although the former was linked to a corresponding drop in the provisioning rates of the males (Cucco & Malacarne 1997) and the latter to increasing brood size (Low *et al.* 2011). Mock *et al.* (2005) studied House Sparrows and found that provisioning rates of males increased in response to supplementation of nestlings. This was hypothesised as being due to a perceived increase in brood value. Males have also invested less than females to this point in the breeding season (Martin 1987), so they may have more intrinsic resources to raise their provisioning rates, particularly if foraging conditions are improved through food supplementation and, hence, foraging time is reduced (Naef-Daenzer & Keller 1999).

Although in my study the provisioning rates of male and female Blue Tits were positively related to each other, the provisioning rates of Great Tit parents were unrelated. I have already suggested that male Great Tits are influenced by different factors from females when provisioning, and they may not reduce their provisioning rates in times of high food availability. Males and females may also have different priorities at this stage in the breeding cycle, with a different relative value placed on the brood. Great Tits are facultatively double-brooded (Verboven *et al.* 2001), and females could be conserving reproductive effort for a second clutch, hence taking opportunities to reduce their provisioning rate (although see Smith *et al.* 1988 for a lack of connection between second broods and male provisioning rate). Sex-specific variation in responses to heavier workload are common, although whether it is the female (Sanz *et al.* 2000a) or the male (Christe *et al.* 1996) which responds to the increased workloads is not consistent.

4.5.3. Food supplementation and DEE

Although female Great Tits did not significantly increase or decrease their provisioning rate in response to food supplementation with peanut cake or peanut cake and mealworms, nor appear to reduce their provisioning rate in response to increased male contribution, the DEE results provide surprising insights into their behaviour. Female Great Tits supplemented with peanut cake significantly reduced their DEE and there was also a significant negative correlation between female DEE and male provisioning rate. Females appeared to be maintaining a consistent provisioning rate across food supplementation treatments and did not adjust their provisioning rate in response to increasing male provisioning rate. They were, however, making significant energetic savings. Partial compensation game theory models (e.g. Houston & Davies 1985) predict that partners should counter a reduction in effort by one partner by raising their own effort without compensating fully, and reduce their own effort partially when the partner increases effort (Hinde 2006). Under this theory females could be reducing DEE as males increase their provisioning rate in response to a shortage of invertebrate food in early broods.

My results also raise intriguing questions regarding the honesty of the cueing system hypothesised to be used by species employing bi-parental nestling feeding to maintain a consistent provisioning rate to nestlings (Johnstone & Hinde 2006, Meade *et al.* 2011). More recent game theory studies (e.g. Hinde 2006, Johnstone & Hinde 2006, Meade *et al.* 2011) have investigated within-partner contributions as a function of increased brood demand, and suggested that parents cue from the provisioning rate of the partner. These studies interpreted a positive response to increased partner contribution as evidence of information-sharing regarding brood value or brood need. Females could be supplying inferior quality food items or even peanut cake itself to maintain their provisioning rates whilst reducing their energy expenditure, appearing to their partner to be maintaining provisioning rate.

The two previous food supplementation studies which measured DEE found that female European Pied Flycatchers did not lower DEE when food supplemented (Moreno *et al.* 1999) but that Black-Legged Kittiwake parents did lower DEE when supplemented (Jodice *et al.* 2002). In the former study, however, the amount of food supplemented was insufficient to sustain both adults and nestlings, so the decrease in DEE in Kittiwakes and Great Tits in my population could be a reflection of *ad libitum* access to food and so a predictable reduction in foraging costs.

4.5.4. Food supplementation and nestling body mass

Considering that Great Tit broods in the area supplemented with peanut cake were smaller and receiving more feeding visits per nestling than larger unsupplemented broods, it would be expected that the body mass of these nestlings would be higher (e.g. Sanz & Tinbergen 1999). Heavier nestlings usually have better chances of surviving post-fledging (Tinbergen & Boerlijst 1990), and heavier fledglings may have a better chance of surviving to breed (Naef-Daenzer *et al.* 2001, MacColl & Hatchwell 2003), becoming heavier breeding adults (Perrins & McCleery 2001), although this is not necessarily the case (Nur 1984c, Magrath 1991). Schwagmeyer and Mock (2008) found that the body mass of nestlings on day 11 was a good predictor of recruitment in House Sparrows. I would expect that any changes in nestling body mass in my study as a result of food supplementation, increased male provisioning rates or reduced brood sizes, may prove to be important for nestling survival and recruitment. There was no effect of parental provisioning rates in either of the nestling body mass analyses (Table 4.7), or in the analysis of the effect of nestling mortality before ringing age on parental provisioning rates (Table 4.5), so there was no support for direct nestling gains resulting from reduced brood size. There was no effect of food supplementation with peanut cake on Blue Tits, but nestling body mass was significantly positively related to caterpillar availability and negatively related to hatch date, as has been found in other studies of both Great and Blue Tits (e.g. Wilkin *et al.* 2009a, García-Navas & Sanz 2011).

An earlier study of nestling body mass and growth rates within this population found no effect of supplementation with peanut cake (supplied pre-laying, during incubation and brood rearing) or peanut cake and mealworms (supplied post-hatching) on nestling body mass between 2006 and 2008 in both Blue and Great Tits (Harrison 2010). There was, however, a significant negative effect of food supplementation with peanut cake (supplied pre-laying, during incubation and brood rearing) on nestling body mass in 2010 and a significant positive effect of supplementation with peanut cake in 2011 in my study. This could have been due to the fact that caterpillar emergence in 2010 was comparatively late and supplemented Great Tits bred early and were significantly mismatched from peak caterpillar availability (Chapter Two). In contrast, caterpillar emergence in 2011 was comparatively early (Chapter Two) and early breeders would have been at more of an advantage. Although caterpillar availability was not a significant factor in the Great Tit nestling body mass model (Table 4.7), the dichotomy in breeding dates between control and supplemented females in 2010 could have produced such an effect. It is striking that male body condition was significantly positively related to nestling body mass in both Blue and Great Tits, particularly given that male body condition was unrelated to provisioning rates. However, males in higher body condition could have been providing higher quality prey items but not delivering at a faster rate. Further research would be needed to determine the quality of prey being delivered to nestlings in order to ascertain the exact nature of the correlation between food delivery rate, prey size, parental energy expenditure and nestling body mass.

In summary, higher mortality and lower brood size in supplemented Great Tits are likely to result from mistiming of breeding with the availability of invertebrate prey. Food supplementation with peanut cake alone was not able to alleviate the consequences of this phenological mismatch and did not reduce nestling mortality. The provision of a supplement which could be fed directly to nestlings, however, mitigated for the effects of such seasonal mistiming and this was evidenced by reduced nestling mortality and larger brood sizes. Blue Tits did not show the same effect of food supplementation on nestling mortality and brood size as they do not exhibit mistiming between caterpillar availability and peak nestling food demands to the same degree as Great Tits (Chapter Two).

Higher nestling mortality in Great Tit broods could be considered as an adaptive response, in which more resources are allocated to individual offspring under conditions of lower food availability. However, reduced energy expenditure of female Great Tits indicates that brood reduction was a strategy to conserve adult resources. Alternatively, females may have switched food type to deliver a more readily abundant source of nutrition (potentially peanut cake itself) which reduced their foraging costs but did not affect provisioning rate. This could be investigated in future studies by use of Stable Isotope Analysis (SIA) of nestling tissue samples.

Male and female Blue and Great Tits respond to different stimuli when regulating their provisioning rates and males are likely to increase their effort if foraging costs are reduced, whereas females reduce their effort. The parent-offspring conflict was further illustrated by the reduction of nestling body mass in a year of severe phenological mismatch. The honesty of intra-pair signalling or cue interpretation needs to be investigated further, with emphasis on male DEE in response to provisioning rate and food supplementation. Brood manipulation experiments, where parents are compelled to work harder to maintain equivalent provisioning rates to a larger brood, may also provide a clearer picture of partner contribution, and the energetic savings made through food supplementation and strategic brood reduction. The size of prey being delivered also needs to be quantified within an energetics framework to enable a complete interpretation of the correlation between provisioning rates and energy expenditure to be achieved. These represent exciting avenues for future research. However, my results indicate that the interplay between provisioning adults may be even more intricate than theoretical models have assumed.

Chapter Five

Energetic and life history consequences of phenological mismatch as a result of food supplementation: a case study

5.1. Abstract

Food availability is closely linked to life history trajectories in breeding animals, but the effects of natural and anthropogenic food resources on seasonal timing and breeding success in birds remain unclear. Food is now provided for birds in gardens and backyards year-round, but the consequences of this increase in food during the breeding season have not been quantified. During the 2010 breeding season, food supplementation in Chaddesley Woods acted as an ecological trap, causing breeding Great Tits to become phenologically mismatched with natural prey. However, rather than increasing parental effort, females reduced their Daily Energy Expenditure (DEE) in the food-supplemented area and passed the costs of the mismatch onto their offspring in terms of increased nestling mortality and reduced nestling body mass. Lower DEE during the nestling phase was also positively correlated with lower DEE during egg laying. This reduction in parental effort across the reproductive period was linked to a significant increase in adult female survival. This constitutes rare evidence of a shift in life history strategy for a short-lived bird as current reproduction was traded off against future reproduction. Such a novel result provokes intriguing questions regarding how phenotypic plasticity can affect not only breeding parameters but also life history strategies.

5.2. Introduction

One of the key tenets of life history theory is that in the face of limited extrinsic resources there necessarily should exist for the individual a tension between two life history traits: effort allocated to a current reproductive attempt, and that apportioned to future reproductive attempts or survival of the individual (Lessells 1991, Stearns 1992). Trade-offs between these two life history traits are proposed to be intrinsically linked to food availability (van Noordwijk & de Jong 1986, Martin 1987) as resources dictate the physiological cost of reproductive activities (reviewed in Ricklefs & Wikelski 2002). In addition to potentially constraining the number of young an adult can produce (Lack 1947, van Noordwijk *et al.* 1995), food availability may act as a cue to initiate and regulate breeding attempts (Källander & Karlsson 1993, Harrison *et al.* 2010). There exist, however, many unanswered questions regarding how food availability may act to cue the onset of breeding in birds. In particular, there is uncertainty regarding which food items may trigger a breeding attempt (e.g. an investigation into the availability of tree buds Bourgault *et al.* 2009, e.g. an investigation into the role of specific invertebrates te Marvelde 2012), and regarding how specific nutrient availability fuels physiological processes (reviewed in Williams 2005). The provision of anthropogenic food resources to wild birds may interact with natural food availability and miscue females into breeding too early with respect to the availability of resources for feeding young (e.g. Nilsson 1994). This is referred to as ‘phenological mismatch’. Such mismatching of reproductive effort with the necessary availability of resources to achieve reproductive success can have damaging consequences for the survival of both adults and offspring (Norris 1993, Nilsson 1994).

5.2.1. The costs of phenological mismatch

In many seasonally breeding avian species the ability of adults to time their reproductive attempt to a peak in the availability of certain prey species is one of the primary determinants of their reproductive success (e.g. van Noordwijk *et al.* 1995, Cresswell & McCleery 2003, Schultz *et al.* 2009). The accuracy of this timing is under increasing selection pressure, however, due to phenological shifts in lower trophic levels as a result of rapid climate change (Visser & Both 2005, Both *et al.* 2009) and can lead to a phenological mismatch or disjunction between the timing of breeding in the birds and peak in availability of their preferred prey (Visser *et al.* 1998). The long-established seasonal decline in reproductive productivity (Perrins 1970) has become more acute as a result, leading to increased selection for early laying and much greater breeding success in early breeders (Verboven & Visser 1998). Selection is also favouring phenotypic plasticity in timing of breeding of birds (Nussey *et al.* 2005) with the phenotypes most able to respond to rapid environmental changes proliferating in the population. Food supplementation can affect not only the date at which birds initiate laying (Svensson 1995), but it also reduces the phenotypic plasticity of responses to natural phenological cues (Chapter Two). It is critical, therefore, to understand both the physiological and life history consequences of the interaction between food availability and timing of breeding to be able to predict avian responses to environmental change (te Marvelde *et al.* 2011c).

5.2.2. Food supplementation and seasonal timing

There is a marked paucity of food supplementation studies that examine how anthropogenic food resources may affect the seasonal timing, reproductive physiology and long-term fitness of breeding adult birds. Such a lack of information is particularly important in the face of equivocal results from previous food supplementation studies (reviewed in Boutin 1990, Robb *et al.* 2008b) and the prevalence of provision of food for birds in gardens and backyards (Chapter One). Food supplementation has been demonstrated to affect not only the accuracy of seasonal timing in breeding Great Tits, but also the plasticity of their phenotypic response to fluctuating seasonal food resources (Chapter Two). Mistimed breeding may be energetically (te Marvelde *et al.* 2011b) and reproductively costly (van Noordwijk *et al.* 1995), and adults whose timing of breeding is mismatched from the seasonal peaks in food abundance, that are necessary for provisioning their chicks, may exhibit lower survival to the next breeding season (Verhulst *et al.* 1995). With the provision of supplementary foods to birds in gardens and backyards becoming increasingly popular (Chapter One, Lin 2005), a widespread reduction in the accuracy of seasonal timing could have profound demographic consequences in terms of lower adult survival or reduced offspring recruitment.

5.2.3. Reproductive energetics and life history trade-offs

The brood-rearing phase is one of the most energetically costly periods in the avian annual cycle, both for long-lived species such as Macaroni Penguins (*Eudyptes chrysolophus*) (Green *et al.* 2009) and short-lived species such as Great Tits (Nilsson & Råberg 2001), in spite of their opposing life history strategies. Providing food for larger brood sizes may incur higher energetic costs for adults (Deerenberg *et al.* 1995), with downstream life history consequences for future adult survival (Reid 1987, Daan *et al.* 1996).

The timing of reproduction has been demonstrated to be important for the energy expenditure of breeding female Great Tits, with those synchronized to natural food availability paying lower energetic costs to feed nestlings than mismatched females (te

Marvelde *et al.* 2011b). Energetic strategies during the brood rearing period normally consist of lowering Daily Energy Expenditure (DEE) in response to high food availability as is the case for Great Tits (Tinbergen & Dietz 1994). The relationship may be reversed in opportunistic aerial foragers such as House Martins (*Delichon urbica*) that increase DEE in response to short windows of beneficial weather conditions and higher food availability (Bryant & Westerterp 1983b). DEE could be forcibly elevated under conditions of low food availability (the 'forcing hypothesis' – Welcker *et al.* 2009), or enabled to rise higher without incurring additional life history costs (the ‘enabling hypothesis’ – Bryant & Westerterp 1983b).

Many studies (reviewed in Dijkstra *et al.* 1990) have been conducted to investigate the downstream costs of reproductive effort for adult survival and future fecundity (known as residual reproductive value, Williams 1966). However, many of these studies do not find a correlation between increased brood sizes and reduced residual reproductive value (reviewed in Golet *et al.* 1998). This is particularly true of short-lived species with large brood sizes such as Willow Tits (*Parus montanus*) (e.g. Orell & Koivula 1988), Blue Tits (e.g. Pettifor 1993) and Great Tits (e.g. Tinbergen & Verhulst 2000), where experiments that have increased brood or clutch size have found no costs to adult survival or future fecundity. Other brood manipulation studies have found a *negative* effect on future adult survival or future fecundity associated with an increase in parental effort, achieved by increasing clutch size, in short-lived species (Gustafsson & Sutherland 1988, de Heij *et al.* 2006). However, unequivocal empirical evidence for a *positive* trade-off between low parental investment in the current reproductive attempt and increased adult survival are rare (but see Dijkstra *et al.* 1990, Hõrak 1995). Results from brood manipulations, however, have been called into question, because the parental response to an artificial manipulation of brood size may not be an optimal one (Lessells 1993).

Highly fecund shorter-lived species such as Great Tits are predicted to invest strongly in the current reproductive attempt because their annual adult mortality is high (Stearns 1977, Gosler 1993). Indeed, studies show that when reproductive costs are raised, the parents increase their energetic investment to meet the demands of the offspring, potentially sacrificing residual reproductive value (Sanz 1997, Sanz & Tinbergen 1999). There is some evidence that parents will also decrease reproductive effort if their workload is experimentally reduced (Smith *et al.* 1988, Verhulst & Tinbergen 1997), although this has rarely been linked to increased inter-annual residual reproductive value in short-lived birds. The ease with which food resources can be assimilated underlies all reproductive costs (Martin 1987), and quantifying the ecological backdrop against which life history trade-offs may occur is critical to understanding the costs and benefits of reproduction (Sibly & Calow 1986).

5.2.4 Food supplementation and DEE study

In order to examine the relationship between food availability, adult energetic and reproductive investment and life history trade-offs associated with parental effort, I conducted a food supplementation study using Great Tits during the 2010 breeding season. I measured adult female DEE using the DLW method during the brood-rearing phase. Breeding pairs were provided with a food supplement in the form of peanut cake from pre-nesting to post-fledging, as this should have represented reduced foraging costs for the whole reproductive period. Provisioning rates were measured using RFID technology in the form of PIT tags. Provisioning rates, and the simultaneously recorded DEE measures, were used to ascertain the relationship between energy expenditure and feeding rates in the female and the feeding rates of her partner. There was a significant mismatch in the food-supplemented area between the timing of the peak in caterpillar availability and that of the period of peak nestling demand at around 10 days old, with females breeding up to 15 days too early (Chapter 2). I assumed that pairs breeding in the supplemented area would have to work harder to meet the demands of their nestlings as the caterpillars were still too small to meet the nutritional demands of the brood. Therefore, I predicted that females in the supplemented area would exhibit higher rates of DEE and lower provisioning rates as they worked harder to find prey for the nestlings. I also predicted that adult survival would be affected as per life history trade-off theory, and that fewer females from the supplemented area would return to breed in 2011.

5.3. Methods

5.3.1. Nestbox study

Please refer to Chapters Two and Four for details of routine monitoring of nestboxes and adult and nestling capture in 2010.

5.3.2. Food supplementation

Please refer to Chapter Two for details of the food supplementation regime. Woodland areas were unsupplemented (Control, CON) or supplemented with peanut cake (PC) from pre-laying to post-fledging in 2010.

5.3.3. Estimating timing of peak caterpillar biomass

Please refer to Chapter Two for details of the methodology used to measure caterpillar biomass and determine timing of the seasonal peak in biomass.

5.3.4. Measurement of female and male provisioning rates

Please refer to Chapter Four for details of how provisioning rate data were obtained and subsequently processed. Due to logistical problems with the PIT tag readers at seven nestboxes and missing data for male provisioning rates at eight nestboxes, there were 15 nestboxes excluded from analyses including provisioning rate data.

5.3.5. Measurement of female DEE

Please see Chapter Four for a detailed explanation of how measures of DEE were obtained from females provisioning nestlings in 2010, and Chapter Three for the methodology used to obtain measures of DEE during egg laying. DEE estimates were obtained for a total of 47 females, 21 breeding in the unsupplemented area and 26 breeding in the supplemented area. For analyses including provisioning rate, data from the 15 females without provisioning rates for both partners and two females with outlying DEE values (probably as a result of analysis processing error due to low blood volume sample size) (Speakman 1997) were excluded, giving a total of 13 females in the unsupplemented area and 17 in the supplemented area.

5.3.6. Measurement of ambient temperature

For details of ambient temperature measurements conducted during the DLW sampling period using Thermochron® DS1921G iButtons®, please see Chapter Three.

5.3.7. Statistical analyses and data filtering

Only first breeding attempts were included in the DLW sampling protocol. Either the mismatch from the caterpillar peak (defined as the half-fall date – see Chapter Two for details) in days, or the absolute mass of caterpillar biomass in grammes from the woodland block was used as a proxy for caterpillar availability. The choice of which of these two variables was used depended on whether hatch date needed to be tested separately in the model. Provisioning rates and female DEE were only examined if provisioning rate data for both parents were available unless otherwise specified. Nestling mortality was defined as the number of nestlings dying before ringing age (at nestling day 11) and was calculated by subtracting the number of surviving young from clutch size, minus any un-hatched eggs. Brood size was defined as the number of nestlings alive at ringing age (at nestling day 11). Female and male body conditions were calculated by using the residuals of the regression of body mass on tarsus length for each sex.

All statistical analyses were conducted using Generalized Linear Models (GLM – glm function) in R version 2.13.1 (R Development Core Team 2011). All response variables were tested for normality using the Shapiro-Wilk test for normality (Crawley 2007) and then checked against a Poisson error structure using a diagnostic distribution plot. All response variables were then checked for under- and overdispersion and corrected where necessary using a quasi-likelihood error structure (Crawley 2005, Zuur *et al.* 2009a). Collinearity was checked in all models using Variance Inflation Factors (VIF).

All interactions between explanatory variables were tested using backward stepwise regression to find the minimal adequate model, and quadratic terms tested where appropriate. This model simplification was conducted using Chi-squared (i.e. Poisson or binomial distribution) or F tests (i.e. normal distribution) to compare the residual deviance of models with and without terms and interactions (Crawley 2007). An alpha level of 0.05 was used throughout. Pseudo R^2 values for GLMs were calculated from the proportion of residual deviance in the model.

Due to high multicollinearity between certain covariates, identified using VIFs (Zuur *et al.* 2009b), GLM model output was supplemented by conducting Hierarchical Partitioning tests which determine the proportion of variance explained by each of the main order effects, independently of each other (Chevan & Sutherland 1991, Jiguet *et al.* 2010). Results are presented for analyses where the hierarchical partitioning differed from GLM output. Significance and Z score values were gained by running 100 replications of a hierarchical partitioning randomization test with program rand.hp in the hier.part package for R.

5.4. Results

5.4.1. Food supplementation and phenological mismatch

Food supplementation led to a significant advance of laying date of female Great Tits, in 2010 (Table 2.1), with supplemented birds initiating laying five days earlier than control females. This advance resulted in a significant mismatch between the peak in nestling demand (at nestling day 10) and the peak in the caterpillar availability (Fig. 5.1 Table 5.1).

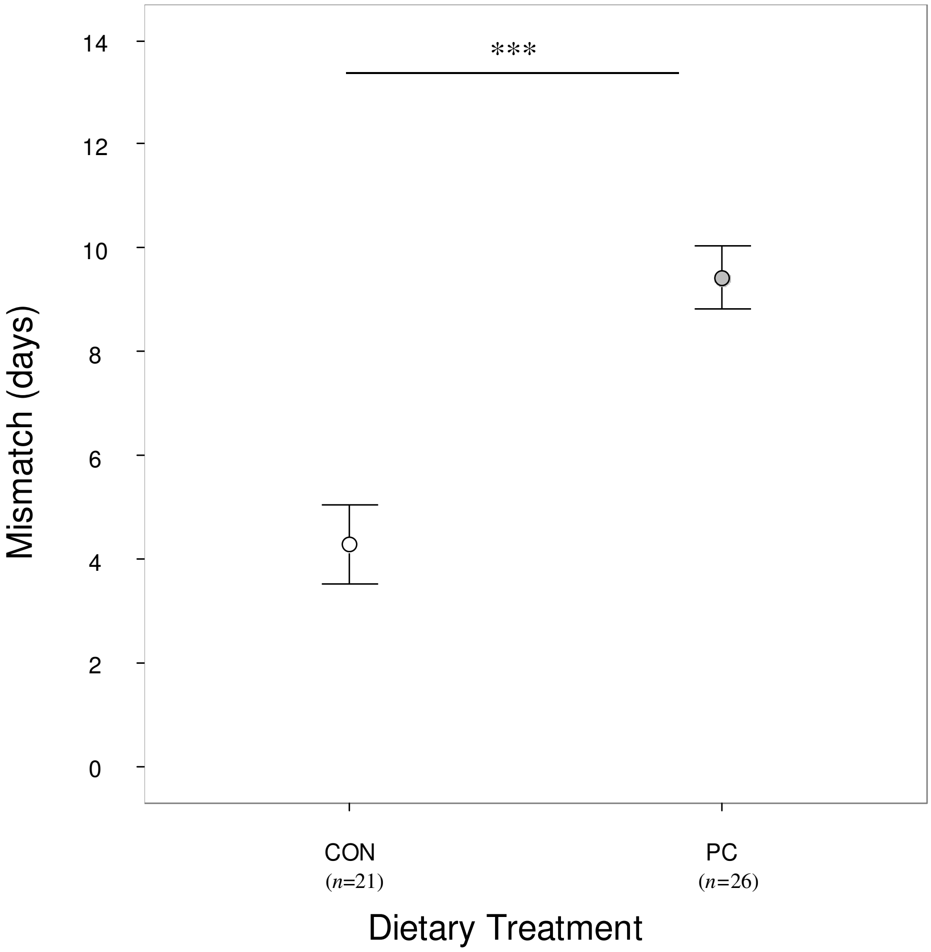

Figure 5.1. Mismatch (mean ± 1 SE from raw data) between date of peak nestling demand (nestling day 10) and peak in caterpillar availability of female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010. Females breeding in the control treatment area (CON) are represented by the open circle and those in the peanut cake treatment area (PC) by the grey-filled circle. *** = $P < 0.001$. Number of broods is shown in parentheses below each axis label. See Table 5.1 for statistical tests and text for details.

Table 5.1. Results from simplified GLM analysis of specified breeding parameters (Response) of pairs of Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 regressed upon significant explanatory variables (Fixed Effect). Chi-squared results from tests (Chisq) detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo R^2 value is presented. For details of statistical tests applied see section 5.3.7.

Response	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	**R^2**
Mismatch	Intercept	10.475	0.333					0.41
	Dietary Treatment	19.044	0.140	1,46	45.2	**< 0.001**	Higher mismatch	
	Clutch Size	-0.925	0.033	1,46	7.9	**< 0.01**	Lower mismatch	
Nestling Mortality	Intercept	0.097	0.617					0.34
	Dietary Treatment	0.359	0.309	1,46	21.5	**< 0.001**	Higher mortality	
	Number Hatched	0.025	0.064	1,46	7.6	**< 0.001**	Higher mortality	
Brood Size	Intercept	9.037	0.115					0.42
	Mismatch	-0.454	0.044	1,46	14.8	**< 0.001**	Lower brood size	
	Nestling Mortality	-0.718	0.015	1,46	3.8	0.06	Lower brood size	
Female DEE	Intercept	95.574	0.037					0.63
	Dietary Treatment	84.953	0.024	1,29	9.9	**< 0.001**	Lower DEE	
	Female Body Condition	4.739	0.016	1,29	3.1	**< 0.01**	Higher DEE	
	Female Provisioning Rate	0.681	0.002	1,29	6.9	**< 0.001**	Higher DEE	
Female DEE (including egg laying DEE)	Intercept	65.110	0.158					0.74
	Dietary Treatment	54.909	0.029	1,19	14.7	**< 0.001**	Lower DEE	
	Egg DEE	0.455	0.002	1,19	7.4	**< 0.01**	Higher DEE	
Nestling Body Mass	Intercept	17.383						0.17
	Brood Size	-0.161	0.074	1,46	0.7	**< 0.05**	Lower mass	
	Dietary Treatment	-1.004	0.345	1,46	8.7	**< 0.01**	Lower mass	
Female Survival	Dietary Treatment	2.342	0.833	1,32	9.4	**< 0.01**	Higher survival	0.21

Tested fixed effects varied for each response variable but all analyses tested for dietary treatment (Intercept = control, ‘PC’ = peanut cake). Additional effects tested were laying date, clutch size, number of hatched young, nestling mortality, hatch date, brood size, female and male age, female and male body condition, mismatch from peak in caterpillar availability, ambient temperature, female DEE (for nestling body mass and female survival models) and female and male provisioning rates. Analyses were corrected for overdispersion and a Poisson or normal error distribution was used.

Females breeding in the supplemented area were significantly more mismatched with the peak in caterpillar availability than control females (Fig. 5.1, Table 5.1). The mismatch from the caterpillar peak declined with increasing clutch size (Table 5.1). There was a significant positive effect of dietary treatment on nestling mortality (Fig. 5.2, Table 5.1), and nestling mortality increased with brood size (Table 5.1) but not with increasing mismatch from peak caterpillar availability (Table 5.1).

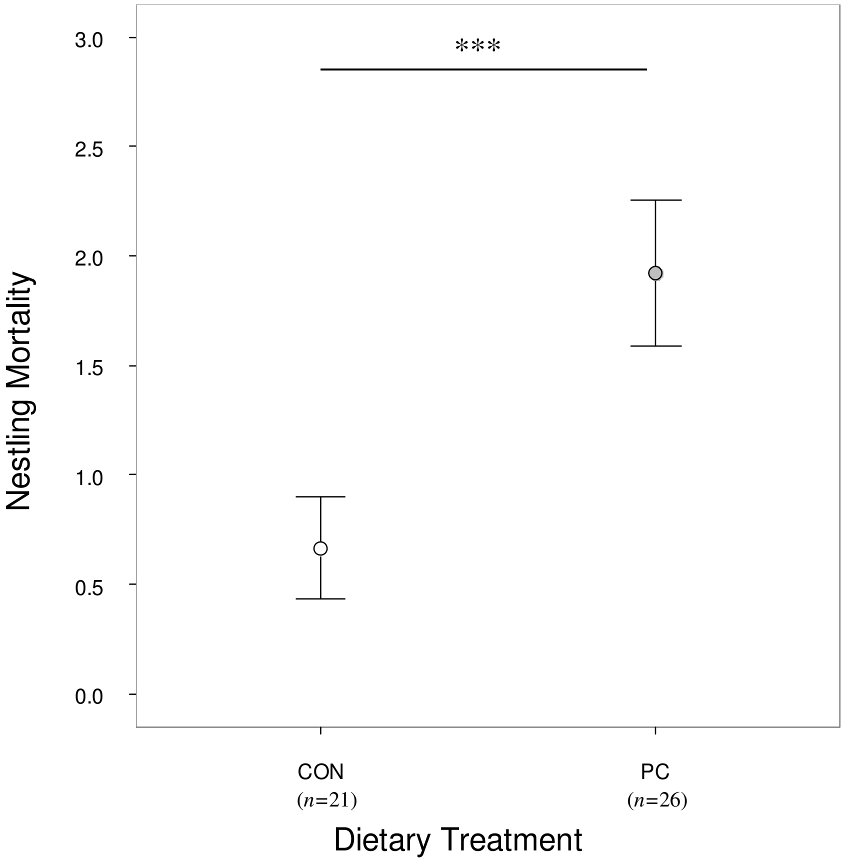

Figure 5.2. Nestling mortality (mean ± 1 SE from raw data) before nestling day 10 of female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010. Females breeding in the control treatment area (CON) are represented by the open circle and those in the peanut cake treatment area (PC) by the grey-filled circle. *** = $P < 0.001$. Number of broods is shown in parentheses below each axis label. See Table 5.1 for statistical tests and text for details.

The mismatch from peak caterpillar availability was then associated with lower brood sizes (Table 5.1), and although nestling mortality was included in the final model, it was approaching significance, rather than having a strong effect (Table 5.1, Fig. 5.3).

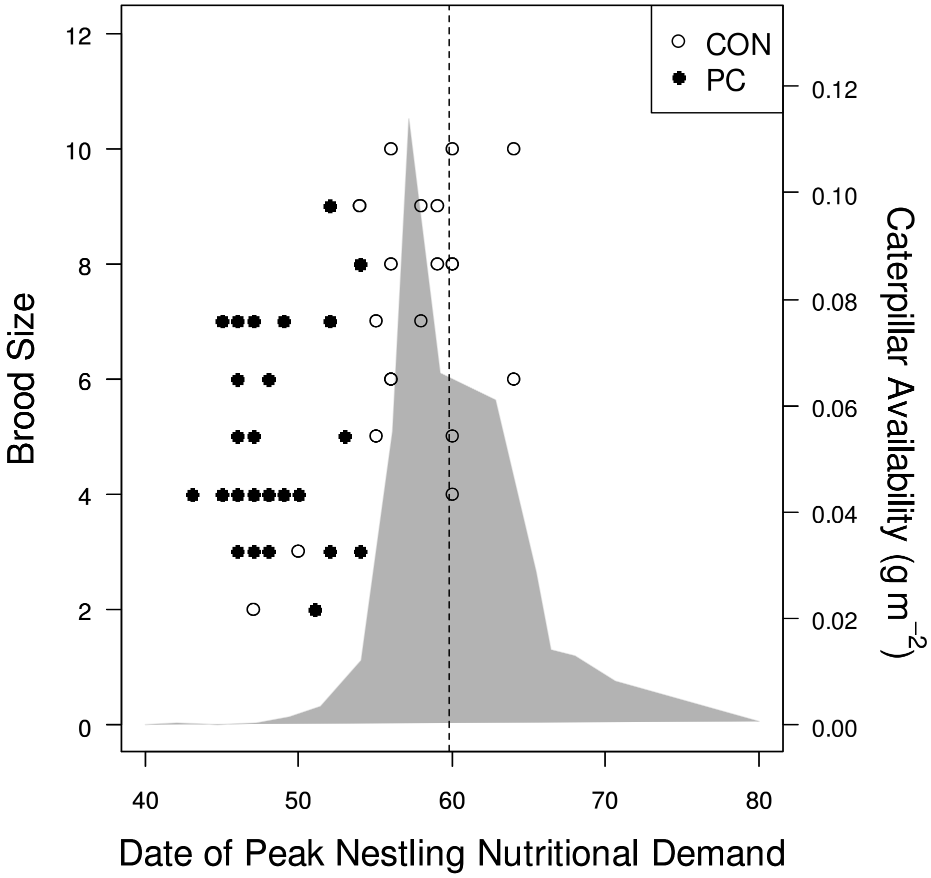

Figure 5.3. The significant relationship between brood size and matching to peak caterpillar availability in unsupplemented Control (CON, open circles) and supplemented (PC, filled circles) female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 showing an increase in brood size with more accurate matching to the peak in caterpillar availability. Date is in April Days where 1 = 1st April. The shaded area represents the mean daily dried caterpillar biomass in grammes as a function of date, and the dashed line represents the caterpillar half-fall date. See Tables 5.1 and 5.2 for statistical tests and text for details.

Table 5.2. Results from hierarchical partitioning analysis of specified breeding parameters (Response) of pairs of Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 in relation to significant explanatory variables (Fixed Effect). *Z* Scores, significance (either significant '*' [$P < 0.05$] or non-significant) and the proportion of the explained variance attributable to each fixed effect (Explained Variance) were calculated from randomisation tests. For details of statistical tests applied see section 5.3.7.

Response	Fixed Effect	*Z* Score	Explained variance (%)	Significance	Direction
Brood Size	Dietary Treatment	4.07	27	*	Lower brood size in PC area
	Mismatch	7.62	49	*	Lower brood size
	Nestling Mortality	2.11	20	*	Lower brood size
Female DEE	Dietary Treatment	4.80	20	*	Lower DEE in PC area
	Caterpillar Availability	2.11	14	*	Higher DEE
	Female Body Condition	3.17	11	*	Higher DEE
	Female Provisioning Rate	2.00	21	*	Higher DEE
Female DEE	Dietary Treatment	3.80	56	*	Lower DEE in PC area
(including egg laying DEE)	Caterpillar Availability	4.44	64	*	Higher DEE
	Egg DEE	2.03	25	*	Higher DEE

Tested fixed effects were dietary treatment, mismatch and nestling mortality for brood size analysis and dietary treatment, caterpillar availability, DEE during egg laying, hatch date, brood size, female provisioning rate, male provisioning rate, female and male age, and ambient temperature for female DEE analysis.

Due to high multicollinearity, dietary treatment and phenological mismatch could not both be retained within the brood size model. Hierarchical partitioning analysis (Table 5.2) demonstrated that both food supplementation treatment and mismatch from peak caterpillar biomass were significant when considered independently of one another, although the mismatch from peak caterpillar availability explained more of the variance.

5.4.2. Food supplementation and Daily Energy Expenditure

Females in the supplemented area exhibited significantly lower DEE than control females, expending 12.1kJ less energy per day than unsupplemented females which equated to a reduction of 12% of their total DEE (Fig 5.4, Table 5.1). Although dietary treatment seemed to be confounded with brood size and the timing to peak caterpillar availability

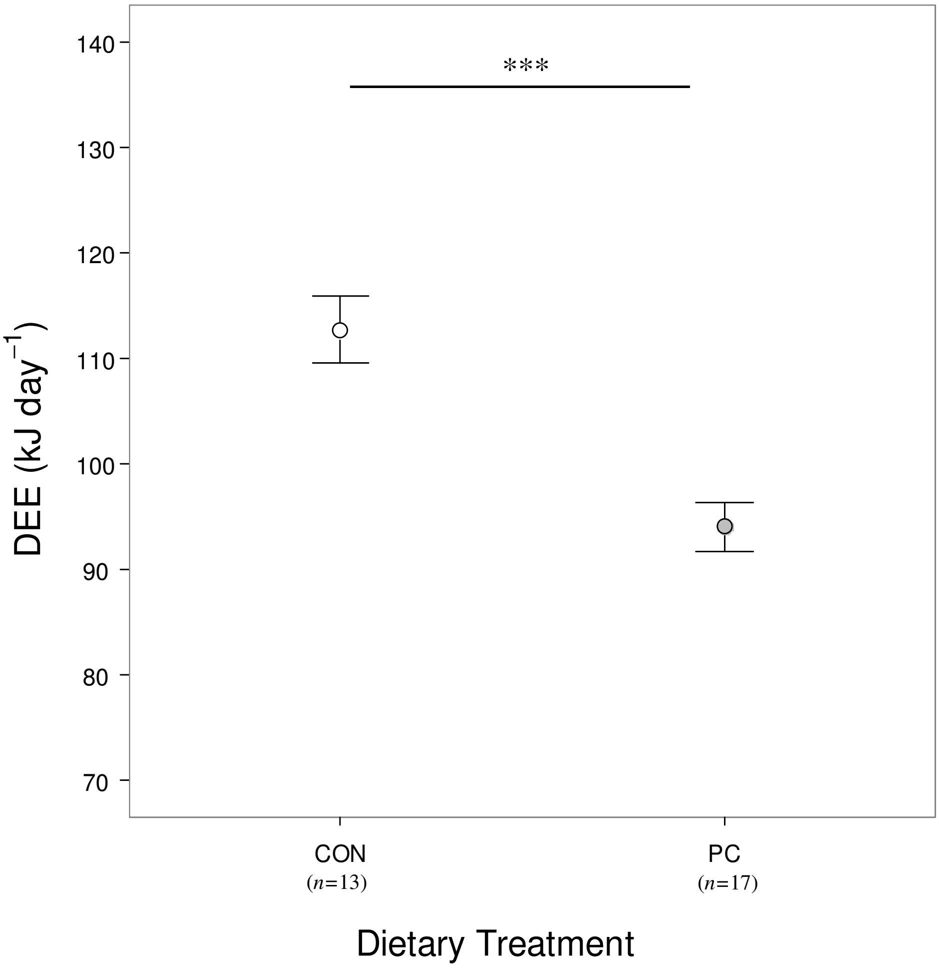

Figure 5.4. Daily Energy Expenditure (DEE) (mean ± 1 SE from raw data) of female Great Tits provisioning nestlings in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010. Females breeding in the control treatment area (CON) are represented by the open circle and those in the peanut cake treatment area (PC) by the grey-filled circle. *** = $P < 0.001$. Number of broods is shown in parentheses below each axis label. See Table 5.1 for statistical tests and text for details.

(Table 5.1), partitioning the variance with hierarchical partitioning demonstrated that both dietary treatment and caterpillar availability had significant effects on female DEE independently of one another (Table 5.2). Food supplementation treatment was negatively related, and caterpillar availability positively related, to female DEE (Fig. 5.4, Table 5.2). Brood size was not a significant predictor in the GLM or hierarchical partitioning analyses, neither were ambient temperature, male provisioning rate or male body condition. Female provisioning rate was significantly positively correlated with female DEE (Fig. 5.5,

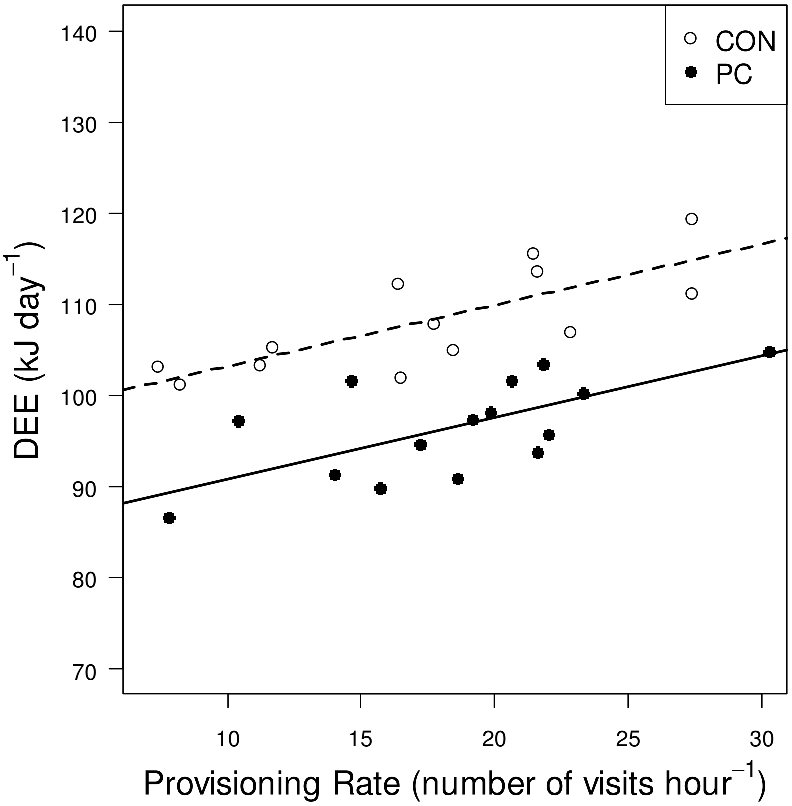

Figure 5.5. Daily Energy Expenditure (DEE) (from model predictions) of female Great Tits provisioning nestlings in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 in relation to mean hourly provisioning rate. Females breeding in the control treatment area (CON) are represented by the open circles and those in the peanut cake treatment area (PC) by the black-filled circles. Regression lines are from linear models of predicted data. See Tables 5.1 and 5.2 for statistical tests and text for details.

Tables 5.1 and 5.2), as was female body condition (Tables 5.1 and 5.2). Females provisioning at higher rates and those in higher body condition expended more energy. Although there was no significant interaction between food supplementation treatment and female provisioning rate in the final model, predictions from the model suggested that supplemented females were expending less energy to achieve a comparable provisioning rate (Fig. 5.5).

Measures of energy expenditure during egg laying were obtained for a subset of the sample, and DEE during egg laying proved to be a significant positive predictor of DEE during brood rearing (Figs 5.6 and 5.7, Table 5.1). Dietary treatment was the only other significant variable in this analysis, having a negative effect on DEE (Table 5.1). In terms of multiples of BMR, the control birds were expending energy at a mean of 3.98 × BMR

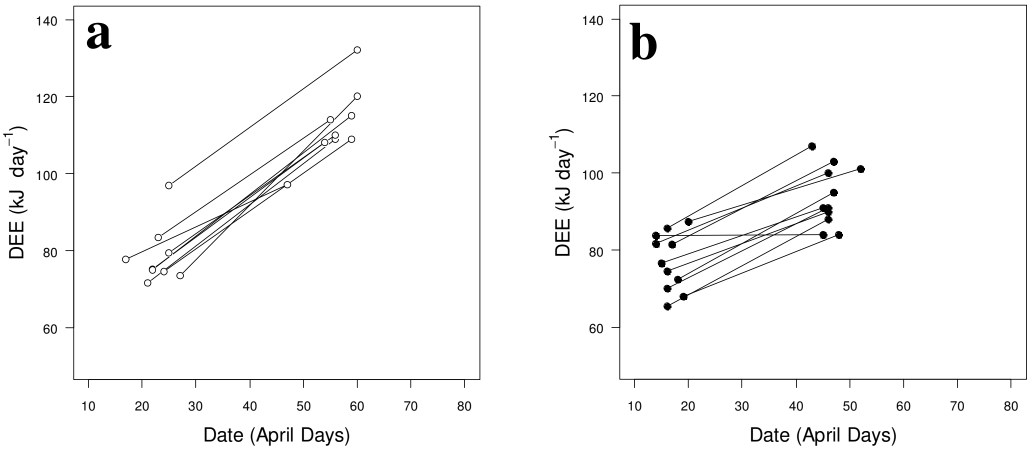

Figure 5.6. Significant relationship between Daily Energy Expenditure (DEE) during egg laying and brood rearing of (a) unsupplemented Control (open circles) and (b) supplemented (filled circles) Great Tit females breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 in relation to date in April days where 1 = 1st April. Data show an increase in DEE between the two phases. See Tables 5.1 and 5.2 for statistical tests and text for details.

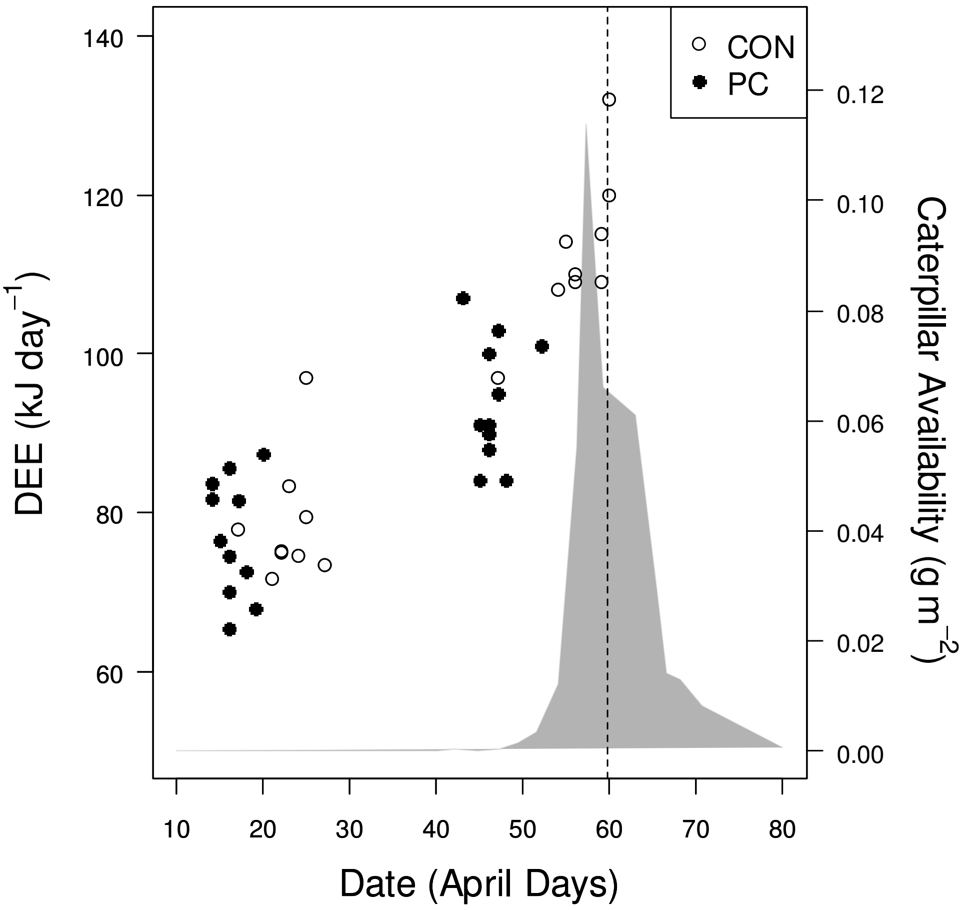

Figure 5.7. Significant relationship between Daily Energy Expenditure (DEE) during egg laying and brood rearing of unsupplemented Control (CON, open circles) and supplemented (PC, filled circles) female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 in relation to accuracy of timing to the peak in caterpillar biomass. Date is in April days, where 1 = 1st April. Data show an increase in DEE between the two phases. The shaded area represents the mean daily dried caterpillar biomass in grammes as a function of date, and the dashed line represents the caterpillar half-fall date. See Tables 5.1 and 5.2 for statistical tests and text for details.

(± 0.07 [1 SE], range: 3.50 – 4.41) and the supplemented birds were expending energy at a mean of 3.5 × BMR (± 0.04 [1 SE], range: 3.02 – 4.34).

5.4.3. Food supplementation and nestling mass

Mean body mass of 11 day-old nestlings was significantly negatively related to dietary treatment and brood size (Fig. 4.11, Table 5.1). The nestlings in the supplemented area were on average 1 g (i.e. 6%) lighter than those in the unsupplemented area (Table 5.1).

5.4.4. Female survival to 2011 breeding season

There were significantly more females from the peanut cake supplemented area observed breeding in 2011 than from the control area (Fig. 5.8, Table 5.1). Females were 2.3 times

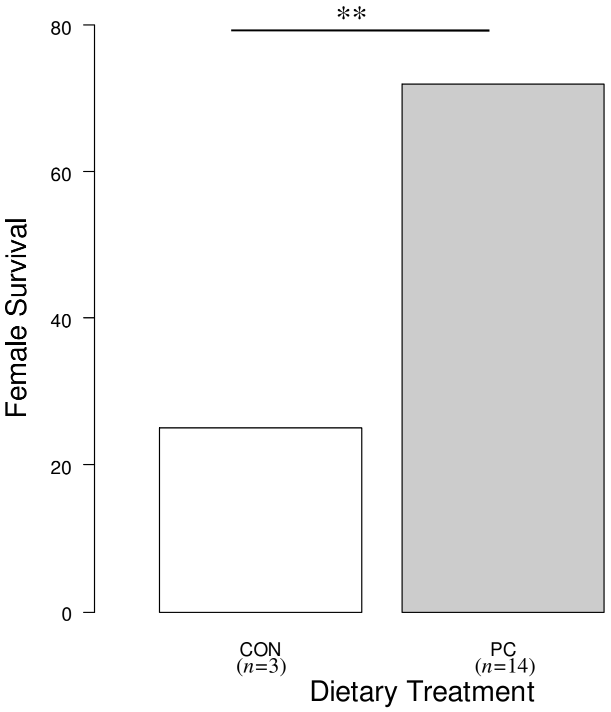

Figure 5.8. Survival probability (%) of female Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 that bred in 2011 in relation to dietary treatment. Females breeding in the unsupplemented area (CON) are represented by the open bar and those in the supplemented area (PC) by the grey-filled bar. ** = *P* <0.01. Number of surviving females is shown in parentheses below each axis label. See Tables 5.1 and 5.2 for statistical tests and text for details.

more likely to survive to breed the following year in the supplemented area than in the unsupplemented area (Table 5.1). Dietary treatment was the only variable which was significant in the female survival GLM analysis and female survival could not be linked to DEE, brood size, provisioning rates, the mismatch from peak caterpillar biomass, female body condition or nestling mortality.

5.5. Discussion

The primary purpose of this food supplementation study was to explore phenological consequences for Great Tits of increased food availability, which potentially mitigated for both the energetic and reproductive costs of the whole breeding period from pre-laying to post-fledging. The extent of the difference in timing of breeding between the two treatment groups in 2010 acted as an apotheosis of previous findings from my Chaddesley Woods study (Chapters Two, Three and Four). This provided an opportunity to examine the mechanisms behind seasonal timing of reproduction and the physiological and life history effects thereof. In 2010 food supplementation advanced the laying date of female Great Tits to the degree that a severe phenological mismatch between peak prey availability and maximum nestling demand occurred (Fig. 5.1, Table 5.1). Although the mismatch from prey phenology could not be linked directly to nestling mortality, there was a highly significant difference in nestling mortality between the two food supplementation treatment groups (Fig. 5.2, Table 5.1), with higher nestling mortality in the food-supplemented area. This led to significantly smaller brood sizes (Fig. 5.3, Table 5.1). Contrary to expectations of maximal reproductive investment from a short-lived breeding bird (Stearns 1977), females in the supplemented area exhibited lower DEE in response to food supplementation and increasing mismatch from the caterpillar peak (Fig. 5.4, Tables 5.1 and 5.2). DEE was positively correlated with female provisioning rate (Tables 5.1 and 5.2) and females in the supplemented area appeared able to maintain equal provisioning rates at lower energetic cost (Fig. 5.5). Female DEE during brood rearing was positively correlated with female DEE during egg laying and significantly lower in the supplemented treatment group in both reproductive phases (Figs 5.6 and 5.7, Table 5.2, Chapter Three). Nestling body mass was significantly lower in the broods in the supplemented area (Table 5.1) and females from the food-supplemented treatment area were twice as likely to survive to breed in 2011 as unsupplemented females (Fig. 5.8, Table 5.1).

5.5.1. Food supplementation and phenological mismatches

Early breeding usually confers a selective advantage for the offspring of Great Tits (e.g. Perrins 1970, Verboven & Visser 1998, Visser & Verboven 1999). Early breeding in 2010 led to a severe phenological mismatch, however, as it was a comparatively late year phenologically (Chapter Two). Although this mismatch was associated with significantly smaller brood sizes, it could not be linked directly to nestling mortality. Between-pair variance in nestling mortality has been shown to increase in years when the number of successful broods is reduced (Henderson & Hart 1993), indicating that individual pair ability may play a significant role in adapting to changing environmental conditions. Finding a unifying contributory factor may not be possible, therefore, and it could be difficult to relate it empirically to a continuous variable such as phenological matching. Such penalties of early breeding are not commonly reported (but see Norris 1993) and, indeed, only rarely in response to food supplementation (e.g. Clamens & Isenmann 1989, Nilsson 1994, Schoech & Bowman 2001).

The mismatch exacerbated an already reduced reproductive output due to lower clutch sizes in areas supplemented with peanut cake (Chapter Three) by increasing nestling mortality, presumably because parents were obliged to forage under suboptimal conditions. Having initiated laying earlier, supplemented females could have maximised their fitness within this reproductive attempt by laying a larger clutch (Cresswell & McCleery 2003). Larger clutches would have been beneficial to delay their mistimed reproductive attempt and improve synchronization, as the additional days required to lay extra eggs would have delayed their hatch date and resulted in the maximum nutritional demand of their nestlings being better timed to the peak in caterpillar availability. Additional eggs would have also increased their chances of offspring recruitment from their larger brood (Boyce & Perrins 1987). The fact that females did not increase clutch size is contrary to theoretical predictions for short-lived 'income breeders' (Drent & Daan 1980) which are expected to maximise their investment in the current reproductive attempt (Stearns 1977, 1992). Selection pressure on early laying could be higher in the Chaddesley population than in other similar Great Tit populations where selection for early breeding is easing off as population mean laying dates have advanced (e.g. Wytham Woods, Oxford, UK – Cresswell & McCleery 2003). The advantage to offspring recruitment of hatching one day earlier could, therefore, substantially outweigh the selective advantage of having one extra nestling in the brood (Pettifor *et al.* 1988, Chapter Two).

The fact that Great Tits are facultatively double-brooded in certain populations (although not in UK populations - reviewed in Verboven *et al.* 2001) is also important, even if incidences of second broods are exceptionally rare in the current study population (Harrison 2010). Indeed second broods are also rare in other UK populations, where second broods have declined as laying dates have advanced in response to warming spring temperatures (Visser *et al.* 2003, Husby *et al.* 2009). The behavioural mechanisms and responses may still exist to invoke a second breeding attempt, or to reduce investment in the current attempt if the chance of a second attempt exists (Verhulst *et al.* 1997a) even if ecological conditions later in the season are prohibitive. Great Tits may still be predisposed to breed as early as possible in case the opportunity to re-nest within the same season arises. The most likely explanation for the degree of mistiming would be that Great Tits have miscued the timing of their breeding attempts following food supplementation (Chapter Two).

5.5.2. Adaptive brood reduction

Brood reduction has been suggested to be an adaptive reaction to unreliable food resources (Stenning 1996). Parents could increase the survival chances of individual offspring by reducing offspring number and dedicating a larger share to each nestling (Gibb 1955, Royama 1966), or increase their own survival chances by reducing costly reproductive investment (Hõrak 1995, 2003). Parents may be unable to avoid brood reduction, however, as has been seen in a food reduction study (Pascual & Peris 1992), where high nestling mortality was related to low food availability in Blue Tits breeding in a woodland impoverished of caterpillars. There is a relationship between high brood mortality in Eurasian Kestrels and poor food availability, and adults breed relatively late in these years (Daan *et al.* 1990). There was significantly lower total caterpillar biomass collected in 2010 (Fig. 2.12), but the daily average biomass did not vary. It is possible that Great Tits used the food supplement as a qualitative cue to predict the quality of food later in the breeding season and adjusted the number of offspring they produced accordingly (Chapter

Three). Despite 2010 not producing the lowest clutch sizes across all the years of the study (Chapter Three), it did yield the lowest brood sizes. Given these circumstances, it seems likely that the nestling mortality was a combined result of the female mistiming of her breeding, and reduced clutch size which was a result of a misconstrued qualitative cue, rather than an indication that natural arthropod food resources were poor across the entire population.

The food supplement could theoretically have compensated the mismatched pairs for the lack of natural arthropod food available for self-maintenance or for brood provisioning (Schoech *et al.* 2008), potentially providing an emergency food resource to supplement the natural food being provided to the brood. The females did not appear to channel the resources from food supplementation into fuelling their reproductive attempt, however, and the costs of the mismatch were passed on to the offspring in terms of higher nestling mortality and lower brood size (Hõrak 1995).

5.5.3. Energetic consequences of phenological mismatch

In complete contrast to my predictions and to the results of similar studies (e.g. Thomas *et al.* 2001, te Marvelde *et al.* 2011b), the mismatched females in the food-supplemented area lowered DEE, rather than elevating it under conditions of lower caterpillar availability. Both food supplementation and the mismatch from peak caterpillar availability had significant independent effects, resulting in lower female DEE (Table 5.2). In common with many other studies (e.g. Hails & Bryant 1979, Bryant 1988, Tatner 1990, Tinbergen & Dietz 1994, Verhulst & Tinbergen 1997), DEE was not related to brood size, although this was in contrast to some brood enlargement studies (e.g. Sanz *et al.* 1998, Nilsson 2002). Therefore, it was not through the mechanism of reduced brood size that these energetic savings were made, but an effect of seasonal timing and food supplementation. Accurate phenological matching to the peak in caterpillar ability or that of other prey has previously been related to *lower* DEE compared with mismatched conspecifics in Great Tits (Tinbergen & Dietz 1994, te Marvelde *et al.* 2011b) and in a range of other species (reviewed in Bryant & Tatner 1991). This indicates that more plentiful natural food resources should reduce the energetic costs of provisioning nestlings to adults.

The observed energy expenditure expressed as multiples of BMR (4.0 × BMR for control females and 3.5 × BMR for supplemented females) was below the predicted energetic ceiling of 4 × BMR, beyond which parents are unable or unwilling to work (Drent & Daan 1980). Breeding birds have been previously recorded to reach levels of more than 7 × BMR, however, although not in tit species (Bryant & Tatner 1991). This suggests that the supplemented females were working well below their optimal capacity. However, the levels of DEE in the supplemented area were similar to other studies of Great Tits feeding nestlings at the same latitude (Sanz *et al.* 2000b), whereas the females nesting in the control area exhibited higher levels of DEE than reported for other sites in Continental Europe at a similar latitude to that of Chaddesley Woods (i.e. 52.36ºN). However, this could be attributable to heavier body mass in the UK subspecies (i.e. *Parus major newtoni*) when compared to the European mainland species of *Parus major major* (Gosler 1993). The multiples of BMR are allometrically corrected for individual mass, and the supplemented birds are closer to estimates from The Netherlands than the control birds (Sanz *et al.* 2000b). In 2011 females in both the supplemented and unsupplemented areas exhibited higher DEE than in 2010 (Fig. 4.8), again indicating that supplemented females in 2010 were working far below the level at which they could.

The first possible explanation for lower energetic costs in mismatched females is that although fewer nestlings inevitably require less effort to feed, the relationship between brood size and the effort allocated per nestling is not linear and, indeed, decreases exponentially with higher brood sizes (e.g. Gibb 1950, Bryant 1988). This indicates that the compensatory energetic saving made by losing one nestling could result in a disproportional reduction in DEE compared to intact broods. Including nestling mortality in the GLM for female DEE (Table 5.1) did not significantly improve model fit ($\chi^2 = 0.44$, df = 1,25, $P = 0.50$), although the intricacy of the relationship could preclude a simple linear predictor and further brood reduction experiments would be required to standardise brood losses and isolate this effect. Other studies have reported mixed responses of female DEE to experimental brood reduction with some illustrating reduced DEE (e.g. Sanz & Tinbergen 1999, Tinbergen & Verhulst 2000) and others no reduction in DEE (e.g. Moreno *et al.* 1995, Verhulst & Tinbergen 1997). The most plausible explanation for lower female DEE in the supplemented area, however, is that the food supplementation reduced energy expenditure through providing easier foraging opportunities.

Although there is a marked paucity of other food supplementation studies that have measured DEE during the nestling phase, a study of European Pied Flycatchers found that supplementation had no effect on the DEE of females rearing nestlings (Moreno *et al.* 1999), whereas a study of Black-legged Kittiwakes found lowered DEE in response to food supplementation (Jodice *et al.* 2002). The results of these two studies reflect far more elegantly the theoretical framework of trade-offs expected from short-lived and longer-lived species than do the Chaddesley data; short-lived passerines do not visibly benefit energetically from easier foraging conditions and invest the energy or time gained into their offspring, but the longer-lived kittiwake adults use the supplement to lower the costs they incur during the current reproductive attempt to increase their chances of survival to the following year.

5.5.4. Female DEE and provisioning rates

Female DEE was positively related to female provisioning rate as in other studies measuring DEE across a range of species (e.g. House Martins, Blue-throated Bee-eaters (*Merops viridis*) – Bryant 1988, Great Tits – te Marvelde *et al.* 2011b). This was in contrast to my previous results from the combined DEE dataset of 2010 and 2011 (Chapter Four) and studies of European Pied Flycatchers and Great Tits (Moreno *et al.* 1997, Sanz *et al.* 1998). Although there was not a significant dietary treatment × provisioning rate interaction, the two treatment groups had a different relationship between DEE and female provisioning rate, with supplemented females seeming to expend less energy to provision at the same rate. There was no difference in provisioning rates between control and supplemented areas across all years of my study, however (Chapter Four).

The combination of higher female DEE with no change in provisioning visits per nestling in the control area suggests that foraging for high quality prey items may be more energetically costly than the act of delivering food to the nestbox. Indeed, short hovering flights are energetically expensive in small passerines such as Zebra Finches (Nudds & Bryant 2000). In conditions of high natural food availability and with large brood sizes, it may be advantageous to pay those energetic costs to increase reproductive output and produce heavier nestlings. Alternatively, the parents from the supplemented area could have resorted to provisioning the nestlings with peanut cake, which does not seem to be something they commonly do within this population based on Stable Isotope Analysis (SIA) results from previous years (Harrison 2010). Claw samples were taken from nestlings as part of routine mist-netting sessions in 2010 (for methods see Harrison 2010), so future SIA would elucidate whether the nestlings in 2010 had a higher proportion of peanut cake in their diet than unsupplemented control nestlings.

Both DEE and provisioning rates have been called into question as techniques for measuring parental investment (Bryant 1988, Tinbergen & Verhulst 2000) and have been much discussed as the mechanism by which life history trade-offs can be measured (Zera & Harshman 2001, Williams 2005). Whilst DEE is frequently unrelated to brood size (reviewed in Bryant 1988), provisioning rate is usually related to brood size (e.g. Nur 1984a, Wright *et al.* 1998, Barba *et al.* 2009). When used in conjunction, however, they can provide an additionally enlightening analysis, as with the results from 2010.

5.5.5. DEE across the reproductive attempt

Although it is widely acknowledged that there is significant inter- and intra-individual variation in DEE and BMR (Tatner & Bryant 1993, Speakman *et al.* 1994), repeated measures of energy expenditure or metabolic rate within the same individuals have been lacking until very recently (Rønning *et al.* 2005, Vézina & Williams 2005). There are only two studies which have measured DEE during egg laying and brood rearing within the same individuals. In a study of Great Tits, there was no correlation between DEE during egg laying and that during brood rearing (te Marvelde *et al.* 2011b), and in a study of captive Zebra Finches (Vézina *et al.* 2006) there was no increase in DEE between egg laying and brood rearing. A further study of Barn Swallows (*Hirundo rustica*), which used different individuals within the same population at each of the reproductive stages, also found no difference in DEE between egg laying and brood rearing (Ward 1996). This has led some researchers to conclude that the energetic requirements of egg laying and brood rearing may be roughly equivalent, although complex energy reallocation strategies are used to offset the increased physiological demands of stages such as egg laying (Vézina *et al.* 2006, Salvante *et al.* 2010). In this instance Zebra Finches reduced locomotor activity to cover the additional costs of producing eggs (Houston *et al.* 1995), without increasing DEE or food intake (Vézina *et al.* 2006). This has important implications for my study, as increased food availability may not necessarily act to enable increases of DEE.

These results are in complete contrast to those from my study population. They are also in complete contrast to a study using heart-rate monitoring in Macaroni Penguins to determine energy expenditure, which indicated that these birds expend more energy during the chick-rearing period than almost all other stages of their annual cycle (Green *et al.* 2009). In both of my treatment groups the DEE of females during egg laying and that during brood rearing was significantly positively correlated, and significantly higher during brood rearing. There was no difference between the treatment groups in terms of the slope of this relationship (Figs 5.6 and 5.7), although the intercepts were lower in the supplemented group. This indicates that females expending comparatively high levels of energy during egg laying were also expending comparatively high levels of energy during brood rearing. These results provide support for the possibility that phenotypes can be divided into two main types: ‘low’ and ‘high’ energy phenotypes (Williams 2001b). ‘High’ energy phenotypes are those where individuals are more able to raise DEE, whereas ‘low’ energy phenotypes are less able to do so, although the consistency between DEE in two reproductive phases could also be evidence of there being higher or lower ‘quality’ individuals (Røskaft *et al.* 1985). The burgeoning evidence of the repeatability of measures of DEE and BMR (Rønning *et al.* 2005, Vézina *et al.* 2006, Bouwhuis *et al.*

2011) also suggest that individuals may be highly consistent in their energy allocation strategies, indicating just how profound an effect food supplementation had in reducing DEE in my study. Food supplementation elicited a proportional reduction in DEE across two reproductive phases and, thus, did reduce the energetic costs of reproduction. These results are unique because such an intra-individual analysis of DEE across two reproductive phases has never been related to food availability or to food supplementation of free-living birds.

5.5.6. Life history trade-offs and resource availability

The culmination of the sequence of indicators that supplemented females were not investing maximally in their offspring is evident in lower nestling body masses in the supplemented treatment group. Heavier nestlings usually have better chances of surviving post-fledging (Tinbergen & Boerlijst 1990), although this does not necessarily equate to higher chances of recruiting into the breeding population (Nur 1984c, Magrath 1991). There is a chance that nestlings could catch up after nestling day 11 with faster growth rates, but the difference in nestling body masses at this stage was marked and compensatory growth is common within broods in response to sibling competition but not across broods (Nilsson & Svensson 1996b, Royle 2000). Unfortunately, too few of the offspring from the 2010 breeding season survived to 2011 to be able to analyse recruitment rates with any statistical rigour; presumably, offspring survival rates were lower as it was a particularly cold winter in 2010. The body masses of nestlings from broods in the supplemented area in 2011 were significantly higher than those from the unsupplemented area (Chapter Four), indicating again that low nestling body masses may have been related to a systematic reduction in reproductive investment.

The most exciting result from the 2010 data was that female survival was significantly higher as a result of food supplementation. In addition to incurring predicted survival costs relating to higher investment during reproduction (e.g. Bryant 1979, Deerenberg *et al.* 1995), breeding adults are hypothesised to improve their survival if they reduce their investment in the current reproductive attempt (e.g. Hõrak 2003). Although much evidence of future fecundity or survival costs exists in relation to *increased* breeding effort (e.g. Gustafsson & Part 1990, Daan *et al.* 1996, de Heij *et al.* 2006), it is very rare to find evidence of increased adult survival from *reduced* reproductive costs (but see Hõrak 1995). This is particularly true of income breeders (Meijer & Drent 1999) where exogenous reserves are of critical importance and future survival prospects of the adult are lower than in capital breeders (Drent & Daan 1980).

In longer-lived capital breeders such as seabirds, parents reduce reproductive effort under conditions of low food availability to maximise their chances of breeding the following year (e.g. Yellow-nosed Albatross – Weimerskirch *et al.* 2001) and raise significantly smaller brood sizes than they are physiologically able to (reviewed in Ricklefs 1990). They may also improve their survival prospects by foregoing breeding altogether, as was found in Black-legged Kittiwakes (Golet *et al.* 1998). The response to more difficult foraging conditions in the Chaddesley population in 2010 is much more characteristic of a longer-lived species, although the passerine studies that have recorded increased adult survival in response to reduced reproductive effort were both on Great Tits (Hõrak 1995, 2003). By food supplementing breeding adults we may have not only changed their seasonal timing and reproductive output, but also altered the perceived value of this reproductive attempt and induced phenotypic plasticity in their life history strategy.

In conclusion, food supplementation prior to and during egg laying with a high-energy supplement advances laying dates but can lead to a severe phenological mismatch between the timing of peak nestling nutritional demand and that of caterpillar availability. This mismatch can then result in higher nestling mortality and lower brood sizes. Mismatched food-supplemented female Great Tits did not appear to invest maximally in their current reproductive attempt in 2010 but traded off offspring quality against their own survival prospects. This raises questions as to how a short-lived species with high annual mortality could effectuate such a decision.

Over 50% of adult female Great Tits die between breeding seasons (Gosler 1993), meaning that most females will breed only once in their lifetime. The effects of senescence in one UK population are estimated to become visible at the age of 2.8 years (Bouwhuis *et al.* 2009), indicating that Great Tits are short-lived among passerines. Some females have been recorded breeding at the age of 9 (Bouwhuis *et al.* 2009), however. There is a lack of evidence of terminal investment associated with increased reproductive effort in the same UK population (Bouwhuis *et al.* 2009) which supports both my findings and those of Hõrak (1995) suggesting that Great Tits will trade off current reproduction for future survival. My results are unique, however, in relating under-investment in offspring to energetic savings, giving empirical evidence of a life history trade-off.

Moult has been suggested by a number of studies (e.g. Siikamäki *et al.* 1994, Nilsson & Svensson 1996a) as being a mechanism by which adults can effect trade-offs between current reproduction and future survival, as feathers grown more slowly are of higher quality (Dawson *et al.* 2000). Alternatively adults may be able to boost their survival prospects by altering their risk-taking strategy (Ghalambor & Martin 2001). Increased reproductive effort could also affect immunocompetence (Ardia *et al.* 2003) or parasite load (Ots & Hõrak 1996), so parents could increase their survival chances by increasing their health status. Females could have placed less value on their offspring due to the higher nestling mortality (Pettifor *et al.* 1988), or valued their young less as they were produced at a time of lower natural resource availability, as is more characteristic of young produced later in the season (Daan *et al.* 1990).

The energetic response of females to low food availability and food supplementation is an intriguing one, and provides a key insight into the life history dynamics in this breeding attempt. It is also the first study to demonstrate that energy expenditure can be the mechanism by which positive life history trade-offs are manifested. Without the energetics data, it would appear that parents were investing highly and provisioning at similar rates but still losing offspring, rather than strategically lowering energy expenditure and conserving resources for future reproduction. This is a unique finding and such a shift in life history strategy has not been demonstrated in a short-lived species such as Great Tits before. It raises new questions surrounding our understanding of life history trajectories and the capital *versus* income breeding dichotomy. Life history trade-offs between fecundity and survival of adults will be explored in greater detail in the next chapter.

Chapter Six

The effects of food supplementation on adult survival and juvenile recruitment

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6.1. Abstract

One of the cornerstones of life history theory is that there should exist a conflict between the level of investment a parent makes in a current reproductive attempt and that which is reserved for subsequent reproductive attempts. Food availability may play a key role in determining the cost of breeding attempts to parents, affecting the quantity and quality of offspring produced and the likelihood of parental inter-seasonal trade-offs occurring. There are, however, few food supplementation studies which have investigated lifetime reproductive success of individuals, so key questions remain unanswered. Measures of adult survival and offspring recruitment in a long-term food supplementation study were examined to determine whether food availability had demographic or life history consequences. The survival prospects of supplemented female Blue and Great Tit adults were found to be improved, but only under specific circumstances. Male Blue and Great Tit adults were unaffected by food supplementation. Similarly, offspring recruitment was unaffected by food supplementation although a strong seasonal decline in recruitment probability conferred an advantage to offspring from the earliest breeding attempts in the supplemented areas. The context of these results is discussed in a life history framework and represents a novel and intriguing re-assessment of the life history strategy of short-lived bird species.

6.2. Introduction

The ultimate expression of an effective individual lifetime reproductive strategy, according to life history theory, is the successful recruitment of offspring into a breeding population (Lessells 1991, Stearns 1992), whether this is achieved in one breeding season or across multiple reproductive attempts. In the latter instance, reduced fecundity in one breeding attempt may theoretically be balanced by an increase in adult survival to allow a subsequent breeding attempt (Williams 1966, Stearns 1976). Following a highly significant reduction in reproductive output detected in food-supplemented Blue and Great Tits (e.g. Harrison *et al.* 2010), life history trade-off theory predicts a rise in the probability that an adult survives to have a second breeding attempt. Alternatively, parents could invest an equivalent amount into the current reproductive attempt and produce fewer offspring of higher quality (Williams 2001a), which may be indicated by increased offspring recruitment into the breeding population (e.g. in Whooping Cranes *Grus americana* – Boyce *et al.* 2005). Resource availability is an integral component of life history trade-offs as the ease with which parents can assimilate resources determines the theoretical relative cost of reproductive investment (van Noordwijk & de Jong 1986, Martin 1987).

The overwinter provision of food supplements can increase the survival prospects of birds (e.g. Jansson *et al.* 1981, Brittingham & Temple 1988, Desrochers *et al.* 1988) and may have carry-over effects to the subsequent breeding season in increasing productivity (Robb *et al.* 2008b) or improving adult breeding body condition (Plummer 2011). Previous food supplementation studies have recorded positive (e.g. Verhulst 1994, Dawson & Bortolotti 2002, Davis *et al.* 2005), negative (e.g. Arcese & Smith 1988, Nilsson 1994) or no effect (Gienapp & Visser 2006) of food supplementation during the breeding season on adult survival. Most food supplementation studies have not been conducted over a sufficient duration to examine the consequences of food supplementation on adult survival or offspring recruitment, instead focussing predominantly on intra-seasonal reproductive output or adult body condition (e.g. Meijer *et al.* 1988, Nager *et al.* 1997). Although a decrease in reproductive output has been recorded in food-supplemented Blue and Great Tits as part of a long-term study (Harrison *et al.* 2010), the demographic consequences of such a decrease in the number of fledglings produced have not been explored.

A decrease in adult survival prospects is commonly associated with an increase in reproductive effort, whether at the inter-specific (Martin 1995, Ghalambor & Martin 2001) or individual level (Bryant 1979, Nur 1984b). Adult survival is also negatively correlated with increased parental effort (Deerenberg *et al.* 1995, Golet *et al.* 2000) and brood enlargement (Reid 1987). This has been interpreted as supporting the general principle that a decrease in adult survival is associated with increased fecundity. Therefore, increased survival prospects of adult Great Tits would be anticipated based on decreased clutch and brood sizes, and lower energy expenditure in both the egg-laying (Chapter Three) and brood-rearing (Chapter Four) phases. Blue and Great Tits are commonly regarded to be income breeders (Drent & Daan 1980), predominantly *r*-selected and exhibiting high fecundity and low annual survival (Cramp 1993, Bennett & Owens 2002); as such, they may be poor models for investigating inter-annual carry-over effects (Harrison *et al.* 2011). Evidence of life history trade-offs exists for both Blue and Great Tits, however, where increased survival prospects may be mediated through the mechanism of carry-over effects such as increased immunocompetence (e.g. Ots & Hõrak 1996), reduced risk-taking (e.g. Ghalambor & Martin 2001) or improved feather quality following earlier moult (Nilsson & Svensson 1996a, Dawson *et al.* 2000). There is a paucity of experimental studies which have manipulated food availability for breeding adults and then monitored adult survival. Such investigations could provide key insights into the relationship between resource availability and the cost of reproduction.

Food availability may be one of the critical determining factors not only of the number of offspring an adult produces, but also the level of reproductive investment an adult is willing to make to ensure the subsequent survival of those offspring to breeding age. There is some debate as to the timing of the peak in post-fledging mortality (Perrins 1965, Lack 1966, Dhondt 1979) and starvation has been suggested as one of the key factors limiting juvenile survival (Perrins 1965, Drent 1984). This suggests that food supplementation post-fledging may have significant benefits for offspring survival. However, Naef-Daenzer *et al.* (2001) used radio telemetry and colour marking of individual Great and Coal Tits (*Periparus ater*) to show that predation is the most significant cause of post-fledging mortality in these species, but that the heaviest and earliest fledglings were less at risk of being predated.

Nestling body mass is one of the most consistent predictors of offspring survival to recruitment (Magrath 1991, Lindén *et al.* 1992). The probability of nestling survival for at least three months post-fledging may increase by as much as 4% for every extra gramme increment in fledging body mass (Garnett 1981). Post-fledging survival has been demonstrated to be density-dependent in species such as Great Tits (Drent 1987), partly due to competition for territories (reviewed in Gosler 1993). First year losses of juvenile Great Tits (either due to dispersal or mortality) after fledging are positively associated with the density of breeding adults (Dhondt 1979, Michler *et al.* 2011) or with the total population density (reviewed in Klomp 1980). Competition with existing territory holders may affect juvenile settlement on territories in the autumn and promote higher dispersal

(Dhondt 1979) or higher mortality due to being on poor territories (Kluijver 1951). The mechanism through which this happens may be body mass, as fledging body mass is important relative to conspecifics (Both *et al.* 1999) rather than as an absolute measure (Perrins 1965, Drent 1984). Fledglings that leave the nest in better condition also exhibit more mobility and larger foraging ranges with parents which may further improve survival prospects (Naef-Daenzer & Grüebler 2008). In better quality habitat with higher food availability, higher densities of juveniles are supported (Klomp 1980). Adult survival has also been shown to be density-dependent, but high natural food availability (Perrins 1966) or food supplementation (Brittingham & Temple 1988) may reduce the competition for food (Dhondt 1979) and hence improve overwinter survival. With increased adult survival and increased juvenile survival, higher breeding densities would be expected in response to food supplementation.

There remain few food supplementation studies that have been conducted long-term and are able to examine the effects of food availability on the post-fledging survival and recruitment of offspring. Studies of natural food availability during the nestling period (e.g. Perrins 1991, van Noordwijk *et al.* 1995) have indicated, however, that it is one of the critical determinants of offspring recruitment and, hence, an important contributor to the lifetime reproductive success of breeding adults. Food availability could act to enhance offspring recruitment in one of two principal ways: through either increasing nestling body mass and improving individual nestling survival (Simons & Martin 1990) or through advancing the onset of egg laying (Chapter Two). There is strong selection for early breeding in many Great Tit populations (Verhulst *et al.* 1995, Cresswell & McCleery 2003) and the earliest fledged offspring have the highest probability of recruitment into the breeding population (Lindén *et al.* 1992, Verboven & Visser 1998). Food supplementation would, therefore, be expected to increase the probability of recruitment, either through enabling earlier hatching or by increasing nestling body mass.

In order to examine the role of food availability on both adult and offspring survival and fecundity/survival life history trade-offs, a long-term food supplementation study was conducted with marked adults. An increase in adult survival to breed in a subsequent year was predicted in both Blue and Great Tits on the basis that the costs of foraging have been reduced by food supplementation. An advance in laying date in both Blue and Great Tits has already been reported in response to food supplementation in this population (Chapter Two), so an increase in offspring recruitment was predicted. Based on theoretical models (e.g. Stearns 1992), the two life history traits (survival and fecundity) were predicted to co-vary negatively in breeding adults.

6.3. Methods

6.3.1. Nestbox study

Please refer to Chapter Two for details of the study site and routine monitoring of nestboxes and to Chapter Four for details of capture and determination of age status of breeding adults and ringing of nestlings between 2006 and 2011.

6.3.2. Food supplementation

Please refer to Chapter Two for details of the food supplementation regime. Woodland areas were unsupplemented (Control, CON), supplemented with peanut cake (PC) or supplemented with peanut cake and mealworms (PCM) from 2006 to 2009, and unsupplemented (CON) or supplemented with peanut cake (PC) in 2010 and 2011.

Although food supplementation decreased the number of fledglings produced (Chapter Four) and hence may have affected fledgling density, the high mobility of dependent (Naef-Daenzer & Grüebler 2008) and independent (Gosler 1993) fledglings meant that the woodland should be considered as one unit in terms of population density. Given that the focus of this study was the difference in offspring survival between treatment blocks, measures of density dependence were not included in analyses. Similarly, occupancy of nestboxes did not vary between treatment blocks (Appendix Three), so there should not have been systematic differences in adult survival in each block as a result of density of conspecifics.

6.3.3. Timing of peak in caterpillar biomass

Please refer to Chapter Two for details of the methodology used to measure caterpillar biomass and determine timing of the seasonal peak.

6.3.4. Nestling mortality

Please refer to Chapter Four for details of how nestling mortality prior to ringing age was monitored.

6.3.5. Statistical analyses and data filtering

Only first breeding attempts were included and known or suspected second broods were excluded from analyses based on criteria specified in Chapter Two. Adult survival (or return rate) was examined in terms of survival to a subsequent successful breeding attempt. Offspring recruitment was confirmed following capture of a ringed fledgling from the study site during a successful breeding attempt. The proportion of breeding adults that was captured was high in 2009 (Blue Tit: ♂♂ – 75%, ♀♀ – 82%, Great Tit: ♂♂ – 76%, ♀♀ – 82%), 2010 (Blue Tit: ♂♂ – 83%, ♀♀ – 92%, Great Tit: ♂♂ – 94%, ♀♀ – 97%) and 2011 (Blue Tit: ♂♂ – 85%, ♀♀ – 99%, Great Tit: ♂♂ – 80%, ♀♀ – 94%) but lower in 2007 (Blue Tit: ♂♂– 45%, ♀♀– 59%, Great Tit: ♂♂– 62%, ♀♀– 62%) and 2008 (Blue Tit: ♂♂– 42%, ♀♀– 59%, Great Tit: ♂♂ – 47%, ♀♀– 53%). There was no systematic difference in capture techniques between breeding areas, however, and capture of breeding adults at nestboxes is an effective method for monitoring the breeding population (Bachau & van Noordwijk 1995) so recapture probability was not considered.

The years of data included in each analysis varied according to the response variables under examination as no caterpillar data were available for 2006 or 2009 and no adults were captured in 2006. All statistical analyses were conducted using Generalized Linear Mixed Models (GLMMs – glmmadmb function in glmmADMB package or glmer in lme4 package) in R version 2.13.1 (R Development Core Team 2011). A Bernoulli binomial error distribution was used for all probability analyses, with a complementary log-log link function to account for a high occurrence of zeros. For analyses of the number of recruits produced per brood, a Poisson error distribution was used after testing for normality using the Shapiro-Wilk normality test (Crawley 2007) and correcting for zero-inflation (Zuur *et al.* 2009a). All interactions between explanatory variables were tested using forward stepwise regression to find the minimal adequate model and chi-squared comparisons of residual deviance were used to compare models with and without terms and interactions (Crawley 2007). Data for 2010 and 2011 were analysed separately to investigate year × dietary treatment interactions, as due to the unbalanced nature of the whole dataset, with just two treatment groups in these two years, year × dietary treatment interactions were not possible. Pseudo R^2 values were calculated where possible for GLMMs, to illustrate model fit, using the R^2 value from a linear model of the correlation between fitted and observed values. Pseudo R^2 values for GLMs were calculated from the proportion of residual deviance in the model.

6.4. Results

An average of 24% of female and 21% of male Blue Tits bred in two or more years, compared with survival rates of 32% and 37%, respectively, from the wider population. An average of 28% of female and 22% of male Great Tits bred in two or more years, compared with population annual survival rates of 43% and 38%, respectively.

6.4.1. Food supplementation and adult survival

Female Blue Tit survival was positively affected by supplementation (Fig. 6.1, Table 6.1). The females breeding in the peanut cake and mealworms treatment area had significantly higher survival than those breeding in the control area (Fig. 6.1, Table 6.1). There was no significant difference between females supplemented with peanut cake and control females, however. There was a significant positive effect of food supplementation on the survival rate of female Great Tits in 2010 but not in any other years (Table 6.1; see Chapter Five). There was a significant positive effect of mismatch from peak caterpillar availability in 2010 on the survival rates of female Great Tits (Fig. 6.2, Table 6.1), and also a significant positive effect of brood size on Great Tit female survival rates in 2010 (Table 6.1). There was lower survival in older female Great Tits and higher survival in those females whose offspring recruited into the breeding population (Table 6.1) in years 2007,

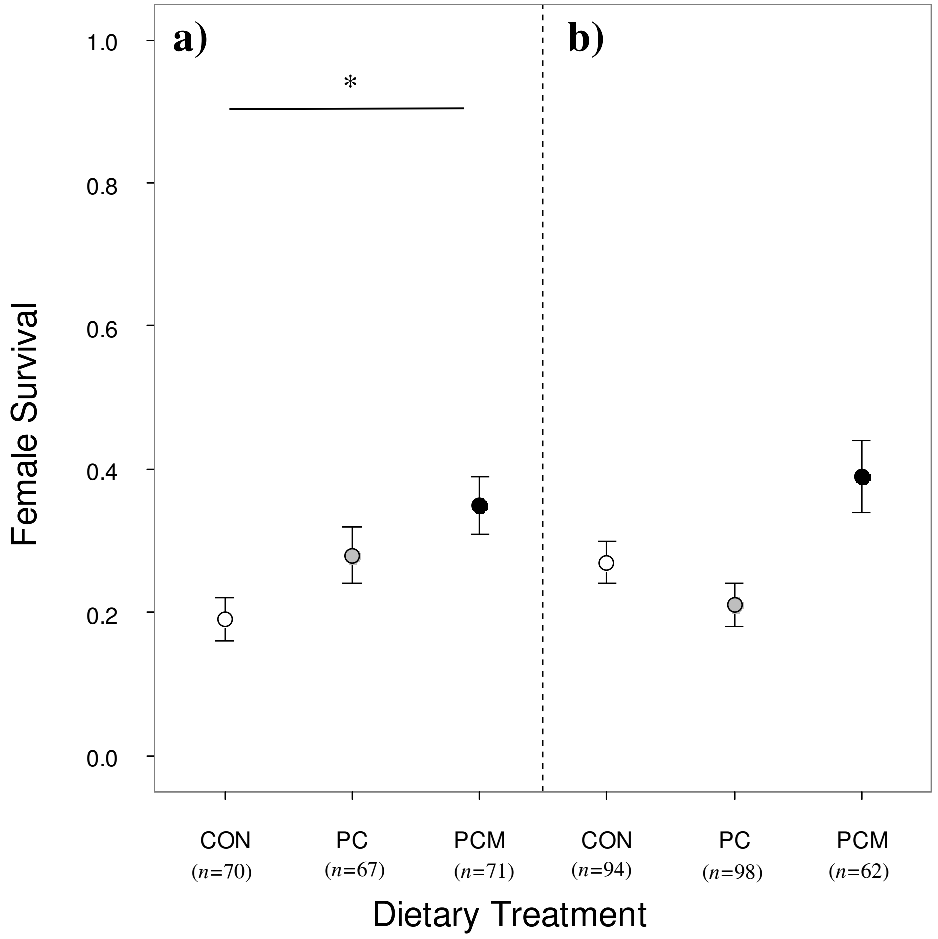

Figure 6.1. Probability of female survival (mean ± 1 SE from raw data) until the following breeding season of (a) Blue and (b) Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2010. Broods in the control treatment area (CON) are represented by open circles, in the peanut cake treatment area (PC) by grey-filled circles and in the peanut cake and mealworms treatment area (PCM) by black-filled circles. * = $P < 0.05$. Number of broods is shown in parentheses below each axis label. See Table 6.1 for statistical tests and text for details.

2008 and 2009, and lower survival in older female Great Tits in 2010 (Table 6.1). There was no significant effect of food supplementation treatment on the survival rate of male Blue or Great Tits (Fig. 6.3, Table 6.1).

Table 6.1. Results from simplified GLM and GLMM logistic regression analysis of specified breeding parameters (Response) of female Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2010 regressed upon significant explanatory variables (Fixed Effect). Estimates represent the increase or decrease in log-odds for each fixed effect relative to the baseline. Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo R^2 value is presented. For details of statistical tests applied see section 6.3.5.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	R^2
Female Survival 2007,2008, 2009	BT	Dietary Treatment: PC	1.774	0.360	1,207	6.6	0.11		0.02
		Dietary Treatment: PCM	2.319	0.342	1,207		**0.01**	Higher female survival	
Female Survival 2010	BT	Hatch Date	1.109	0.098	1,64	1.1	0.29		0.03
Female Survival 2007,2008 2009	GT	Female Age	0.457	0.257	1,253	154.7	**0.01**	Lower female survival	0.09
		Offspring Recruitment	1.828	0.255	1,253	5.4	**< 0.05**	Higher female survival	
Female Survival 2010	GT	Dietary Treatment: PC	4.410	0.679	1,95	9.7	**< 0.05**	Higher female survival	0.27
		Brood Size	1.329	0.135	1,95	1.2	**< 0.05**	Higher female survival	
		Mismatch	1.311	0.084	1,95	10.1	**< 0.01**	Higher female survival	
		Female Age	0.338		1,95	4.6	**< 0.05**	Lower female survival	
Male Survival 2007,2008 2009,2010	BT	Dietary Treatment: PC	0.519	0.374	1,223	4.2	0.08		0.23
		Dietary Treatment: PCM	0.539	0.357	1,223		0.08		
		Year			3,223	9.8	**0.01**	2010 lowest 2007 highest	
Male Survival 2007,2008, 2010	GT	Hatch Date	0.860	0.048	1,237	4.4	**< 0.01**	Lower survival	0.60
		Mismatch	0.814	0.076	1,237	80.8	**< 0.01**	Lower survival	

Data from 2010 were treated separately for both Blue and Great Tit females to account for unbalanced datasets. Random effect was nestbox in all GLMM models or woodland block if insufficient data existed to test at the level of nestbox. Tested fixed effects were dietary treatment (‘PC’ = peanut cake, ‘PCM’ = peanut cake and mealworms), year, brood size, nestling mortality, mismatch from the peak in caterpillar biomass, number of recruits from each brood and female age.

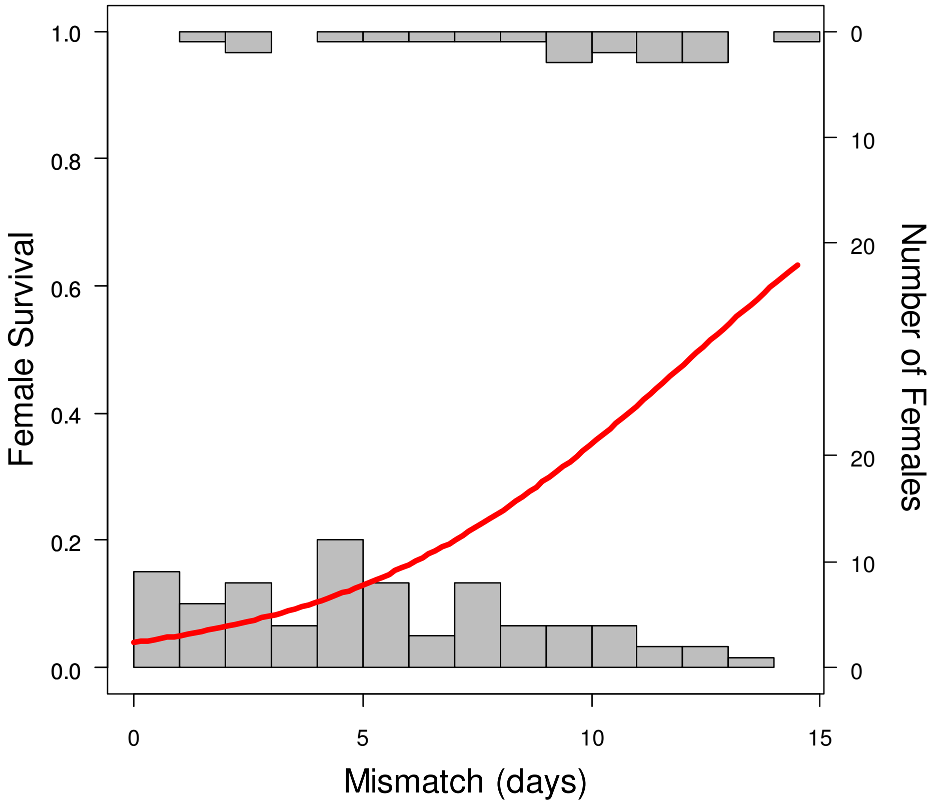

Figure 6.2. Probability of breeding female Great Tits surviving until the following breeding season in Chaddesley Woods National Nature Reserve in Worcs., UK in 2010 in relation to mismatch from the peak in caterpillar availability. The curve represents survival probability predicted from a logistic regression and the histograms represent the frequency of females that survived (top axis) or did not survive (lower axis) associated with each level of mismatch. See Table 6.1 for statistical tests and text for details.

There was a highly significant effect of advancing hatch date on the probability of male Great Tits returning to breed in a subsequent year (Fig. 6.4, Table 6.1), with a 14% reduction in probability of surviving to breeding in the following year for each day that

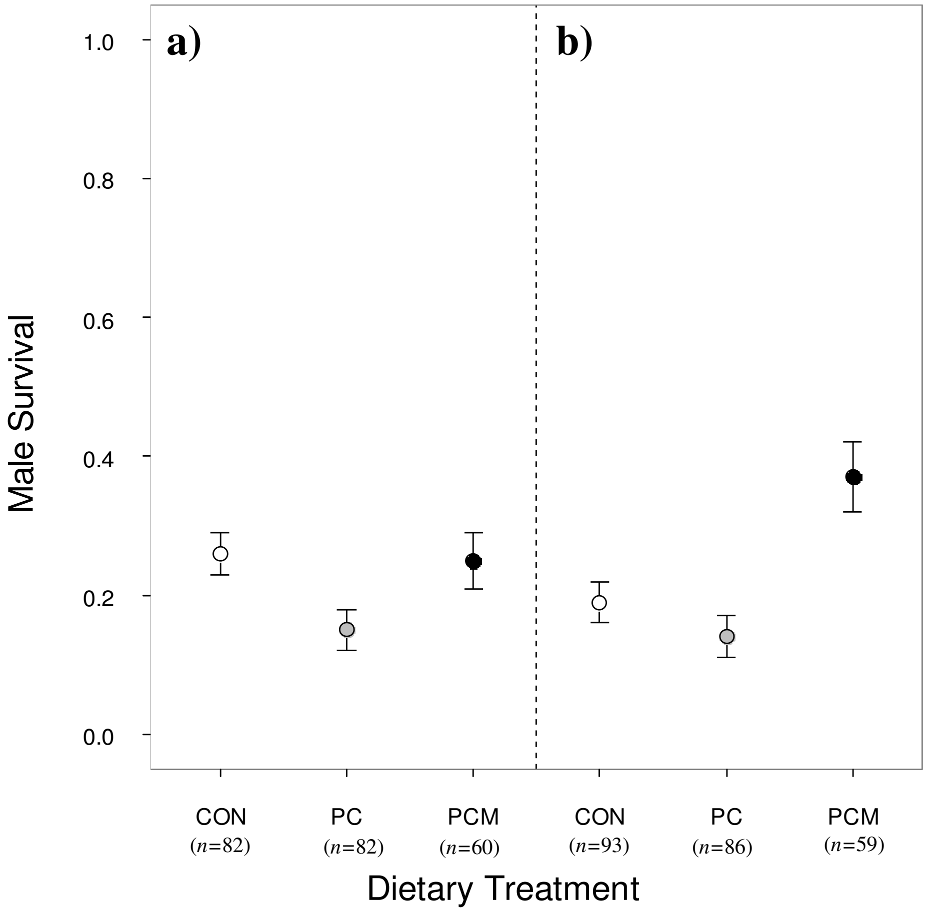

Figure 6.3. Probability of male survival (mean ± 1 SE from raw data) until the following breeding season of (a) Blue and (b) Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2007 to 2010. Broods in the control treatment area (CON) are represented by open circles, those in the peanut cake treatment area (PC) by grey-filled circles and those in the peanut cake and mealworms treatment area (PCM) by black-filled circles. Number of broods is shown in parentheses below each axis label. See Table 6.1 for statistical tests and text for details.

hatch date was advanced. There was also a significant negative effect of mismatch from the peak in caterpillar availability in male Great but not Blue Tits (Fig. 6.5, Table 6.1). There was significant inter-annual variation in survival rates of male Blue Tits, with

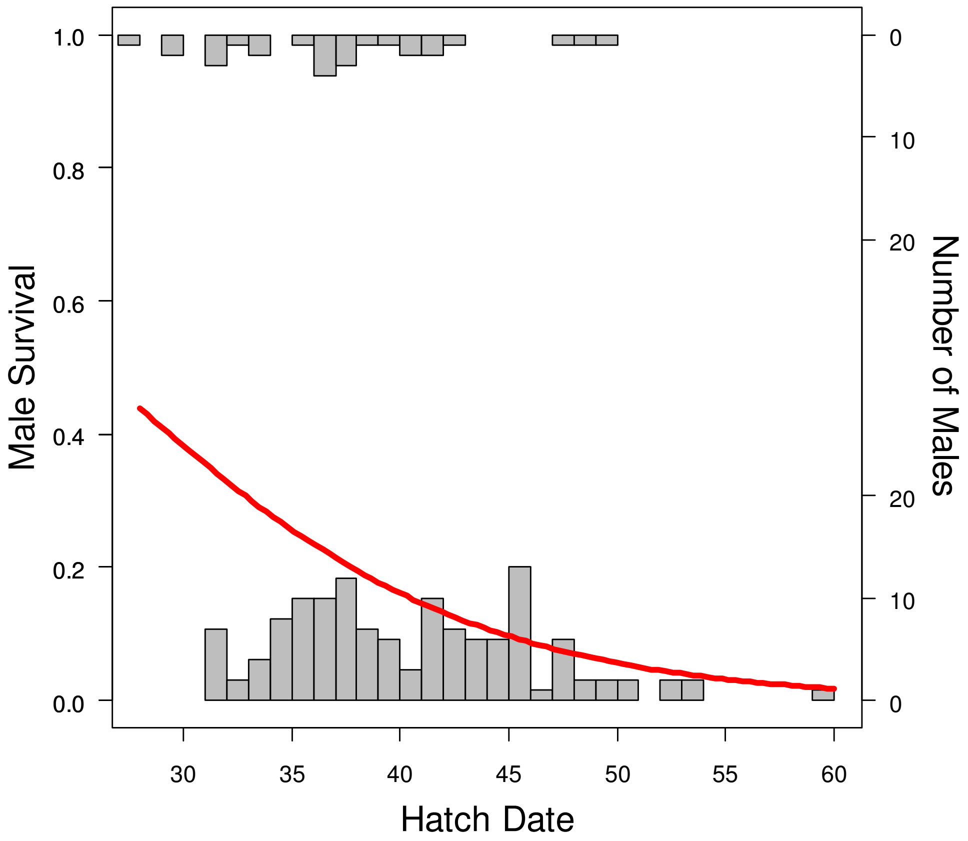

Figure 6.4. Probability of breeding male Great Tits surviving until the following breeding season in Chaddesley Woods National Nature Reserve in Worcs., UK in 2007, 2008 and 2010 in relation to hatch date. The curve represents survival probability predicted from a logistic regression and the histograms represent the frequency of males that survived (top axis) or did not survive (lower axis) associated with each hatch date. Hatch date is in April Days where 1 = 1st April. See Table 6.1 for statistical tests and text for details.

survival to breeding in the following year being highest in 2007 (Table 6.1). There was no significant inter-annual variation in survival of male or female Great Tits, or in female Blue Tits to breed in the subsequent year (Table 6.1).

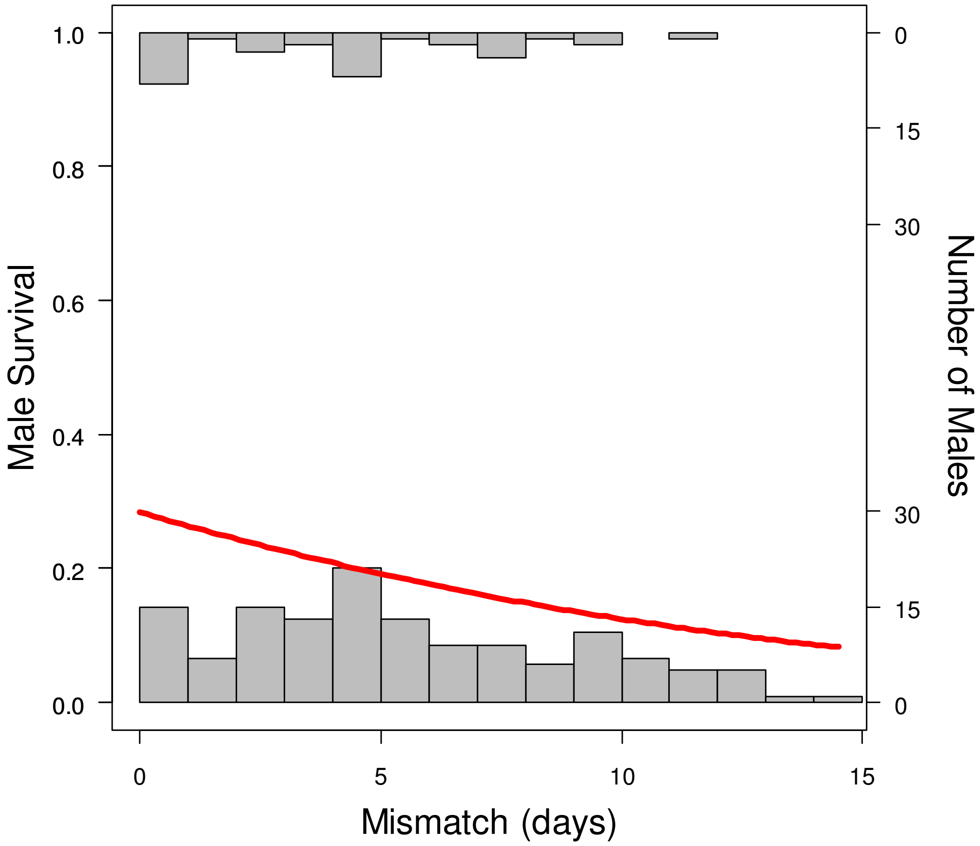

Figure 6.5. Probability of breeding male Great Tits surviving until the following breeding season in Chaddesley Woods National Nature Reserve in Worcs., UK in 2007, 2008 and 2010 in relation to mismatch from the peak in caterpillar availability in days. The curve represents survival probability predicted from a logistic regression and the histograms represent the frequency of males that survived (top axis) or did not survive (lower axis) associated with each level of mismatch. See Table 6.1 for statistical tests and text for details.

6.4.2. Food supplementation and offspring recruitment

There was no significant effect of food supplementation treatment on either the probability of a brood producing a recruit or the number of recruits per brood produced by both Blue and Great Tits (Fig. 6.6, Table 6.2).

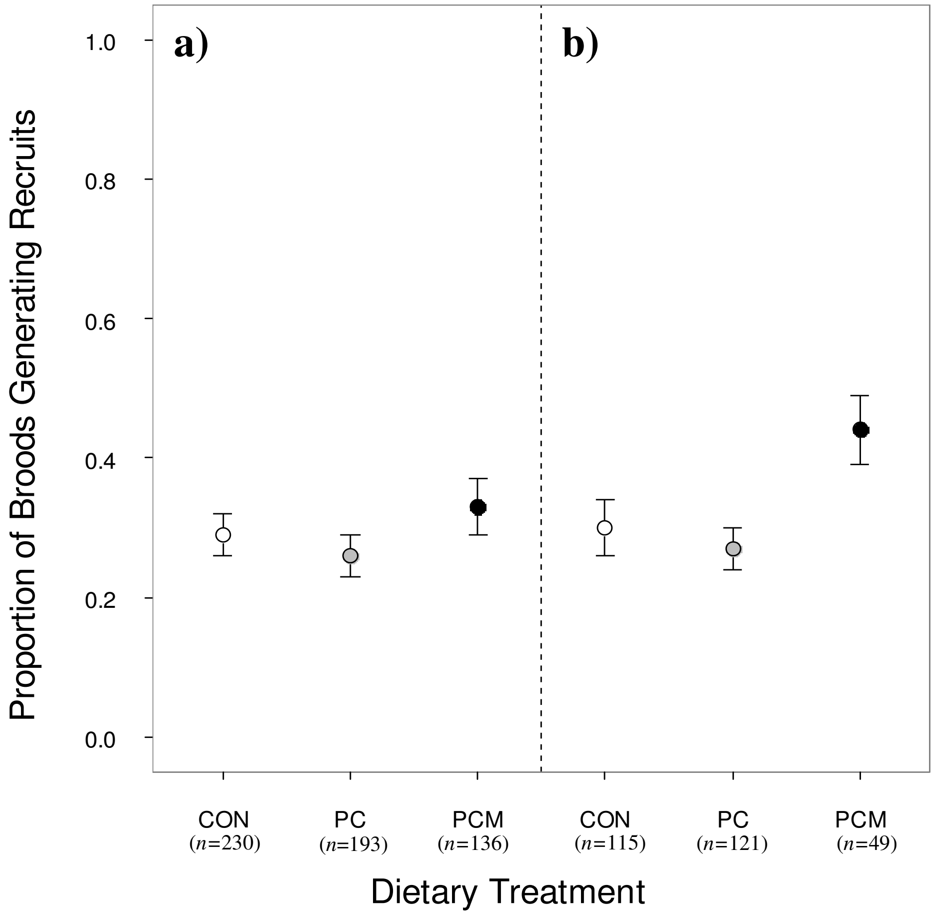

Figure 6.6. Proportion (mean ± 1 SE from raw data) of (a) Blue and (b) Great Tit broods successfully recruiting at least one fledgling into the breeding population in Chaddesley Woods National Nature Reserve in Worcs., UK from 2006 to 2010. Broods in the control treatment area (CON) are represented by open circles, in the peanut cake treatment area (PC) by grey-filled circles and in the peanut cake and mealworms treatment area (PCM) by black-filled circles. Number of broods is shown in parentheses below each axis label. See Table 6.2 for statistical tests and text for details.

Table 6.2. Results from simplified GLMM analyses of specified breeding parameters (Response) of female Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2007 and 2010 regressed on significant explanatory variables (Fixed Effect). The first two models analysed the influence of fixed effects on the probability of a brood producing a recruit ('Offspring Recruitment') and the second two models analysed the influence on the number of recruits produced per brood ('Number of Recruits per Brood'). Chi-squared results (Chisq) from sequential removal of each term from the model detail the relative contribution of each fixed effect. Only significant contributors to each model are displayed. A pseudo R^2 value is presented. For details of statistical tests applied see section 6.3.5.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	**df**	***Chisq***	***P***	**Direction**	**R^2**
Offspring Recruitment 2006-2010	BT	Hatch Date	0.945	0.014	1,486	16.7	**< 0.001**	Lower recruitment	0.03
Offspring Recruitment 2007, 2008, 2010	GT	Hatch Date	0.895	0.042	1,412	9.1	**< 0.01**	Lower recruitment	0.10
		Mismatch	0.886	0.056	1,412	4.7	**< 0.05**	Lower recruitment	
		Nestling Mortality	0.776	0.110	1,412	6.6	**< 0.05**	Lower recruitment	
		Year			3,412	8.1	**< 0.05**	2007 lowest 2008 highest	
Number of Recruits per Brood 2006-2010	BT	Intercept	0.656						0.02
		Nestling Mortality	-0.165	0.134	1,486	4.7	**< 0.05**	Lower number of recruits	
Number of Recruits per Brood 2006-2010	GT	Intercept	0.590						0.01
		Dietary Treatment: PC	0.439	0.357	1,412	0.69	0.41		
		Dietary Treatment: PCM	0.766	0.411	1,412	0.4	0.53		

Random effect was nestbox for the first two models and woodland block for the second two models. Tested fixed effects were dietary treatment (Intercept = control, 'PC' = peanut cake, 'PCM' = peanut cake and mealworms), year, brood size, nestling mortality, mismatch from the peak in caterpillar biomass, number of recruits from each brood and female age. Any models in which the mismatch from peak caterpillar biomass was a significant predictor excluded data from 2009. Analyses corrected for zero-inflation and overdispersion where required and used a binomial (first two models) or Poisson (second two models) error distribution. Estimates represent the increase or decrease in log-odds for each fixed effect relative to the baseline for binomial models.

There was significant inter-annual variation in the probability of recruitment from broods of Great Tits with recruitment being highest in 2008 and lowest in 2007 (Table 6.2). There was little inter-annual variation in the rate of recruitment from broods of Blue Tits (Table 6.2). There was a significant negative effect of advancing hatch date on the probability that a brood of Blue or Great Tits produced recruits (Figs. 6.7 and 6.8, Table 6.2).

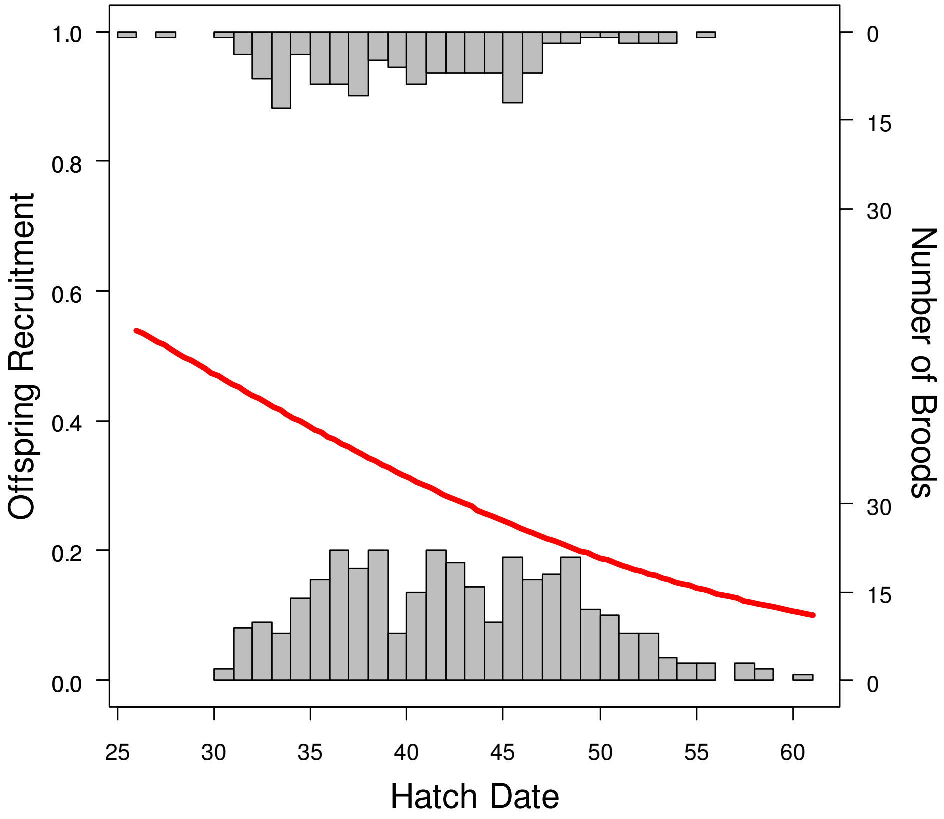

Figure 6.7. Probability of broods of Blue Tits successfully recruiting at least one fledgling into the breeding population in Chaddesley Woods National Nature Reserve in Worcs., UK from 2006 to 2010 relation to hatch date. The curve represents the probability of a brood generating a recruit predicted from a logistic regression and the histograms represent the frequency of broods that generated recruits (top axis) or no recruits (lower axis) associated with each hatch date. Hatch date is in April Days where 1 = 1st April. See Table 6.2 for statistical tests and text for details.

This translated into a 5% reduction in recruitment probability per day advance in hatch date in Blue Tit broods and a 10% reduction in recruitment probability in Great Tit broods.

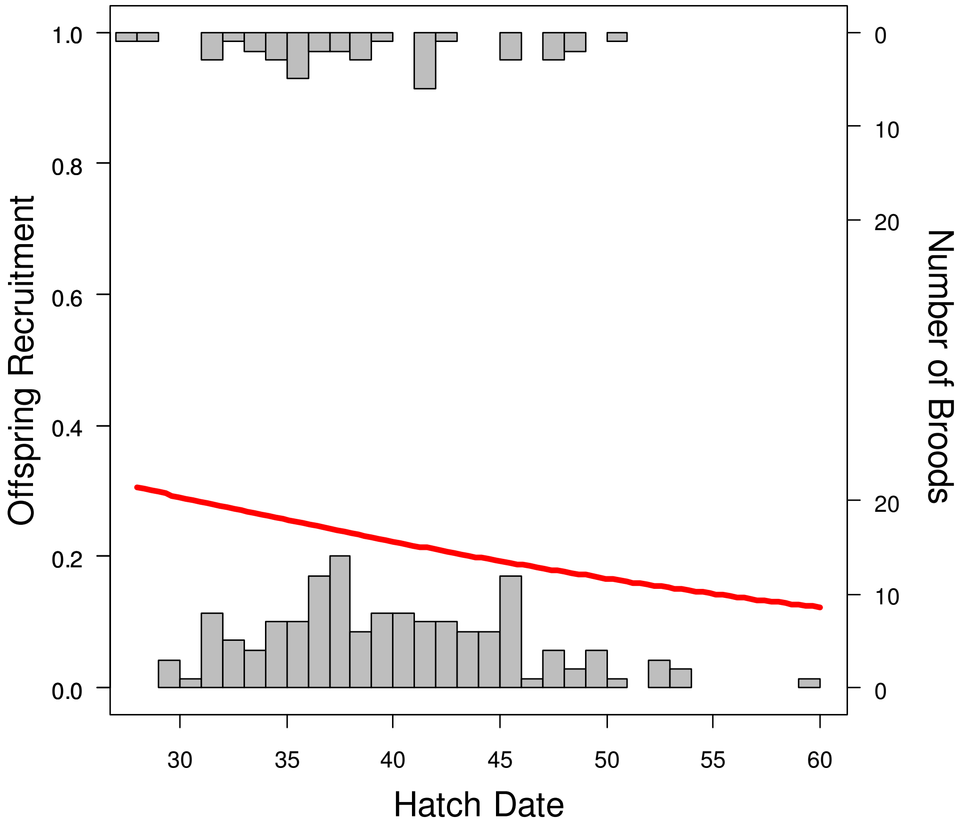

Figure 6.8. Probability of broods of Great Tits successfully recruiting at least one fledgling into the breeding population in Chaddesley Woods National Nature Reserve in Worcs., UK in 2007, 2008 and 2010 in relation to hatch date. The curve represents the probability of a brood generating a recruit predicted from a logistic regression and histograms represent the number of broods that generated recruits (top axis) or no recruits (lower axis) associated with each hatch date. Hatch date is in April Days where 1 = 1st April. See Table 6.2 for statistical tests and text for details.

There was a significant negative effect of increasing mismatch from the peak in caterpillar biomass on the recruitment probability of Great Tit broods (Fig. 6.9, Table 6.2). This translated into an 11% reduction in recruitment probability per additional day of mismatch from the caterpillar peak. There was no effect of mismatch from the peak in caterpillar

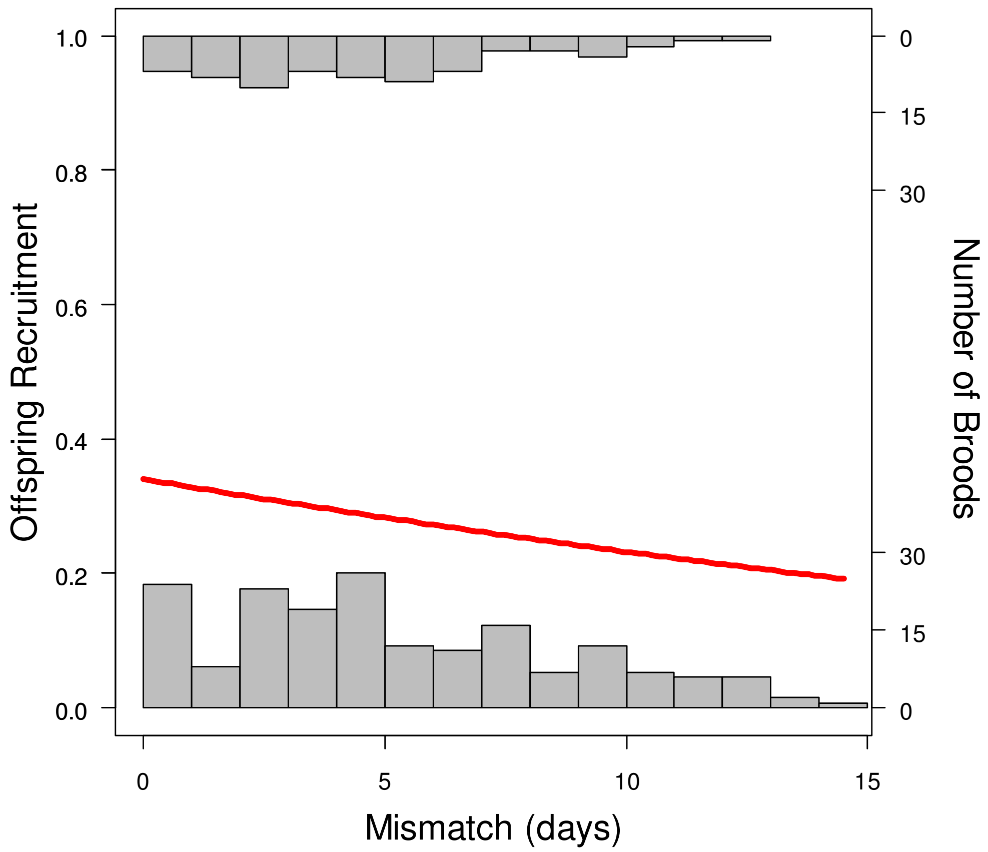

Figure 6.9. Probability of broods of Great Tits successfully recruiting at least one fledgling into the breeding population in Chaddesley Woods National Nature Reserve in Worcs., UK in 2007, 2008 and 2010 in relation to the number of days mismatch from peak caterpillar availability. The curve represents the probability of a brood generating a recruit predicted from a logistic regression and histograms represent the number of broods that generated recruits (top axis) or no recruits (lower axis) associated with each level of mismatch. See Table 6.2 for statistical tests and text for details.

availability on the recruitment probability of Blue Tit broods. There was a significant negative effect of nestling mortality before ringing age on the recruitment probability of Great Tit broods (Fig. 6.10, Table 6.2), and on the number of Blue Tit recruits produced per brood (Table 6.2), but not on the probability that a Blue Tit brood would produce a

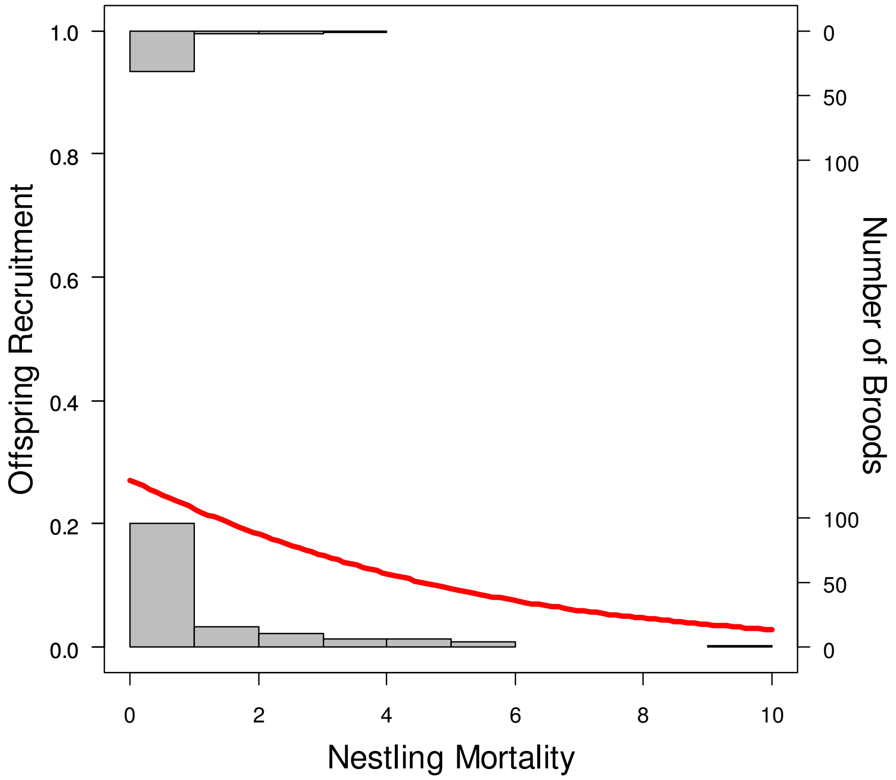

Figure 6.10. Probability of broods of Great Tits successfully recruiting at least one fledgling into the breeding population in Chaddesley Woods National Nature Reserve in Worcs., UK in 2007, 2008 and 2010 in relation to the number of nestlings dying before nestling day 8. The curve represents the probability of a brood generating a recruit predicted from a logistic regression and histograms represent the number of broods that generated recruits (top axis) or did not generate recruits (lower axis) associated with each level of nestling mortality. See Table 6.2 for statistical tests and text for details.

recruit (Table 6.2). The mean annual nestling survival rate was 5% in Blue Tits and 7% in Great Tits and recruitment rates were 3% in Blue Tits and 6% in Great Tits.

6.5. Discussion

In accordance with predictions of improved survival in conditions of more stable food availability (Martin 1987), food supplementation with peanut cake and mealworms significantly increased the chances of Blue Tit females returning to breed for a subsequent year (Fig. 6.1, Table 6.1). There was no effect of food supplementation on the survival rates of male Blue Tits (Fig. 6.3, Table 6.1), and no effect on male or female Great Tits in most years (Figs 6.1, and 6.3, Table 6.1). However, in 2010 supplemented female Great Tits demonstrated higher return rates than unsupplemented conspecifics (Table 6.1, Chapter Five). The survival of female Great Tits in 2010 was also positively correlated with brood size and with the mismatch from peak caterpillar availability (Fig. 6.2, Table 6.1). There was a seasonal decline in male return rates and a negative effect of the mismatch from peak caterpillar availability on the survival probability of male Great, but not Blue, Tits (Figs 6.4 and 6.5, Table 6.1).

There was no effect of food supplementation on either the probability of offspring recruitment (Fig. 6.6, Table 6.2), or on the number of offspring recruited from each brood in either species (Table 6.2). There was a seasonal decline in recruitment probability in both species (Figs 6.7 and 6.8, Table 6.2), and a negative effect of increasing phenological mismatch on the recruitment probability of Great Tit broods (Fig. 6.9, Table 6.2). Both species exhibited an effect of nestling mortality on recruitment; in Great Tits there was a negative association with the probability of a brood generating a recruit (Fig. 6.10, Table 6.2), while in Blue Tits there was a negative association with the number of recruits produced per brood (Table 6.2).

6.5.1. Food supplementation and adult inter-seasonal survival

Resource availability is one of the key constraints on adult survival (Gosler 1993), and, therefore, food supplementation during breeding might aid in increasing adult survival from one breeding season to the next (e.g. Verhulst 1994). Blue Tit females in the Chaddesley population appeared to benefit from supplementation with peanut cake and mealworms, exhibiting improved adult survival in spite of no apparent change in reproductive output (Chapters Three and Four). The unexplained variance in all of the female survival models was very high (Table 6.1, with the exception of Great Tit females in 2010), however, so these results should be treated with caution. Based on the lower brood sizes and reduced energetic investment of Great Tits (Chapters Three and Four), I had predicted an increase in survival of Great Tits across all years of my study, and the lack of such a response is surprising. This inter-specific difference in survival in response to food supplementation could be related to the difference in annual survival between the two species. Adult Blue Tits have lower inter-annual survival rates than Great Tits; within my population Blue Tits have an annual survival rate of 34% compared to 44% in Great Tits. This is consistent with other populations with an average Blue Tit survival rate of 30% compared with 52% in Great Tits (summarised in Cramp 1993). It would appear that even a brief increase of a nutrient-rich food supplement such as mealworms at a critical point in the annual cycle affects the survival of female Blue Tits. Great Tits may not be investing in their reproductive attempts to the same degree and may be conserving their effort for future reproduction (Chapter Five), so a short-term increase in a high-protein supplement may not have such a visible effect on their inter-annual survival rates.

6.5.2. Adult survival and the nutritional quality of food supplements

In all the previous food supplementation studies which have included measures of adult survival, food supplements such as mealworms (Nilsson 1994, Verhulst 1994) and dog food (Arcese & Smith 1988) were high in protein. Therefore, any differences between these studies were unlikely to be due to the quality of the supplement being provided. The nutritional quality of the supplements provided clearly had an effect on the survival probability of the Chaddesley Blue Tits as peanut cake alone did not improve female survival (Fig. 6.1). A previous study of adult Great Tit survival (e.g. Gienapp & Visser 2006) found no response to food supplementation when Great Tits were supplemented with mealworms during the egg-laying period. However, a study of Blue Tits (Nilsson 1994) showed a negative response to food supplementation when the mealworm supplement was provided prior to the egg-laying period. Given that the provision of mealworms to Blue Tits at this earlier point in the reproductive cycle did not improve adult survival, it could indicate that the critical point at which the costs of reproduction impinge on female adult Blue Tit survival is during brood rearing. These costs can then be reduced through supplementation of a protein-rich food resource (e.g. mealworms).

The survival of supplemented female Great Tits was only significantly improved in 2010 (Chapter Five), when supplemented females bred significantly earlier than controls and experienced high brood reduction. There was no supplementation with mealworms in that year, however, so it is not possible to ascertain whether there would have been a difference between the two food supplements on the survival of female Great Tits. Following the effects of the peanut cake and mealworm supplements in reducing nestling mortality (Chapter Four) and maintaining the same brood sizes as control birds, it may be that the mealworm supplement could have mitigated for the low caterpillar availability in

2010 during brood rearing in the earliest broods and increased adult investment in their offspring.

6.5.3. Adult survival and reproductive effort

Reduced adult survival is often thought to be a result of an increase in reproductive effort (e.g. Bryant 1979, Deerenberg *et al.* 1995, Daan *et al.* 1996). However, it is far less common to find an increase in survival prospects in response to a reduction in parental effort, (but see Hõrak 1995 for a study of Great Tits, and Golet *et al.* 1998 for a study of Black-legged Kittiwakes), even if it is one of the key tenets of life history theory (Williams 1966). The increase in survival of female Great Tits in 2010 is particularly striking as it is associated with a sequential under-investment in offspring and increased nestling mortality (Chapter Five). Female Great Tits seem able to pass the costs of reproductive attempts on to their offspring when breeding conditions become difficult (i.e. when food availability is low). Food supplementation has a greater effect on individuals when environmental conditions are more harsh or when territory quality is low (Svensson & Nilsson 1995, Nager *et al.* 1997). Thus, the food supplement in my study could have provided the mechanism through which females were able to effectuate this life history strategy. Alternatively, female Great Tits that were breeding exceptionally early and were severely mismatched from the peak in caterpillar availability may have been able to initiate moult earlier and thus improve their overwinter survival prospects (Nilsson & Svensson 1996a, Dawson *et al.* 2000). It has also been suggested by Ghalambor and Martin (2000, 2001) and by Tilgar and Kikas (2009) that parental risk-taking may be reduced in broods that have sustained high losses where the reproductive value of the young may be reduced, thereby providing another mechanism to boost adult survival chances.

6.5.4. Food supplementation and covariance of life history traits

Food supplementation may have influenced the expected negative relationship between reproductive effort and parental survival as no relationship could be established between brood size and survival of male or female Blue and Great Tits. There was, however, a positive relationship between brood size and adult survival in female Great Tits in 2010 (Table 6.1) and a positive relationship between the probability of offspring recruitment and survival of female Great Tits in 2007 to 2009 (Table 6.1). There can be positive covariance between life history traits if certain individuals have access to more resources than others (van Noordwijk & de Jong 1986, Dhondt 2001, Bize *et al.* 2008). Indeed, this is predicted to drive individual variation in lifetime reproductive success (Stearns 1992). In this instance, however, an interaction between dietary treatment and brood size (for 2010) or dietary treatment and offspring recruitment (for years 2007 to 2009) would be expected but was not found. The statistical significance of the interactions between brood size and adult female survival (2010) and offspring recruitment and adult female survival was marginal (Table 6.1), but could have been more potent with more statistical power. The positive relationship between offspring recruitment and survival of female Great Tits in 2007 to 2009 across all treatment areas could also have been due to intrinsic differences in female physiology, such as resistance to oxidative stress (Bize *et al.* 2008) or on their ability to exploit extrinsic resources (van Noordwijk & de Jong 1986).

Fluctuations in natural food availability between breeding territories could make it difficult to establish consistent covariance in life history traits (van Noordwijk & de Jong 1986). Food supplementation could further confound the direction of covariance in life history traits, particularly when there is an interaction between supplementation and the timing of breeding to the peak availability of natural food (e.g. Chapter Five). Food supplementation was assumed to provide an increase in resources and, hence, supplemented parents were expected to exhibit positive covariance between reproductive traits (e.g. increased offspring production and improved adult survival). The interaction between food supplementation and natural food availability (i.e. shifting the seasonal timing of breeding adults away from the peak in caterpillar biomass) has been demonstrated to result in negative covariance between fecundity and adult survival in female Great Tits (see Chapter Five).

6.5.5. Differential sex-related costs of breeding

Neither females nor males paid a residual reproductive cost of advanced breeding. This was contrary to the findings of Nilsson (1994) who showed reduced adult survival in experimentally advanced broods. In fact, male Great Tits exhibited a seasonal decline in survival, as has been found previously in a Dutch population of female, but not male, Great Tits (Verhulst *et al.* 1995). Again, this could be through the mechanism of food supplementation, as earlier broods tended to be in food-supplemented areas. There were no interactions between dietary treatment and hatch date in any of my statistical models (Table 6.1), however, so this relationship could not be established with certainty. Male Great Tits were also susceptible to reduced survival in the face of increasing mismatch between the timing of breeding and the peak in caterpillar availability, with lower male survival rates in mismatched broods. This is contrary to predictions for female survival in relation to phenological mismatch where the costs of increasing mismatch are expected to be passed on to offspring rather than incurred by the female herself (Visser *et al.* 2011b). This is consistent with results from 2010 (Chapter Five).

Male Great Tits usually have higher survival rates than females (Gosler 1993) although the average male survival rate in the Chaddesley population is lower in males than females. Female and male life histories may, therefore, have a differential relationship with extrinsic factors such as food supply as a result of this. It is difficult to isolate consistent patterns in differential survival responses to food availability and reproductive investment between the sexes. In other populations, male survival has been shown to be density-dependent (e.g. Clobert *et al.* 1988), unaffected by food supplementation (e.g. Verhulst 1994, Dawson & Bortolotti 2002), and affected (e.g. Daan *et al.* 1996) or unaffected by increased reproductive effort (e.g. Bryant 1979, Nur 1984b).

6.5.6. Food supplementation and recruitment of offspring

Food supplementation has been related to substantial increases in the probability of offspring recruitment: seven-fold in a study of European Pied Flycatchers (Verhulst 1994) and nine-fold in a study of Carrion Crows (*Corvus corone*) (Richner 1992). This effect could be a result of supplementation leading to an increase in offspring survival and recruitment following an increase in nestling body mass (Simons & Martin 1990), or leading to earlier fledging (Verboven & Visser 1998, Chapter Two). Increases in survival probability of offspring as a result of food supplementation commonly follow an increase in nestling body mass in species as diverse as Cactus Wrens (*Campylorhynchus brunneicapillus*) (Simons & Martin 1990) and Northern Goshawks (*Accipiter gentilis*) (Dewey & Kennedy 2001) although these increases may not be consistent between years. In Great Tits, nestling body mass is one of the most common predictors of offspring survival (Tinbergen & Boerlijst 1990, Both *et al.* 1999, Monrós *et al.* 2002), so the increased body mass of Great Tit nestlings from areas supplemented with peanut cake in certain years of my study (Chapter Four) should have been a driving factor for higher recruitment rates from supplemented areas in those years.

There is strong selection pressure for early breeding in both Blue and Great Tits (Svensson 1997, Cresswell & McCleery 2003), and the earliest fledged offspring in a population of Great Tits showed the best chance of recruiting (Verboven & Visser 1998), possibly due to earlier establishment of their position in flock hierarchies (Gosler 1993). Hatch date was a significant predictor of the probability of each brood recruiting offspring into the Chaddesley population (Figs 6.7 and 6.8), but a relationship with food supplementation treatment could not be established. There was high unexplained variance in the recruitment models (Table 6.2), however, indicating that more years of data would be required to illustrate such a relationship. In previous years of this supplementary feeding programme, there was significantly higher mid-term survival prospects for Great Tits fledging from the area supplemented with peanut cake and mealworms than from the other two treatment areas (Harrison 2010). Although the raw data seemed to indicate that recruitment probability would reflect this (Fig. 6.6), other variables took precedence in the final model (Table 6.1). It was noticeable that the lower Great Tit brood sizes (Chapter Four) did not translate into fewer recruits, so over the course of the study food supplementation appears not to have affected lifetime reproductive success of adults or the demographic recruitment rates. Larger brood sizes among Great Tits commonly produce higher numbers of recruits (e.g. Tinbergen & Sanz 2004). Therefore the fact that the smaller Great Tit broods in the supplemented area were not at an additional disadvantage implies that there may have been a compensatory effect of food supplementation, potentially mediated through increases in nestling body mass (Chapter Four).

6.5.7. Offspring recruitment and brood reduction

Recruitment probability in Great Tits was negatively related to nestling mortality, so in a year such as 2010 when nestling mortality rates were very high (Chapter Four), recruitment rates were lower. The number of Blue Tit recruits per brood was also negatively related to nestling mortality. This is in contrast to the increase in survival probability which life history might associate with reduced brood or clutch size (e.g. Boyce *et al.* 2005). Reduced offspring survival or recruitment in response to higher nestling mortality or to experimental brood reduction has been reported previously in Great Tits (Hõrak 2003) and Eurasian Magpies (*Pica pica*) (Husby 1986). However, studies of species such as Tree Swallows (*Tachycineta bicolor*) (Shutler *et al.* 2006) have reported no effect of experimental brood reduction on offspring survival and positive effects have been documented in other studies of Great Tits (e.g. Smith *et al.* 1989). Brood reduction in the Chaddesley Great Tit population appears not to be an adaptive parental response to improve the survival prospects of the remaining offspring, but part of a longer term strategy where parents ensure their own survival to reproduce in a subsequent year (e.g. Chapter Five).

6.5.8. Food supplementation and offspring dispersal

Dispersal from the natal site is an intrinsic component of offspring recruitment and, although movements away from the woodland complex in my study are not monitored, previous analysis of movements within the study woodland concluded that dispersal may be influenced by food supplementation (Harrison 2010). Female offspring are acknowledged to be more likely to disperse further than male offspring in Blue and Great Tits (Greenwood *et al.* 1979, Verhulst *et al.* 1997b, Tufto *et al.* 2005), but female Great Tit offspring from food-supplemented areas appeared to be less likely to disperse (Harrison 2010). The sex ratio of Great Tit broods has not been determined, but should there be a higher proportion of males in broods in supplemented areas, this could indicate that a very high proportion of the female offspring from supplemented areas is remaining in the population. Blue Tit broods in the area supplemented with peanut cake and mealworms are more male-biased than in unsupplemented areas, and those supplemented only with peanut cake (Smith 2011). Whether this has implications for the sex ratios of recruits will be an avenue for further exploration (Donald 2007).

6.5.9. Food supplementation and residual reproductive value trade-offs

There were no obvious indications of trade-offs between the number and quality of offspring produced. This can be argued from the finding that brood size was not a factor in any of the recruitment models and when nestling mortality was significant, it had a negative effect. It appears that both Blue and Great Tit adults used the food supplements to boost their own survival prospects rather than increase investment in offspring so trade-offs worked to increase residual reproductive value. This is contrary to predictions for these species which are considered exemplars of short-lived highly fecund species (Drent & Daan 1980). Improved adult survival in most years could not be related to decreases in fecundity (in the form of nestling mortality), unlike in some previous studies (e.g. Great Tits – Hõrak 1995, Black-legged Kittiwakes – Golet *et al.* 1998). Increased nestling mortality in 2010 may have been a mechanism by which the investment in the current reproductive attempt could be reduced. There exist few food supplementation studies that have monitored both offspring recruitment and adult inter-seasonal survival (but see Schoech *et al.* 2008). Of those which did, results are varied, with American Kestrel adults exhibiting higher survival at the expense of offspring survival (Dawson & Bortolotti 2002), higher adult survival and higher recruitment in European Pied Flycatchers (Verhulst 1994) and in Parasitic Jaegers higher adult survival and higher offspring fledging success (Davis *et al.* 2005). In contrast, the survival of food-supplemented adult Song Sparrows was found to be lower with increased nestling body mass but not increased offspring survival (Arcese & Smith 1988) and survival of food-supplemented adult Blue Tits was lower as was their fledging success (Nilsson 1994). In the former study it was suggested that parental survival was reduced due to increased defence of territories with bird feeders (Arcese & Smith 1988), whereas in the latter study food supplementation miscued females into breeding too early in relation to peak prey availability (Nilsson 1994). The key difference between the studies reporting positive results in terms of adult and offspring survival and those reporting negative results is that of timing of supplementation. When food supplements were provided only during the brood rearing phase there was no negative effect of supplementation on adult survival rates (Verhulst 1994, Dawson & Bortolotti 2002, Davis *et al.* 2005). However, when food supplements were provided pre-laying, adult survival rates were lower (Arcese & Smith 1988, Nilsson 1994). This suggests that providing supplementary food during the period when reproductive decisions are made regarding the timing of breeding and the number of offspring produced could have long-term negative consequences for lifetime reproductive success.

Food availability may have complex effects on life history fecundity/survival trade-offs within a breeding season, but there are clear inter-seasonal trade-offs in breeding Great Tits in certain years. Although Blue and Great Tits may be considered as having similar life history strategies (Stearns 1977), Great Tits may be further towards the *K* end of the *r/K* selection continuum as they have slightly higher annual survival and lower fecundity (Bennett & Owens 2002). Therefore, Great Tits may be more able to alter their parental investment strategy to trade off current reproduction against residual reproductive value, particularly when resource availability is more stable under a regime of food supplementation during the breeding season. Blue Tit parents appear to invest maximally in their current reproductive attempt, but their survival and chances of future reproduction may be increased by supplementation with high-protein food items at key points in the reproductive cycle.

Chapter Seven

General Discussion

7.1. Aims of thesis

In this thesis I have explored the role of protracted food supplementation and natural food availability during the breeding season in influencing the reproductive parameters, energy budgets and life history strategies of breeding Blue and Great Tits. My ultimate goal was to understand how food supplementation may affect parental investment in reproduction and whether any energetic savings from this predictable food resource were translated into increased fecundity or improved inter-seasonal adult survival.

The reasons for undertaking this study were based on the fact that although there is a wealth of literature regarding the effects of food supplementation on avian species at various points in the annual cycle (reviewed in Boutin 1990, Robb *et al.* 2008a), there have been relatively few long-term food supplementation studies (although see Schoech *et al.* 2008 for details of a seven-year study where supplementation of Florida Scrub-Jays with a high-fat, high-protein supplement advanced breeding, increased clutch size and improved offspring survival). Isolating the role of food availability in the life history strategies of breeding birds is, therefore, problematic. Furthermore, the uncontrolled provision of supplementary foods to birds in gardens (e.g. in UK) and backyards (e.g. in USA and Canada) is growing in popularity at least in the Western World and is now being conducted year-round (summarised in Chapter One). In addition, it is difficult to quantify to what extent anthropogenic feeding of birds occurs on a global scale (Jones & Reynolds

2008), particularly if the definition of supplementary feeding is extended to include indirect supplementary feeding. For example, human waste sites can provide a critical resource for scavengers such as gulls (*Larus* spp.) in the UK (Rock 2005), caracaras (*Caracara* spp.) (Yorio & Giaccardi 2002) or seabirds following trawlers (Garthe *et al.* 1996). The demographic consequences of such an increase (or decrease if supplementary food is withdrawn) in food resources available to birds could be dramatic. Conducting multiple year food supplementation studies could inform us of the longer-term demographic consequences of food supplementation on individuals. This could enable an appreciation of whether such widespread supplementation is beneficial to the bird populations in the long-term. In addition to the paucity of food supplementation studies examining long-term effects, there is a dearth of studies analysing its physiological effects. Our comprehension of the underlying mechanisms that drive life history variation in response to fluctuating resource availability is mainly theoretical as a consequence (e.g. van Noordwijk & de Jong 1986, Williams 2005).

7.2. Summary of results

Reproductive success

In Great Tits, both the availability of a key seasonal food resource (i.e. caterpillars) and food supplementation during the breeding season were found to have profound effects on reproductive success and parental investment. Blue Tits were predominantly unaffected by food supplementation, however, but some of the key breeding parameters were affected by natural food availability.

Laying date

Food supplementation with peanut cake advanced the laying date of Great Tits and, thus, negatively affected the accuracy of seasonal timing of their breeding attempt to the natural peak in caterpillar biomass. It also negatively affected their phenotypic plasticity in laying date (Chapter Two). However, Blue Tits did not significantly advance their clutch initiation dates in response to food supplementation with peanut cake (when individual variation was accounted for), although their hatch dates were advanced. However, they succeeded in maintaining the synchrony of their breeding attempts with the seasonal peak in natural food availability (Chapter Two). These intra-specific differences were in spite of regular use of feeders by both species ascertained from video footage and Stable Isotope Analysis (Harrison 2010).

Brood size

The brood size of Great Tits was significantly reduced in the areas supplemented with peanut cake only, as a result of smaller clutch sizes (Chapter Three) and higher nestling mortality compared with birds breeding in unsupplemented areas (Chapter Four). The addition of mealworms to the peanut cake treatment during the nestling phase compensated in some way for this negative effect and there was no difference in nestling mortality or brood size compared with unsupplemented areas (Chapter Four). In Blue Tits, there was no significant difference in clutch size, nestling mortality or brood size between food-supplemented and unsupplemented birds (Chapters Three and Four). However, their brood sizes were significantly positively related to caterpillar biomass, so natural food availability played a key role in their fecundity.

Egg volume and egg mass

Egg volume and egg mass of Blue Tits were not affected by food supplementation with peanut cake. However, in Great Tits, those that laid their eggs earliest in supplemented areas laid significantly lighter eggs. This led to a reversal in the seasonal decline of egg mass recorded in the unsupplemented areas (Chapter Three), indicating a penalty of early breeding in Great Tits.

DEE during egg laying

Increased DEE in the earliest breeders provided evidence of a higher cost to early breeding (Chapter Three) in Great, but not Blue, Tits. The peanut cake supplement did not compensate for some of this cost (Chapter Three). Both female Blue and Great Tits reduced DEE during egg laying in response to food supplementation with peanut cake, and did not reallocate the energy saving into producing larger clutches (Chapter Three).

Parental provisioning rates

There were sex-specific responses to food supplementation during the brood-rearing period which were consistent between the two species. Male Blue and Great Tits *increased* their provisioning rate to nestlings when provided with peanut cake, but were unaffected by caterpillar availability. In contrast, female Blue and Great Tits *decreased* their provisioning rates in response to increasing caterpillar availability but were unaffected by food supplementation with either peanut cake or peanut cake and mealworms (Chapter Four).

Female DEE during brood rearing

Female Great Tits reduced their DEE during the brood-rearing phase in response to food supplementation with peanut cake and increased partner provisioning effort. Their provisioning rates were unaffected by food supplementation with peanut cake and increased partner effort (Chapter Four).

Nestling body mass

Great Tit nestling body mass was unrelated to caterpillar availability, but was greater in the peanut cake-supplemented areas in a year in which breeding occurred relatively early (i.e. 2011, Chapter Two) and lighter in a year in which breeding occurred relatively late (i.e. 2010, Chapters Two, Four and Five). Body masses of Blue Tit nestlings were not correlated with food supplementation with peanut cake, but were positively related to caterpillar availability (Chapter Four).

Phenological mismatch between peak caterpillar availability and peak nestling demands

In 2010 female Great Tits passed the cost of a severe phenological mismatch as a result of food supplementation with peanut cake on to their offspring in terms of higher nestling mortality, lower brood size and lower nestling mass (Chapter Five). Female Great Tits exhibited lower DEE and higher survival to the subsequent breeding season (Chapter Five).

Adult survival

The survival of female Great Tits was unaffected by food supplementation with either peanut cake or peanut cake and mealworms, but that of female Blue Tits was higher when females were supplemented with peanut cake and mealworms (Chapter Six). Male survival probability was unrelated to food supplementation but in light of the seasonal decline in survival of Great Tits, it may have conferred a seasonal advantage (Chapter Six).

Probability of offspring recruitment

Offspring recruitment was unrelated to food supplementation with either peanut cake or peanut cake and mealworms, but there was a seasonal decline in recruitment prospects in both species, so, again, food supplementation may have conferred a seasonal advantage (Chapter Six). This potential increase in recruitment probability may be offset for phenologically mismatched food-supplemented Great Tits as offspring recruitment was linked to caterpillar availability (Chapter Six).

7.3. Food availability and seasonal reproduction

In common with other research on seasonally breeding bird species (e.g. Song Sparrows – Hochachka 1990, Great Tits – Verboven & Visser 1998, European Starlings – Christians *et al.* 2001), I have shown that in the absence of supplementary feeding there were many advantages of early breeding in both Blue and Great Tits. These advantages were manifested in terms of larger clutch sizes in both species, heavier eggs in unsupplemented Great Tits, lower nestling mortality and larger brood size in Blue Tits, heavier nestlings in both species, improved survival of Great Tit males, and higher offspring recruitment in both species. However, there were also some significant intra-seasonal costs associated with early breeding in Great Tits, evinced both at the level of parental effort (i.e. increased female DEE during egg laying) and increased nestling mortality leading to lower brood sizes.

There was a substantial inter-specific difference in the degree to which food supplementation advanced laying and, consequently, hatch dates. The result of this advancement in Great Tits was significant phenological mismatch between the peak in nestling nutritional demands and that in caterpillar availability. Food-supplemented Blue Tits succeeded in maintaining the synchrony between their timing of breeding and the caterpillar peak through a more moderate shift in hatch date. Food supplementation has been demonstrated to affect individual Blue Tits differentially in other populations (e.g. Plummer 2011), with individual consumption of supplements varying and effects such as subsequent survival being more evident in some individuals than others. This effect was reinforced in my study as variance in laying dates increased with food supplementation, indicating certain individuals may respond more than others to supplementation. Theoretically this could permit females of lower 'quality' or body condition to breed earlier which could alter the phenotypic quality of the population; if poorer 'quality' females breed early and benefit from the seasonal advantages of earlier breeding, they could recruit more poorer 'quality' offspring into the breeding population.

In years such as 2010 when early breeding was associated with decreased fecundity and lower nestling body mass, selection for offspring recruitment could favour late-breeding birds although, unfortunately, there were too few recruits from 2010 to 2011 to analyse this in isolation. One of the most enduring phenological questions is why such great variation in laying dates exists when natural selection clearly favours earlier breeding (Verboven & Visser 1998, te Marvelde *et al.* 2011c). A shift in favoured selection from early to late breeders could indicate a mechanism by which such variation is maintained, albeit under artificial circumstances.

Great Tits paid fecundity costs of breeding too early within a given season (i.e. higher nestling mortality and lower brood sizes), but gained inter-seasonal benefits in the form of improved male survival and higher offspring recruitment. In common with other studies demonstrating strong selection for accurate timing of peak nestling nutritional demand to the seasonal peak in caterpillar availability (e.g. van Noordwijk *et al.* 1995), the recruitment of Great Tits in my study was negatively related to the degree of this phenological mismatch. Significant negative responses to food supplementation in Great Tits in some parameters (e.g. brood size, nestling body mass) seemed to diminish the importance of caterpillar availability in analytical models (e.g. Tables 4.3 and 4.7), indicating that the inaccuracy of their seasonal timing to peak caterpillar availability may have decoupled the relationship between natural food availability and reproductive success (Pascual & Peris 1992, Naef-Daenzer *et al.* 2000).

The provision of mealworms reduced the negative effects of peanut cake on the mortality of Great Tit nestlings and on their brood sizes, suggesting that peanut cake is not a suitable food supplement to sustain the increased costs of early breeding and of phenological mismatch in this species. However, the addition of a live food source may be able to compensate for this once the nestlings have hatched. Female provisioning rates in both species remained closely linked to the natural caterpillar availability, and were not affected by food supplementation, lending support to the assertion that peanut cake as a supplement is lacking in nutrition for nestlings.

The nutritional value of peanut cake also poses a problem when examining the role of food availability in triggering clutch initiation. Although the lack of increase in clutch size in food-supplemented females of both species might indicate that food does not constrain the onset of reproduction, peanut cake may not have contained sufficient nutrients to produce this effect. Thus, female Great Tits could have been stimulated into laying early by the removal of an energetic constraint, but the lack of other nutrients in the peanut cake prevented them from producing additional eggs. However, the initiation of egg laying was still substantially later than the commencement of food supplementation in all years. Therefore, my conclusion is that food supplementation provided a quantitative cue as to the likely timing of the caterpillar emergence. In this instance, the sudden appearance of a new food resource on the 7th March of each year of the study resulted in breeding females behaving as if they were already late in their timing of breeding with respect to the predicted peak in natural food availability. This led to them apparently accelerating their breeding attempt to construct nests (Smith 2011), laying smaller clutches and curtailing incubation (Harrison 2010) in the process.

Very few food supplementation studies have also monitored natural food availability to determine how a shift in the timing of breeding could relate to seasonal peaks in prey such as invertebrates. My study achieved this, but my results indicate some subtle and intriguing results. Therefore, there remains much to be explored by varying the quality of food supplements within long-term multi-generational studies.

7.4. Food supplementation and energy expenditure

One of the foremost aims in this thesis was to link food supplementation to energy expenditure in order to examine whether breeding female Blue and Great Tits were benefitting energetically from food supplementation. In both the egg-laying and brood-rearing phases of reproduction food-supplemented females reduced their DEE. The analysis of energy expenditure provided fresh insights into the relative energetic costs of these breeding phases. Although some work has concluded that egg laying is an energetically costly phase in the annual cycle (Ricklefs 1974), Great Tits expended significantly less energy than during brood rearing (Chapter Five). This is the first time that such a result within free-living birds has been shown, and provides firm empirical evidence that not only is chick rearing significantly more expensive energetically than egg laying, but that female DEE during egg laying is positively correlated with DEE during brood rearing. Thus, females that were expending more energy laying eggs (i.e. the earliest breeding female Great Tits) were also expending more energy during brood rearing. This effect was independent of food supplementation treatment and provides additional evidence that females of 'higher phenotypic quality' (or those more able to exploit resources) may be able to breed earlier than 'lower quality' females (van Noordwijk & de Jong 1986, Verhulst *et al.* 1995, Christians *et al.* 2001). This was unrelated to the age of the female in my study, as age did not feature significantly in the DEE analyses. Unfortunately, measures of the energy expenditure of female Blue Tits during brood-rearing could not be obtained due to logistical difficulties in obtaining blood samples of sufficient volume. Were this to be possible, however, the comparison between DEE during egg laying and brood rearing in Blue Tits should provide additional insights into parental effort at different points in the reproductive cycle.

The measurement of DEE provided illuminating new evidence of the mechanisms underlying reproductive effort and parental investment. Increased (supplementary) food availability was able to reduce the energetic costs of egg laying so that although earlier laying was still relatively energetically costly (in Great Tits), it was less expensive than in birds breeding in areas which did not receive supplementary food (i.e. controls). Analysis also demonstrated that it may have been females in lower body condition that benefitted most from food supplementation with peanut cake, enabling them to initiate laying earlier. Such evidence supports the suggestion that there is a body condition threshold that females need to reach to start egg laying (Drent & Daan 1980, Rowe *et al.* 1994) and illustrates that food supplementation may permit females to overcome such constraints.

Brood rearing was more energetically demanding than the egg laying phase, and, although female provisioning rates seemed to indicate that females were meeting these demands, they actually reduced their DEE when food supplemented and also in response to increased male brood provisioning effort. Without the additional energy expenditure information, it would have appeared that female and male provisioning rates were unrelated and that food supplementation did not play a role in determining female provisioning effort. The validity of using measures of energy expenditure or metabolic rate for life history studies has been much questioned (e.g. Bryant 1988, Moreno *et al.* 1999), but in my study the DEE data provided clues as to how breeding females were actually using the supplementary food when more traditional metrics such as egg size, clutch size and adult provisioning rates would have been misleading.

7.5. Food availability and life history trade-offs

Both natural food availability and food supplementation were found to have significant but distinctly different effects on parental investment, offspring recruitment and adult survival of both Blue and Great Tits. The peanut cake supplement in particular was associated with a sequence of negative effects on the fecundity of Great Tits. It did facilitate an apparent shift in life history strategy in 2010 to boost residual reproductive value (i.e. by increasing adult female survival to the next breeding season) to the detriment of current reproductive value (i.e. higher nestling mortality and lower nestling body mass). This change in life history could provide evidence of a tendency towards a conservative bet-hedging strategy, where an individual maximises its fitness under varying environmental conditions over the long-term by reducing fitness in the short-term (Amundsen & Slagsvold 1998, Olofsson *et al.* 2009, Simons 2011). However, it would require multiple generations of data to determine if this is a bet-hedging strategy (Partridge & Harvey 1988, Simons 2011). To the best of my knowledge, this is the first time that an explicit change in life history strategy has been empirically demonstrated in a short-lived passerine in response to mistimed breeding and impoverished natural food availability. Such an alteration of life history strategy may not be expected in Blue Tits, however, because exceptionally early breeding as a result of food supplementation results in higher female mortality rather than reduced reproductive value (e.g. Norris 1993, Nilsson 1994).

Neither Blue nor Great Tit females used any energy savings from food supplementation to produce more offspring, nor did they appear to invest more in offspring, but channelled the energetic savings into improving their own survival. This suggests that the peanut cake supplement reduced the costs of self-maintenance for adults (e.g. reducing foraging effort), but that the time or energy saved was not reallocated to increasing fecundity, counter to expectations for short-lived highly fecund species (e.g. Song Sparrows – Arcese & Smith 1988, nuthatches [*Sitta* spp.] – Ghalambor & Martin 2000). However, food-supplemented male Blue and Great Tits increased their parental investment in terms of provisioning rate. This is possibly because the cumulative cost of reproduction is less for males than for females and so male survival is less threatened by increased investment in reproduction (Trivers 1972). Within my study population female survival was higher than that of males of both Blue and Great Tits (Chapter Six). Male survival is higher than female Blue and Great Tits in some other populations (e.g. Wytham Woods: Perrins & McCleery 1985, Gosler 1993), but lower in others (e.g. in rural and urban populations in Estonia: Hõrak & Lebreton 1998), so it is not clear whether increased investment in female survival represented a sex-specific shift in life history strategy. It is also worth reiterating that female Blue Tit survival only improved when they were supplemented with mealworms, which appears to indicate that the cost of reproduction is high in this species, but that a supplement of an appropriate nutritional value can reduce costs and improve survival prospects. Bearing in mind that most food supplements provided to birds in gardens are not part of their normal diet (e.g. peanuts [*Arachis hypogaea*], peanut cake, nyger seed [*Guizotia abyssinica*]), such differing results for two commonly provided supplements demand a re-examination of what we are feeding to birds.

7.6. Inter-specific responses to food supplementation

The similarity in energetic response to food supplementation, but their different translation into reproductive outputs may indicate diverging life history strategies or selection pressures between these two similar species. Great Tits used the food supplementation to advance their laying date and they paid fecundity, but not survival, costs in doing so. The ultimate result of this was no significant change in offspring recruitment and no increase in female survival in most years of study. Therefore, as a life history strategy it may not have increased their lifetime reproductive success. Conversely, female Blue Tits increased their survival without decreasing the number of offspring produced, and in this way may have significantly increased their lifetime reproductive success.

Although the drive to initiate laying earlier may have been due to selection pressure or a life history strategy, Great Tits may also have simply used food availability as a quantitative cue and misread information that predicted the timing of seasonal changes in natural food availability. This represents one of the potential downsides of the methodology used, in that the commencement of food supplementation could have acted as a seasonal trigger to accelerate breeding, a trigger to which Blue Tits were largely insensitive. Great Tits may have also used the food supplement as a qualitative cue to indicate the future quality of food resources during brood rearing and adjusted their clutch size downwards accordingly. Again, Blue Tits seem to be using different cues because their clutch sizes were unaffected by food supplementation.

7.7. Demographic effects of food supplementation

Previous work within this population showed that although food-supplemented Great Tit parents produced fewer fledglings than control birds, as a composite outcome of lower clutch sizes and higher nestling mortality, those fledging from the peanut cake and mealworms treatment area had higher mid-term survival (Harrison 2010). In terms of offspring recruitment, however, there were no significant differences between the food treatment groups, so from a demographic perspective the breeding population of Great Tits would be unaffected by long-term food supplementation. This lack of a difference could, however, mask differential dispersal, and further work is needed to determine whether food supplementation affected the sex ratio of recruits and also dispersal.

7.8. Future directions

My study has shown that food supplementation can have a dramatic effect on parental investment (i.e. decreased fecundity) of breeding Great Tits. However, food supplementation does not seem to provide any benefits to Blue Tits in terms of enabling an increase in parental investment. The primary limitation of this study, however, was that only one type of food supplement was tested in the early breeding season. Clearly, it would be of considerable interest to investigate the effects of different food supplements and also to examine the effects of the timing of food supplement provisioning. With such studies it will be possible to verify whether the reported detrimental effects of food supplementation on reproductive output in these species are restricted to peanut cake. Varying the start date of supplementation will enable a test of whether it is the sudden increase in food resources that triggers an accelerated breeding season in Great Tits, or whether it is the absolute amount of food available.

Although my study has explored some of the physiological impacts of food availability on the reproductive performance and survival of breeding birds, there remain disconcertingly few physiological studies that have manipulated food availability. The use of more precise indicators of health and body condition of both nestlings and parents using immunocompetence challenges could provide even more information on the physiological effects of food supplementation. Integrating these measures with those of stress responses and hormone levels could also enlighten our understanding of how life history traits are influenced (Zera & Harshman 2001, Williams 2005). Provisioning birds with added nutrients such as antioxidants (e.g. vitamin E – Plummer 2011) may provide benefits during the breeding season by reducing oxidative stress such as could be caused by consumption of fat-based supplements. Examining the oxidative stress levels of breeding adults could provide clear evidence of whether fat-based products such as peanut cake constitute a good dietary supplement for garden birds during the breeding season.

Peanut cake is composed of 50% beef tallow, which is not part of the natural diet of most garden birds: of the 39 species defined as ‘garden birds’ by British Trust for Ornithology (BTO) Garden BirdWatch data (BTO 2012), only nine (or 23%) of them could be considered as regular consumers of animal fats. These species either regularly eat small mammals such as Tawny Owls (*Strix aluco*) or Eurasian Sparrowhawks (*Accipiter nisus*), or prey on the nestlings of other species through the breeding season, such as Carrion Crows, Eurasian Magpies and Great Spotted Woodpeckers (*Dendrocopos major*). Clearly there is little precedent for consumption of animal fats by passerines in particular during the breeding season, so there could be a variety of unanticipated side-effects associated with the feeding of animal fats, such as those contained within peanut cake, to garden birds. Again, only detailed studies of the effects of food supplementation during the breeding season on their physiology will clarify whether it would be preferable to provide supplements that reflect the normal diet of a breeding bird (e.g. through 'wildlife gardening' – Soper 2006).

The two areas which constitute the greatest unknowns in the avian annual cycle, especially of short-lived species, are post-fledging survival and moult. Individual marking and radio-tracking of fledglings could answer the questions as to whether post-fledging food supplementation can aid birds as they reach nutritional independence and whether it reduces dispersal. Breeding early enables parents to moult earlier and produce better quality plumage which is then linked to improved overwinter survival (Dawson *et al.* 2000). Given that supplemented parents bred earlier and that some parents began moulting whilst the food supplement was still present in the study site, it is important to understand whether this confers advantages in terms of earlier timing of moult. This would provide key information on the fecundity/survival trade-off mechanisms in Blue and Great Tits.

Finally, there is a clear need for further research into the effects of supplementary feeding on the physiology and life history strategies of other avian taxonomic groups. Taking the UK as an example, the implications of my study may also be applicable to feeding of species common in our gardens, such as Common Blackbirds (*Turdus merula*) (e.g. Magrath 1992), European Nuthatches (*Sitta europaea*) (e.g. Enoksson 1990) and House Sparrows (Vaclav *et al.* 2003). With species such as Common Blackbirds and Dunnocks having markedly different life histories from Blue and Great Tits, however, targeted research would be required to examine how food supplementation affects their life history strategies. There is an imperative to clarify how different types of anthropogenic food affect avian physiology, breeding parameters and lifetime reproductive success. My results may also have implications for studies involving wider scale diffuse feeding of birds (e.g. of farmland birds – Siriwardena *et al.* 2007) or for indirect food supplementation of birds. With more than 50% of the human population now living in towns or cities (United Nations 2009), birds may come to rely ever more on anthropogenic foods, which, consequently, may dictate which species thrive and which decline (Fuller *et al.* 2008). With so many members of the public keen to engage in wildlife-friendly activities such as garden bird feeding, the responsibility lies with researchers to clarify which supplements are most suitable and when they should be provided.

7.9. Conclusions

Food supplementation with a fat-based supplement has negative consequences for the timing and success of breeding attempts in Great Tits, resulting in reduced fecundity. These effects do not persist into the long-term, however, so there are likely to be no demographic consequences to this reduction in fecundity. Food supplementation appears to affect some individuals more than others, and could encourage birds in lower body condition to alter their reproductive strategies. Blue Tits are largely unaffected by food supplementation, with respect to both reproductive timing and fecundity. This may be because they use different cues to trigger their reproductive effort from those used by Great Tits. The provision of a live invertebrate supplement, which can be consumed by adults and also fed to nestlings, may significantly improve reproductive performance of Great Tits and survival of Blue Tit adults. Food supplementation has a profound effect on the energy expenditure of breeding females and provides new insights into the complexity of life history strategies and ecological interactions of seasonally breeding bird species. These insights suggest that we may need to revise our dichotomous opinion of income and capital breeders (Drent & Daan 1980). It is also necessary to reconsider that even at the more extreme *r*-selected end of the *r/K* continuum, species may employ far more intricate and subtle life history strategies than previously surmised. Only through the integration of physiological techniques, ecological interactions and life history frameworks can we fully appreciate how an organism interacts with its environment and our potential impact thereupon.

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Appendix One

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Appendix Two

Breeding parameters from the full six-year dataset

Table A.2.1. Results from GLMM analyses of specified breeding parameters (‘Response’) of Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK between 2006 and 2011, regressed on significant explanatory variables (‘Fixed Effect’). Tested fixed effects included dietary treatment, where Intercept = unsupplemented (control), PC = supplemented with peanut cake and PCM = supplemented with peanut cake and mealworms. All models used either Poisson or negative binomial error distributions and corrected for over- or underdispersion where necessary. Nestling mortality models also correct for zero-inflation. Akaike’s Information Criterion (AIC) values for comparative model fitting or log-likelihood (Log-lik.) measures of model fit are included where appropriate and chi-squared results from likelihood ratio tests (Chisq) detail the relative contribution of each fixed effect. Only significant contributors to each model are shown. The *P* values for significant terms are highlighted in bold. For details of statistical tests please see: Chapter Two for laying date and hatch date analyses; Chapter Three for clutch size analyses; and Chapter Four for nestling mortality and brood size analyses.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	**AIC / *Log-lik.***
Laying Date	BT	Intercept	26.01	0.02				750
		Dietary Treatment: PC	24.42	0.02	8.84	**< 0.01**	Earlier lay date in PC treatment	
2006-2011		Year			510.78	**< 0.001**	2006: latest year 2009: earliest year	
Laying Date	GT	Intercept	25.70	0.03				515
		Dietary Treatment: PC	22.19	0.02	37.31	**< 0.001**	Earlier lay date in PC treatment	
2006-2011		Year			286.16	**< 0.001**	2006: latest year 2009: earliest year	
Hatch Date	BT	Intercept	48.41	0.06				240
		Dietary Treatment: PC	55.47	0.08	3.01	0.08	Later hatching in PC treatment	
		Year			273.57	**< 0.001**	2006: latest year 2011: earliest year	
		Clutch Size	0.14	0.01	9.88	0.51		
2006-2011		Dietary Treatment × Clutch Size	-0.80	0.01	5.55	**< 0.05**	Earlier hatching with larger clutch size in PC area	

Hatch Date	GT	Intercept	42.04	0.05				207
		Dietary Treatment: PC	38.08	0.02	42.51	**< 0.001**	Earlier hatching in PC treatment	
		Year			15.29	**< 0.01**	2006: latest year 2009: earliest year	
2006-2011		Clutch Size	0.48	0.004	74.03	**< 0.01**	Later hatching with larger clutch size	
Clutch Size	BT	Intercept	16.622					151
		Dietary Treatment: PC	16.086	0.027	1.46	0.23		
		Laying Date	-0.216	0.003	36.54	**< 0.001**	Lower clutch size	
		Year			38.87	**< 0.001**		
2006-2011		Egg Removal	-1.324	0.032	6.92	**< 0.01**	Lower clutch size	
Clutch Size	GT	Intercept	12.280					164
		Dietary Treatment: PC	11.447	0.036	3.79	**0.05**	Lower clutch size	
		Laying Date	-0.105	0.004	6.11	**< 0.05**	Lower clutch size	
		Year			13.29	**< 0.05**		
2006-2011		Egg Removal	-1.809	0.039	16.78	**< 0.001**	Lower clutch size	
Nestling Mortality	BT	Intercept	0.00	1.00				-762.19
		Dietary Treatment: PC	0.004	0.1513	11.10	**< 0.001**	Higher mortality	
		Dietary Treatment: PCM	0.003	0.1825		0.10		
		Year			59.7	**< 0.001**	2006: lowest 2009: highest	
		Number Hatched	0.003	0.032	46.21	**< 0.001**	Higher mortality	
2006-2011		Hatch Date	0.002	0.015	17.74	**< 0.001**	Higher mortality	
Nestling Mortality	GT	Intercept	0.019					-558.37
		Dietary Treatment: PC	0.053	0.145	47.37	**< 0.001**	Higher mortality	
		Dietary Treatment: PCM	0.036	0.184		**< 0.001**	Higher mortality	
		Year			66.14	**< 0.001**		
2006-2011		Number Hatched	0.005	0.033	48.94	**< 0.001**	Higher mortality	

Brood Size	BT	Intercept	15.645					NA
		Dietary Treatment: PC	14.533	0.026	8.4	**< 0.01**	Lower brood size	
		Dietary Treatment: PCM	14.909	0.029	2.8	0.10		
		Year			19.7	**< 0.01**		
		Hatch Date	-0.122	0.005	2.4	0.13		
2006-2011		Year × Hatch Date			21.8	**< 0.001**		
Brood Size	GT	Intercept	8.560					NA
		Dietary Treatment: PC	7.869	0.034	6.2	**0.01**	Lower brood size	
		Dietary Treatment: PCM	8.089	0.039	2.2	0.15		
		Year			31.1	**< 0.001**		
		Hatch Date	0.007	0.007	0.01	0.91		
2006-2011		Year × Hatch Date			23.4	**< 0.001**		

Table A.2.2. Inter-annual comparisons of breeding parameters of Blue and Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK. Comparisons are for laying dates, mismatch between the timing of peak caterpillar availability and peak nestling nutritional demand from the caterpillar peak, and of variation in total caterpillar biomass. Data are from Tukey post-hoc tests of models in Tables 2.1, 2.2 and 2.3. *** = *P*<0.001, ** = *P*<0.01 and * = *P*<0.05. See Chapter Two for details of statistical tests applied and analysis of results.

Year Comparison	**BT Laying Date**	**GT Laying Date**	**BT Mismatch**	**GT Mismatch**	**Total Caterpillar Biomass**
2006 vs 2007	1.59 ***	1.53 ***			
2006 vs 2008	1.24***	1.31 ***			
2006 vs 2009	1.94***	1.77 ***			
2006 vs 2010	1.20 ***	1.12 **			
2006 vs 2011	1.71 ***	1.624***			
2007 vs 2008	-1.28 ***	-1.17 **	1.19	-1.43 *	0.41 ***
2007 vs 2009	1.22 ***	1.16 *			
2007 vs 2010	-1.33 ***	-1.37 ***	1.14	-1.66 ***	0.35 ***
2007 vs 2011	1.07	1.06	-1.83 ***	-1.92 ***	0.03
2008 vs 2009	1.56 ***	1.35***			
2008 vs 2010	-1.03	-1.17 ***	-1.04	-1.16	-0.06
2008 vs 2011	1.38***	1.24 ***	-2.18 ***	-1.34	-0.38 ***
2009 vs 2010	-1.61***	-1.58 ***			
2009 vs 2011	-1.14 *	-1.09			
2010 vs 2011	1.42***	1.45 ***	-2.09 ***	-1.16	-0.32***

Appendix Three

Nestbox Occupancy

One of the fundamental questions that needed to be established as part of my study was whether there was any difference in nestbox occupancy between food supplementation treatments. Higher occupancy in the food-supplemented areas could have indicated that a wider sample of the population was breeding in these areas and could have affected the interpretation of other results.

Methods

A nestbox was defined as being occupied if eggs were laid in it, and no account was made for subsequent successful hatching or rearing of the brood. Total occupancy was examined in relation to year, to the woodland block in which the nestbox was located, and to the rotating dietary treatment which was assigned to the woodland block in each year (see Chapter Two for nestbox monitoring and supplementation methods). The data were further separated into species (i.e. Blue or Great Tit) and each parameter re-examined at the species level. All analyses logistic regression were conducted in R version 2.13.1 (R Development Core Team 2011), using Generalised Linear Models (GLMs) with a binomial error distribution. Models were corrected for underdispersion where necessary.

Results

There was no consistent difference in occupancy of nestboxes between the food supplementation treatment areas, either at the total population level, or the individual level (Fig. A.3.1, Table A.3.1). There was significant inter-annual variation in nestbox occupancy

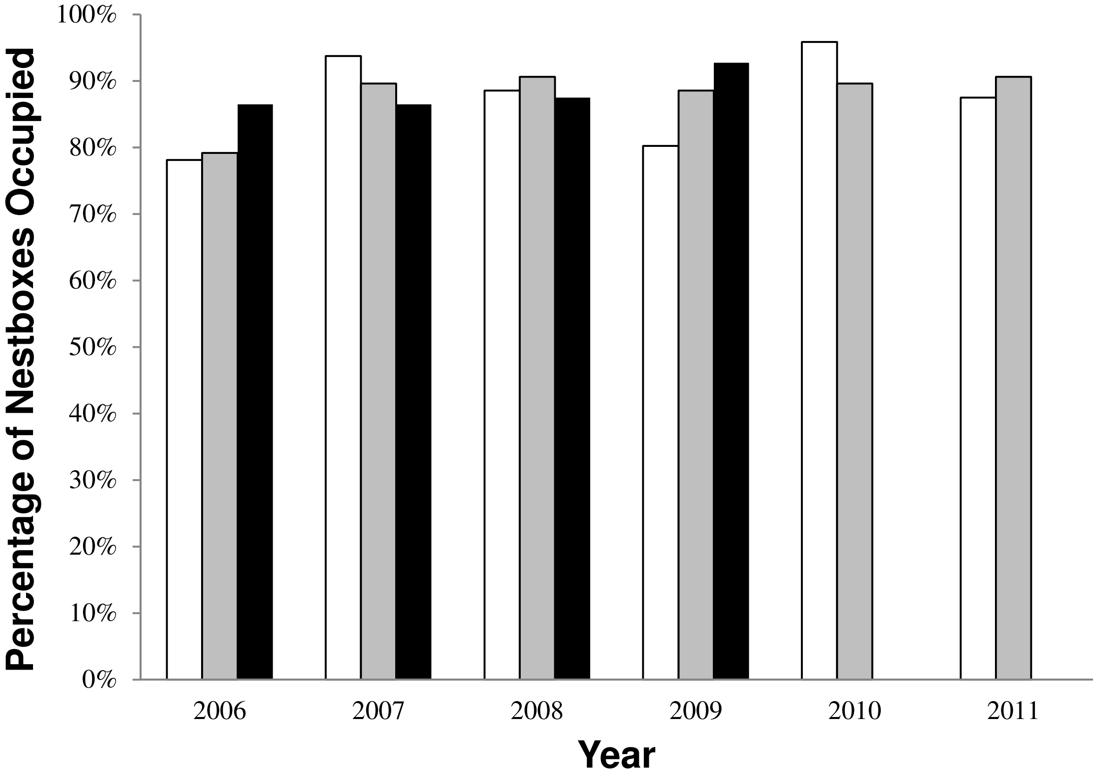

Figure A.3.1. The percentage of nestboxes occupied by either Blue or Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2006 to 2011. Occupied nestboxes in the unsupplemented (control) areas are represented by open bars, those in the peanut cake-supplemented areas by grey-filled bars, and those in the peanut cake and mealworms-supplemented areas by black-filled bars. Please refer to Chapter Two for details of nestbox monitoring and food supplementation.

Table A.3.1. Results from GLM analyses of specified occupancy parameters (‘Response’) of Blue (BT) and Great (GT) Tits breeding in Chaddesley Woods National Nature Reserve, Worcs., UK between 2006 and 2011, regressed on significant explanatory variables (‘Fixed Effect’). Each explanatory variable was tested separately. All models used a binomial error distribution and were corrected for underdispersion where necessary. Tested fixed effects were year, woodland block (‘Area’, where CP = Coalpit Coppice, CW = Chaddesley Wood and SHW = Santery Hill Wood) and food supplementation treatment (Dietary Treatment, where ‘CON’ = control (unsupplemented) and ‘PC’ = supplemented with peanut cake). Chi-squared results from likelihood ratio tests (Chisq) detail the relative contribution of each fixed effect. The P values for significant terms are highlighted in bold and a *pseudo* R^2 value is presented for model comparison.

Response	**Sp.**	**Fixed Effect**	**Estimate**	**SE**	***Chisq***	***P***	**Direction**	R^2
Total Occupancy	NA	Year			1.18	**< 0.05**	Highest: 2010 Lowest: 2009	0.53
Total Occupancy	NA	Area: CP	0.92	0.020	0.83	**< 0.05**	Highest: CP Lowest: CW	0.37
		Area: CW	0.85	0.030				
		Area: SHW	0.86	0.030				
Total Occupancy	NA	Dietary Treatment: CON	0.87	0.024	0.01	0.77		0.01
		Dietary Treatment: PC	0.88	0.031				
Total Occupancy	BT	Year			7.38	**< 0.001**	Highest: 2007 Lowest: 2010	0.81
Total Occupancy	BT	Area: CP	0.47	0.054	1.93	0.17		
		Area: CW	0.50	0.081				
		Area: SHW	0.54	0.079				
Total Occupancy	BT	Dietary Treatment: CON	0.51	0.072	0.10	0.75		0.01
		Dietary Treatment: PC	0.49	0.091				
Total Occupancy	GT	Year			17.03	**< 0.01**	Highest: 2010 Lowest: 2006	0.69
Total Occupancy	GT	Area: CP	0.44	0.075	8.382	**< 0.05**	Highest: CP Lowest: SHW	0.34
		Area: CW	0.37	0.116				
		Area: SHW	0.32	0.120				
Total Occupancy	GT	Dietary Treatment: CON	0.37	0.080	0.261	0.61		0.01
		Dietary Treatment: PC	0.39	0.101				

(Table A.3.1), in both Blue and Great Tits. There was no significant variation in nestbox occupancy by Blue Tits across the woodland blocks (Table A.3.1), but Great Tits had significantly higher nestbox occupancy in the Coalpit Coppice area than in the Santery Hill Wood area (Table A.3.1). The proportion of each species breeding in the whole study site as a proportion of the total number of occupied nestboxes changed over time, with a higher proportion of Blue Tits in the early years of the study (Figure A.3.2).

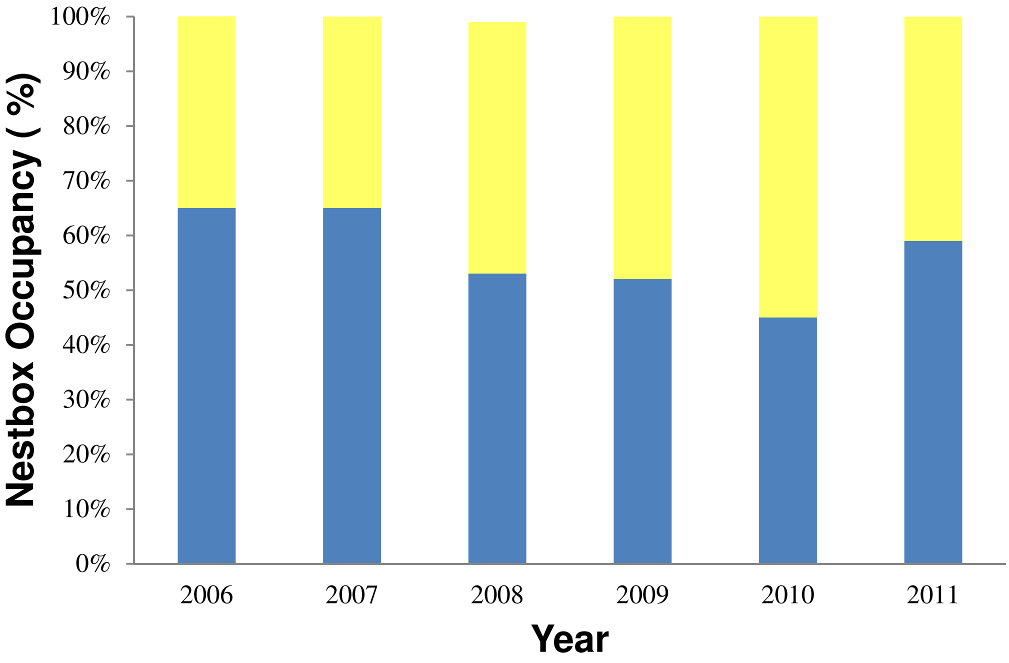

Figure A.3.2. The percentage of nestboxes occupied by either Blue or Great Tits breeding in Chaddesley Woods National Nature Reserve in Worcs., UK from 2006 to 2011. The proportion of nestboxes occupied by Blue Tits is represented in blue and that occupied by Great Tits is represented in yellow. Please refer to Chapter Two for details of nestbox monitoring.

The proportion of Great Tits increased until 2011 when the proportion of Blue Tits increased again.

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