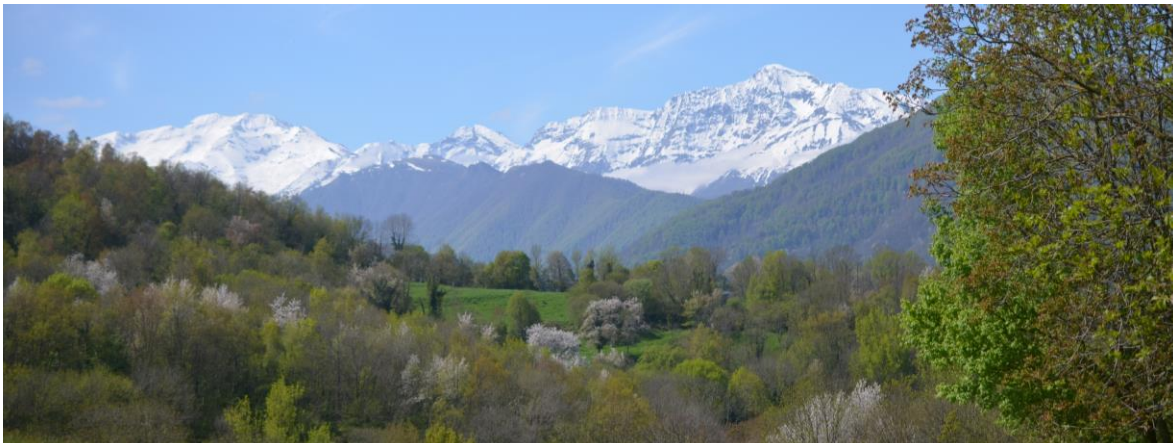
Parental Investment across an Altitudinal Gradient in Blue Tits (*Cyanistes caeruleus*)



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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: …………………………………………………………

To Helen and Philip Hull

Abstract

Environmental gradients can help shed light on the evolution of life history strategies such as parental investment. Parental investment is crucial for the fitness of many species. In this thesis, I examine reproductive investment dynamics in the Eurasian blue tit (*Cyanistes caeruleus*) in the French Pyrenees and assess potential differences in reproductive measures across an altitudinal gradient that creates variation in environmental “harshness”. Further, I investigate fine-scale aspects of bi-parental care, such as investment tactics in current reproduction, and sex differences in contributions to offspring care. To do so, I used a mixture of observational and experimental data, collected over a total of six breeding seasons from over 500 blue tits nests. I showed that breeding conditions are “harsher” due to colder temperatures with increasing elevation, leading to changes in reproductive timing and output. I found that increasing altitude leads to decreased hatching success. Nevertheless, clutch size and brood mortality is comparable across the gradient. A shift to a lower, but qualitatively comparable reproductive output may be part of a slower “pace of life” strategies pursued at high relative to low altitudes. From experimental data, I also found that parental investment is positively linked across different phases within one reproductive attempt. Finally, in line with theory, a temporary brood manipulation revealed that parents balance the benefits and costs of reproduction by partially compensating for changes in brood size. Parents also responded in similar ways to brood size. Overall, the findings presented in this thesis highlight the importance of mechanisms to fine-tune reproduction to maximise reproductive fitness. I suggest that initial reproductive decisions such as timing and amount of offspring produced heavily shape the success of a reproductive attempt. These results have implications for current versus future reproductive trade-offs in life history theory, in particular for short-lived species.

Acknowledgements

“*When the student is ready the teacher will appear.*” (Buddhist proverb)

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Eurasian blue tit (*Cyanistes caeruleus*, Linnaeus 1758)

The study species being characteristically aggressive when caught.

Chapter One

General introduction

1.1 General framework

A key area and driver of evolutionary biology is how animals, including humans, invest in reproduction due to the potent influence on individuals' fitness. Reproductive or parental investment is defined as "*any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring*" (Trivers 1972) and other fitness contributors such as parental survival and growth (Clutton-Brock 1991). Reproductive investment is integral to species' life histories (Stearns 1992), particularly as animals must balance the costs and benefits of reproduction to maximise life-time fitness (Williams 1966). Studies of parental investment thus have the potential to greatly enhance our understanding of underlying mechanisms driving evolutionary processes. In the following, I will highlight the historical origins of the concepts of reproductive investment and parental care, different parental care systems, existing gaps in our understanding of the forms and maintenance of bi-parental care today and which of these gaps I will address in this PhD thesis, along with the methodology to do so.

1.1.1 Reproductive investment

Surprisingly, even though key to species' life histories, the field of reproductive investment is less than a century old. In the 1930s, Ronald Fisher was one of the first to acknowledge its importance in shaping natural selection in his book entitled "*The Genetical Theory of Natural Selection*", built on the works of Charles Darwin and the founder of modern genetics; Gregor Mendel. Fisher was a pioneer in highlighting that reproductive investment should vary depending on the expected current and future fitness returns (Fisher 1930; Roff

1992; Stearns 1992; Houston and McNamara 1999). Further, Angus Bateman (1948) developed a principle in fruit flies (*Drosophila melanogaster*) that stated that lower reproductive variance should be found in females than males as their reproductive success does not benefit from a larger number of mates, but also that females invest more in offspring and are in turn more important for an offspring's reproductive success than males. Later, a theoretical model by C. Smith and Fretwell (1974) demonstrated that investment in offspring number and quality is inversely related, and that higher investment leads to greater offspring fitness. Around this time, Robert Trivers also explored reproductive investment based on human sexual behaviour in the book "*Parental investment and sexual selection*" (1972). Trivers expanded on Bateman‘s principle showing a difference in reproductive investment between the sexes; with females investing higher in the production of eggs than males invested in sperm and thus being key to sexual selection. This was in stark contrast to Fisher's idea that the cost of reproductive investment should be the same between the sexes (Fisher 1930). In support of Bateman's and Trivers' arguments, parental care (a form of reproductive investment) exhibits unequal costs and responsibilities between the sexes (Kokko and Jennions 2008; Klug et al. 2013).

1.1.2 Parental care

Parental care was first defined by Clutton-Brock (1991) as "*any form of parental behaviour that appears likely to increase the fitness of a parent's offspring*". However, in this thesis I use a more specific, updated definition of parental care proposed by Royle and colleagues (2012) as being "*parental behaviour that 1) occurs post-fertilization (or after the production of daughter cells if reproduction is asexual), 2) is directed at offspring, and 3) appears likely to increase offspring* *lifetime reproductive success*". Thus, behaviours that have possibly not evolved to increase offspring fitness are excluded from this latter definition.

Parental care is rare, though can be found in diverse forms across the animal kingdom (Clutton-Brock 1991; Alonzo 2010; Royle et al. 2012). Parental care ranges from simply provisioning gametes with one-off nutrient transfers or selecting optimal sites for oviposition to more sophisticated parent-offspring interactions such as food provisioning after birth (Royle et al. 2012). Such parental care can last for extensive periods; for example in mammals young are often tended for until and past entering adulthood (Gubernick 2013). In humans this parental care period is exceptionally long for primates – up to 20 years (Howell 1979; Hawkes et al. 1998; Hill and Kaplan 1999). Amphibians such as alpine salamanders (*Salamandra lanzai*) also have a relatively long dependency period with young spending up to four years *in utero* (Miaud et al. 2001). In general, offspring will benefit from a longer duration of parental care, however this will also prevent parents from reproducing again sooner rather than later (Trivers 1972; Maynard Smith 1977; Clutton-Brock and Vincent 1991). Thus, parental care clearly carries costs. In extreme cases such as social spiders (*Stegodyphus dumicola*), the mother sacrifices her body to be eaten by the offspring – a process called matriphagy (Junghanns et al. 2017). This matriphagy occurs after the female has invested multiply in egg production, egg sac tending and regurgitation feeding. This example illustrates that parental investment decisions must be made at different stages of a breeding attempt to minimise reproductive costs.

Trade-offs between current versus future reproductive investment choices have been demonstrated (Tinbergen and Both 1999, and references within). For example, in burying beetles (*Nicrophorus orbicollis*) those females experimentally forced to produce more offspring experienced lower lifetime fecundity and died at a younger age than controls (Creighton et al. 2009). Females, which were given larger carcasses to raise their young on, also invest higher in current than future reproduction. Contrastingly, less attention has been paid to trade-offs in parental care between different time points of one breeding attempt. These intra-seasonal trade-offs may however be crucial for our understanding of overall life history strategies. The few empirical studies to date have focused on long-lived bird species such as common terns (*Sterna hirundo*; Heaney and Monaghan, 1995) and lesser black-backed gulls (*Larus fuscus*; Monaghan et al., 1998). Negative trade-offs between stages of a single breeding attempt were found in these studies. For instance, in the lesser black-backed gull study females that were induced to lay an additional egg, though obtained the same number of chicks as controls, reared these at a lower rearing quality and thus had lighter chicks. However, shorter-lived species should invest higher in a current breeding attempt than longer-lived species, as their future reproductive chances (residual reproductive value) are lower, leading to possible diverging results than those found in long-lived species (Stearns 1992). To my knowledge, no empirical studies so far have directly tested these trade-offs within a breeding attempt in short-lived species (though see evidence of additive female fitness consequences in Visser and Lessells, 2001). Furthermore, early high investment by one partner, such as mothers having to produce the offspring, may impact other caregivers such as fathers at later stages of rearing, adding another facet to the story (Russell et al. 2007, 2008; Savage et al. 2013a).

1.1.3 Systems of parental care

Offspring can be reared by differing number of caregivers and this has consequences for their fitness and that of the caregivers (Clutton-Brock 1991). On one side of the care spectrum, a single parent is solely responsible for rearing young. For instance, paternal care is common in fish performing care (Klug et al. 2013). In black-striped pipefish (*Syngnathus abaster*), males carry the entire cost of pregnancy by brooding the eggs until hatching, leading to sex-role-reversal (Wilson et al. 2001; Cunha et al. 2017). In invertebrates and mammals, maternal care can be more frequently found (Klug et al. 2013). Maternal care is obligatory in mammals, as the offspring are dependent on milk produced by the mother after birth (Royle et al. 2012). However, at the other extreme a small minority of mammals and birds perform cooperative breeding (Cockburn 2006; Lukas and Clutton-Brock 2013); where caregivers other than the genetic parents help raise the offspring (Royle et al. 2012). In eusocial insect systems such as honey bees (genus *Apis)* cooperative breeding can even lead to sterilisation of some individuals in the social group (Naeger et al. 2013). Central on the spectrum lies bi-parental care. A tenth of mammal species fall into this care system (Reynolds et al. 2002), with male contribution being shown to increase litter size, decrease female lactation period and thus enable more frequent breeding (West and Capellini, 2016; though also see (Stockley and Hobson 2016). Around 80 % of extant bird species perform bi-parental care (Kendeigh 1952; Cockburn 2006).

Bi-parental care leads to a fascinating interplay between conflict and cooperation as two unrelated individuals together raise genetic offspring. Each member benefits from minimising their investment cost and taking advantage of their partner working harder in rearing (Trivers 1972). This evolutionary “game” has gripped the attention of many theoreticians over the last 40 years. Starting in the 1980s, Houston and Davies (1985) developed a ‘sealed bid’ model, where parents invested at a fixed level after making a single choice in investment (also see Chase 1980). These theoreticians built on previous models of genetically fixed parental investment (Maynard Smith 1977), though instead viewed parental care as a facultative behavioural reaction (Chase 1980). They found that the evolutionary stable strategy (ESS) was partial compensation, predicting that any change in one parent’s care level will be matched by the partner partially. For example, if a female drops her feeding rate the male will increase his rate, though insufficiently to match the original total provisioning level. This strategy should limit the occurrence of cheating at the population level. McNamara and colleagues (1999) updated these early bi-parental models by incorporating negotiation at different points during rearing. Thus, the partners respond directly to each other’s efforts. Partial compensation was again found to be the ESS. Average results of a meta-analysis of 54 bird empirical studies support this theoretical ESS, though many exceptions exist (Harrison et al. 2009). One explanation for these exceptions may be that parents match each other’s care levels when an asynchrony in information levels exists between the sexes (Hinde 2006; Johnstone and Hinde 2006; Meade et al. 2011). Thus, the parent with less information may use their partner’s rearing effort as a sign of brood demand and thus match their effort. Another explanation for deviations from traditional predictions may be that most classic models do not consider how environmental variation may affects the cost and benefit of parental investment and thus cooperation between the sexes over offspring care.

1.1.4 Environmental variation

One of the first parental investment models incorporating environmental variation found that under higher variability parental fitness should benefit from investing equally in each offspring (McGinley et al. 1987). However, empirical studies often find a large variation in offspring sizes in more variable environments, maybe due to developmental constraints such as maturation rate. In harsher and more heterogenous conditions life history theory predicts that parents should invest higher into each offspring to increase their survival chances, rather than into producing more offspring (Smith and Fretwell 1974; Lloyd 1987; Stearns 1992). In support of this, Atlantic salmon (*Salmo salar*) show a conservative bet-hedging by producing less but higher quality offspring in such variable environments (Einum and Fleming 2004). These reproductive investment strategies may be part of a larger “pace of life” strategy characterising species’ life histories, which is dependent on environmental conditions (MacArthur and Wilson 1967; Wilbur et al. 1974; Stearns 1976, 1977; Ellis et al. 2009). It was shown in the erpobdellid leech (*Nephelopsi obscura*), that lower environmental predictability such as in temperature, led to higher mortality risk and plasticity, with individuals flexibly shifting their reproductive investment strategy along the “pace of life” gradient to match their environment (Baird et al. 1986). Specifically, a slow “pace of life” is characterised by longer developmental periods, lower reproductive rates, though higher levels of parental care to increase offspring recruitment and life expectancy in more variable environments (Gaillard et al. 1989; Ricklefs and Wikelski 2002; Réale et al. 2010). In support, heightened parental care has been found to increase fitness in more variable environments (Bonsall and Klug 2011).

The effect of environmental variation on parental investment is of particular interest under current climate change. Climate change is leading to increases in global temperature and variance of weather patterns including a growing record of exceptional climatic events (Intergovernmental Panel on Climate Change 2014). These two effects combined are having an impact on key reproductive investment decisions (Parmesan 2006). For example, species are shifting offspring production to match optimum environmental conditions; which has been recorded in a diverse array of taxa, presumably as an adaptive response to climatic changes (e.g. resident and migratory birds - Charmantier et al. 2008; Hüppop and Hüppop 2011; fish - Crozier and Hutchings 2014; insects - Andrew et al. 2013; amphibians and reptiles – Urban et al., 2014; though see Lyon et al., 2008). These shifts may be viewed as parental effects, by which mothers and fathers, through their own capacity to plastically invest in offspring, might be able to generate early, non-genetic channels, such as the hormonal content of eggs or nesting site choices, which inform the next generation before or soon after birth of prevailing environmental conditions (Cheverud and Moore 1994; Badyaev and Uller 2009; Wolf and Wade 2009). These maternal effects might hasten the speed of evolution by generating offspring plastically suited to their environment (Mousseau et al. 2009); though the reverse may also be true by shielding genotypes from selection (Räsänen and Kruuk 2007) - the debate is still ongoing. The direct knock-on effects of these parental mechanisms on population-level reproductive decisions and fitness in a changing climate have been hard to decipher, as measuring climate change effects require decades of data collection.

Instead of longitudinal studies, more short-term, variable environmental systems may be used to understand climate change responses. Environmental gradients offer an opportunity to study the interaction between parental investment strategies and environmental harshness, and consequently its effect on reproductive fitness. Extreme, harsh environments may provide organisms with greater selective challenges, leading to a stronger role of phenotypic plasticity in reproductive strategies and further in directing species' evolution (Rotkopf and Ovadia 2014). Some evidence comes from microbial communities, which live in extreme habitats (acid mine drainages, saline lakes or hot springs). These populations evolve faster than their counterparts in more lenient conditions (Li et al. 2014). Latitudinal gradients have been the norm to study environment-dependent life history trade-offs, mostly focusing on temperate north-south clines. Congruent with theory, female coho salmon (*Oncorhynchus kisutch*) lay fewer, though larger eggs to combat stronger competition for lower resources and increased predator risk at more southern latitudes (Fleming and Gross, 1990). Similar quantity-quality trade-offs have been demonstrated in various other taxa solely investing in eggs (Fox et al. 1997; Armbruster et al. 2001; Johnston and Leggett 2002; Khokhlova et al. 2014), and in species such as birds with more extensive parental care (Smith et al. 1989; Järvinen 1996; Encabo et al. 2002). However, much variation exists in the specific investment choices made and reproductive trade-offs may not only occur in a two-dimensional manner. For example, a meta-analysis looking at 135 different gallinaceous species found that the typical increase in clutch size observed with latitude and thus seasonal environments (Lack 1947; Jetz et al. 2008) may be confounded by an interaction with altitudinal gradients (Balasubramaniam and Rotenberry 2016).

Altitudinal gradients are a potent alternative proxy of climate change to latitudinal systems in investigating how parental investment strategies change with environmental variation. Compared to latitudinal gradients, they have smaller geographic ranges and thus minimise differences in day length and in genetic backgrounds of populations. More generally, montane environments constitute up to 25 % percent of the earth‘s surface, providing habitat for a vast amount of species (Meybeck et al. 2001; Spehn and Körner 2005). These environments are also one of the most vulnerable to climate change, leading to species range shifts, contractions and extinctions (Parmesan 2006; Sorte and Jetz 2010). Altitudinal gradients are characterised by drops in air temperature and oxygen levels, frequent extreme weather events such as sudden snow storms and summers being shorter with decreased plant and insect productivity, paralleling climate change (Rolland 2003; Körner 2007). These environmental changes affect species living at high altitudes (see review by Laiolo and Obeso, 2015). In plants, life history shifts have been found with altitude such as decreased body size, less investment into reproduction, though more into vegetal growth (Young et al. 2002; Hautier et al. 2009). Other examples come from animal taxa such as insects; grasshoppers (*Omocestus viridulus*) at high elevation have longer egg and juvenile developmental periods (Berner et al. 2004). In humans a shift to a slower “pace of life” has also been demonstrated; Andean Nuñoa women living above 4000 m have lower reproductive fitness and their children experience slower developmental time (Little and Baker 1976).

Birds invest greatly in reproduction with an extremely high prevalence of bi-parental care (80 %; Cockburn, 2006; Royle et al., 2012), leading to complex life history strategies. Phenotypic alterations with altitude have already been demonstrated in birds. Pre- and post-hatching parental investment, body fat levels and other morphological characteristics (e.g. wing length), and survival rates have been shown to change with altitude (Altshuler et al. 2004; Bears et al. 2009; Lu, Xin et al. 2011; Evans Ogden et al. 2012; Bastianelli et al. 2017). Empirical work points to a slower life history strategy, including longer developmental periods with altitude. In a meta-analysis of paired low versus high elevation bird populations, Boyle and colleagues (2016) found that the annual number of breeding attempts and early investment (clutch size) consistently decreased with altitude. For example, the number of fledglings per female was halved in dark-eyed juncos (*Junco hyemalis*) breeding at high compared to geographically close low altitudinal sites, though higher offspring survival rates existed at higher altitudes (Bears et al. 2009). Across 24 pairs of avian species, Badyaev and Ghalambor (2001) showed that male contribution to nestling feeding increased with altitude, at the cost of sexual traits. However, Boyle et al.'s meta-analysis (2016) found much variation at later stages of reproductive attempts in parental care and survival, which does not conform to the traditional slow "pace of life" suggested for harsher montane environments. This may be due to evolutionary constraints such as slow generation times and range edges of species (Laiolo and Obeso 2015). Overall, our understanding of the underlying mechanisms and the role of avian parental investment decisions in life history evolution exhibit large gaps, in particular in the face of climate change.

1.2 PhD aims

The overall aim of this PhD is to investigate how parental investment strategies change with environmental harshness along an altitudinal gradient (a proxy for climate change) and what potential consequences this will have on fitness. In line with this, the overarching questions asked in this PhD are:

(a) Do parents, in particular mothers, change their reproductive investment depending on environmental harshness; i.e. along the altitudinal gradient? Does the altitudinal gradient highlight differences in investment strategies with the progression of the season? If so, do these changes in reproductive investment have consequences on reproductive output, specifically the quality and quantity of offspring? (Chapter Two)

(b) Do parents change their reproductive investment depending on environmental cues? In particular, do potential budburst cues play a role in adjusting reproductive timing with food availability? (Chapter Three)

(c) Can reproductive investment choices be balanced across different time points of a breeding attempt? Are there potential links between early and late investment choices? (Chapter Four)

(d) How do bi-parental systems coordinate reproductive investment in line with changing reproductive costs such as environmental harshness and brood demand? (Chapter Five)

(e) How can reproductive investment and parental care models be improved with these new thesis findings? What hypothetical impacts do these reproductive investment choices have in a changing world? (Chapter Six)

1.3 Study species

I aim to investigate these questions with a combination of observational and experimental studies. Specifically, I make use of a frequently used model organism, the Eurasian blue tit (*Cyanistes caeruleus).* The blue tit is a small cavity nesting, passerine bird occurring in the Western Palearctic (Föger and Pegoraro 2004). The IUCN (International Union for Conservation of Nature) red list assigns the blue tit Least Concern conservation status with large, even increasing populations sizes (BirdLife International 2016). It is a 12 g passerine, blue and yellow in colour, with small sexual dimorphism (Föger and Pegoraro 2004). Preferred habitats for breeding are mixed deciduous forests as opposed to coniferous stands. Generally this species occurs in lowlands, but the record for breeding has been set at 3500 meters above sea level in the Caucasus mountain range (Cramp and Perrins 1993; Föger and Pegoraro 2004). This socially monogamous species has been thoroughly studied since the 1850s due to its wide distribution and accessible breeding in artificial nest boxes and large, manipulable clutch sizes (5-15 eggs; Krüper 1853; Nur 1986). In this species only the female builds nests and incubates the eggs, though both parents provision the chicks (Cramp and Perrins 1993). Thus, conveniently I can manipulate maternal investment strategies at the early stages in this model organism and also investigate parental costs for both parents at the rearing stage, plus test for partner responses.

1.4 Study system

Within my fieldwork, I utilise a novel 1000 m altitudinal study system, located in the French Pyrenees mountain range. The French Pyrenees are characterised by relatively short, though steep valleys with mixed forests gradually turning into beech and fir stands above 900 m (Ninot et al. 2017). The tree line and the transition to mountain pastures is situated at ca. 1500 m, depending on the geological profile (Prodon et al. 2002). Additionally, since the second half of the 20th century, abandonment of land and farming practice is leading to increases in forested areas (Gibon and Balent 2005; Mottet et al. 2006). The focus population breeds in an established nest box population (N = ca. 640) across a 450-1500 m altitudinal gradient. I have aimed to distribute the nest boxes evenly across the altitudinal gradient, however due to characteristics of the terrain (e.g. steep slopes) some irregularities and minor gaps exist (Fig. 1.1). Woodcrete nest boxes were installed before the first breeding season in 2012 with a distance of more than 50 m between neighbouring boxes. In addition, handcrafted bamboo poles are used to lift down nest boxes. Nest boxes are shared with other passerine species; mainly great tits (*Parus major*), coal tits (*Periparus ater*), marsh tits (*Poecile palustris*), and occasionally nuthatches (*Sitta europaea*). A full characterisation of the study system can be found in Chapter Two.

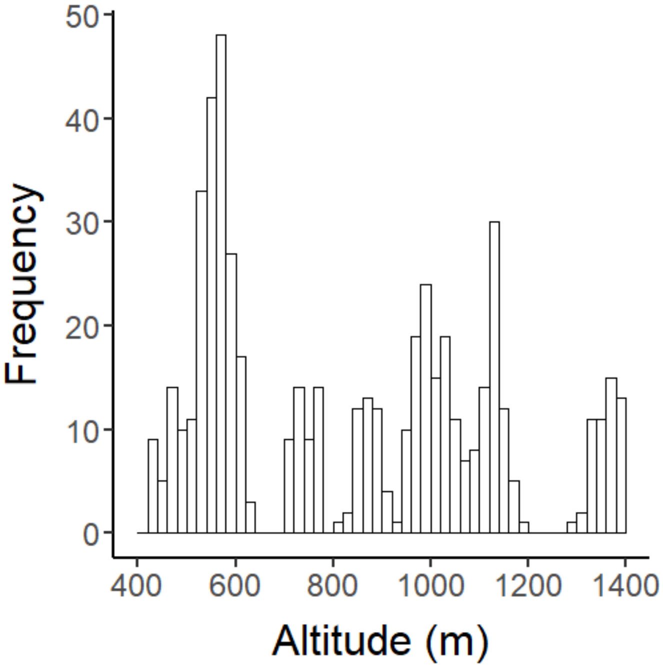

Figure 1.1: Frequency of nest boxes situated along the altitudinal gradient (50 m intervals).

1.5 Thesis outline

The first data chapter (Chapter Two) will investigate general breeding parameters of our blue tit system. Specifically, I will investigate the associations among altitude, breeding phenology, fecundity, productivity and nestling mass, from egg laying until fledging. Purely observational, climatic and reproductive data will be collated across six breeding seasons; including average daily temperature, clutch size, hatching and fledging numbers, fledging mass, and reproductive timing; i.e. first egg lay date. As part of a characterisation of the altitudinal gradient, I will first investigate if average daily temperature shifts with elevation using temperature logger data. A gradual altitudinal decline in temperature is predicted (Körner 2007). Furthermore, lay date has been found to be closely linked to temperature, and further to environmental phenology, i.e. tree and caterpillar development, which has consequences on later chick survival (McCleery and Perrins 1998; Sanz 2002). Thus, I predict that a delayed start of reproduction will be observed with increasing altitude, which should consequently affect later breeding parameters. Further, as the productive period is shorter at high altitude (Rolland 2003; Körner 2007), I predict that the reproductive output such as fledging numbers should be negatively affected. Specifically, I investigate phenological plasticity in response to altitude and year in this population. Second, I then investigate the effects of lay date and altitude on clutch size and hatching success, as a means of quantifying the phenotypic correlation between lay date and clutch size across the altitudinal gradient, and its effects on hatchability. Finally, I test the effects of lay date on fledging success and nestling mass to provide insights into phenological mismatch in this population, and whether such metrics of success are modified by phenology-fecundity associations.

The third chapter will look at if environmental cues such as budburst are used to differing degrees along the altitudinal gradient. As aforementioned, lay date has been found to be linked to environmental phenology, e.g. tree development, which has consequences on later chick survival (McCleery and Perrins 1998; Sanz 2002). To investigate if females can predict optimal prey availability and thus if hatch date is correlated with this, budburst will be used as a proxy. As temperature is lower at higher altitudes (Körner 2007; Chapter Two), I predict budburst to be delayed compared to lowlands. As a consequence, at higher altitudes there should be higher selective pressures to make use of environmental cues to time breeding, as the productive season is shortened, resulting in fewer reproductive opportunities. I also predict that budburst should be tracked more closely by higher elevation birds due to the vegetation being more homogenous than at low elevations thus facilitating environmental cue use. To decipher this relationship, I will look at observational phenological and reproductive data, specifically at how well budburst and lay date are matched with altitude and whether this temporal relationship affects reproductive output such as fledgling numbers and mass. Additionally, I will investigate if strategies are used to improve the association between budburst and hatching after laying.

The fourth chapter will focus in on how parental investment choices are linked across different phases of a single breeding attempt. I will investigate how experimentally manipulated investment choices in early breeding phases (the number of eggs laid) will affect later investment levels at the rearing stage. The rationale behind this experiment is that most studies have ignored the costs of egg laying and incubation to females (Oppliger et al. 1996; Monaghan and

Nager 1997). Both can contain a cost in various bird species, in particular for future fitness of the female (Reid et al. 2000; Visser and Lessells 2001; Nager et al. 2001). These early costs should impact later investment in offspring (Savage et al. 2013a), which should shift care contributions of the parent directly affected (females), and their partner (males). However, these costs may also affect future reproductive abilities and especially in short-lived species such as blue tits. Hypothetically, high early investment may lead to high investment at the rearing stage, as residual reproductive value is reduced if key resources are depleted faster and future survival is reduced (Stearns 1992). Thus, I predict that heightened early investment by females will affect later investment choices in the rearing phase, however decreased and increased investment are possible. To test these two predictions, females are made to lay additional eggs, though incubation and rearing costs are kept constant, as in control groups, which were not made to lay additional eggs. This is achieved by a cross-fostering approach (see Chapter Four for more detail). Later investment in the rearing stage is investigated by observational data on provisioning of both parents.

The fifth chapter will concentrate on the rearing stage and highlight how different environmental drivers influencing parental care. In particular, I will look at whether contributions of females and males change depending on altitude, year, caterpillar availability and intrinsic nest characteristics such as brood age and size. I predict that if environmental harshness (altitude) increases it will be harder for parents to provision at equivalent levels to their lowland counterparts. On the other hand, I predict that parents may respond in line with the “pace of life” framework, with high altitude individuals shifting to a slower pace resulting in higher parental care in fewer offspring (Hille and Cooper 2015; Boyle et al. 2016). Additionally, traditional theory does not consider differential task division between the sexes, such as nest sanitisation by the female and predator defence task by the male (Maynard Smith 1977; Klug et al. 2013). This should result in differential investment strategies. As part of this, I will first explore natural nestling provisioning. As brood size is a key fitness trait, it must be a crucial factor for investment choices. However, the sexes may have different optimal brood sizes, depending on previous and future investment choices. Thus secondarily, a temporary brood manipulation is performed to reveal possible underlying differences in provisioning strategies between the sexes, in response to artificially increased or decreased brood sizes, compared to controls, and if responses change with altitude. I will test classic models of bi-parental care predicting: (a) comparable provisioning contributions of males and females independently of ecology; (b) partial compensation response rules by both sexes; and (c) these partial response rules to be manifest as overall increases in nestling mass.

Globally these observational and experimental data chapters aim to investigate underlying drivers and mechanisms of bi-parental care in birds. Reproductive costs of both parents during the pre- and post-hatching stages will be manipulated naturally with use of the altitudinal gradient and through directed experiments to investigate underlying reproductive strategies. To conclude, the sixth chapter will constitute an overall discussion aiming to tie all the results together found during this PhD. I aim to highlight the novelty of these results for the field of parental care. I will be indicating overarching parental investment (care) strategies for this particular system. Impacts on potential species' evolutionary processes and endurance under climate change prognoses will be discussed.

Chapter Two

Extreme plasticity in breeding phenology across an altitudinal gradient: implications for understanding phenological mismatch

2.1 Abstract

There is a pressing need to understand whether and how populations respond to changing climates. To date, much of our understanding stems from longitudinal studies of sufficient duration to encapsulate climate shifts. While such studies provide essential insights, they obviously require significant time, and the magnitude of any effect measured is contingent upon the magnitude of inter-annual variation in climate; which is often modest. Here I use a 1000 m altitudinal gradient in the French Pyrenees to generate representative 2-3 °C differences in temperature faced by breeders in a population of blue tits (*Cyanistes caeruleus*). During the six years of study, I found that breeding phenology typically varied by ca. nine days within altitudinal zones, but was on average 11 days earlier at low versus high altitudes. Early breeding was generally associated with larger clutch sizes, which in conjunction with reduced nestling mortality, led to more young being fledged. However, compared with birds breeding at low elevation, those breeding at high elevations also laid larger clutches than expected for their lay dates. As a consequence, despite low elevation birds showing reduced probability of hatching failure and brood failure compared with high elevation birds, breeding success was similar across elevations. My results suggest that constraints on mean population plasticity are unlikely to explain phenological mismatches; and lead me to hypothesise that the answer lies with the relative quickening of development of ectothermic prey with warming springs, compounded by current selection on negative phenology-fecundity associations of endothermic predators.

2.2 Introduction

Recent meta-analyses indicate that organisms of diverse taxonomy are responding to climate change by advancing the timing of key life events, particularly reproduction (Thackeray et al. 2010, 2016). Phenological responses within populations appear to be largely plastic (Phillimore et al. 2010), and such plasticity is suggested to play a significant role in allowing populations to adapt in real time to changing climate (Parmesan 2006; Both et al. 2006, 2009a; Visser 2008; Visser et al. 2012; Gienapp et al. 2013). Nevertheless, whether plastic advances in breeding phenology (timing) are sufficient or adaptive will depend additionally on associated changes to reproductive investment, including fecundity and any subsequent levels of care. Despite this, less is known about potential constraints to plasticity or climatic impacts on adaptive associations among breeding phenology, key life history traits and metrics of success (Visser et al. 2015; Visser 2016). In order to address these shortcomings, the obvious general association between the location of a population and its climate will often need to be de-coupled. There are two potential ways of achieving such decoupling in natural systems: intensive longitudinal study encapsulating sufficient climatic variation; and the use of altitudinal gradients to generate representative levels of climatic variation in the short term and to test responses by individuals from the same population in conjunction with their downstream consequences for investment and success.

Testing adaptive responses to climatic variation for fecundity and subsequent levels of care is more challenging than testing impacts on breeding phenology because fewer taxa are amenable to quantitative assessment of such measures. Birds offer an important model in this regard because fecundity and subsequent care is variable and easily measured. Current evidence from longitudinal studies in such taxa, often spanning several decades, suggests that advancing lay date is generally often associated with increased clutch size (Potti 2009; Dunn & Møller 2014). This might be interpreted as adaptive because the ability to advance breeding more in response to warming springs is likely to generate improved match with peaks in prey availability (Visser et al. 2006; Charmantier et al. 2008). On the other hand, higher fecundity generally leads to reductions in *per capita* prey acquisition rates, potentially compounding any effects of mismatches between breeding phenology and prey availability. Interestingly, quantitative genetic approaches suggest a negative genetic correlation between phenology and fecundity (Sheldon et al. 2003), suggesting that an advance in lay date might often be associated with an incidental increase in clutch size. Compensating for increased clutch size as a consequence of advanced breeding phenology would require increased parental effort, but whether or not this is the case is not well known (Dunn and Winkler 2010). Thus, it is currently unclear whether or not commonly reported negative associations between phenology and fecundity are adaptive, or contribute to documented detrimental effects of climate change (Dunn and Møller 2014).

While longitudinal studies are unquestionably invaluable, opportunities to establish such studies are now more limited and the time taken to do so is prohibitive with respect to the need for answers. A potentially viable alternative approach is to use altitudinal gradients to generate representative variation in climate among individuals within a single population. Altitudinal gradients have been commonly used to test for ecological impacts on key fitness-related traits.

For example, a recent meta-analysis of bird species breeding across altitudinal gradients showed that breeding phenology was considerably later at higher elevations, and that clutch sizes tended to be smaller (57 % of 98 species); with average reductions of ca. 6 % (Boyle et al. 2016). These findings mirror the results of longitudinal studies: that warmer weather leads to both advanced phenology and fecundity. However, almost all previous altitudinal studies have conducted comparisons of the same species across different populations, meaning that varying degrees of local adaptation could cloud assessment of plastic responses to climatic variation. In order to provide a more realistic analogy of climate change impacts, associations between breeding phenology, fecundity, levels of care and productivity need to be investigated across altitudinal gradients within the same population.

Here I investigate the associations among altitude, breeding phenology (timing), fecundity, productivity and nestling mass in a nest box population of blue tits breeding along a 1000 m altitudinal gradient in the French Pyrenees. This altitudinal range is associated with an average 2-3 °C difference in mean daily (24 h) temperature during the breeding season. I am confident that any variation in parameters measured across our gradient is owed to plasticity because the median distance between sites is ~5 km, the habitat is contiguous between sites, and I have observed several instances of dispersal across our elevational gradient. The blue tit is a 12 g passerine in which the breeding female is responsible for all forms of care, and her male partner contributes to offspring provisioning. Previous longitudinal studies have suggested this species to be able to advance lay date, plastically, in response to advancing springs, and to show associated increases in clutch size (Potti 2009; Ahola et al. 2009).

However, generally no increased fledging success has been recorded with advancing springs, suggesting that selection for larger clutches may be maladaptive due to associated increasing costs of egg production and rearing, which may be enhanced due to a larger prey mismatch (Dunn 2004; Dunn and Winkler 2010).

Specifically, I first describe annual and altitudinal variations in breeding phenology as a means to investigate the maximal scope for mean phenological plasticity at the population level (see Phillimore et al. 2010). I predict that due to previously observed large decreases in temperature associated with altitudinal gradients (Körner 2007), variation in breeding timing will be driven to a larger degree by altitude than year. In a second step, I then investigate the effects of lay date and altitude on clutch size and hatching success, as a means of quantifying the phenotypic correlation between lay date and clutch size across the altitudinal gradient, and its effects on hatchability. Specifically, I predict that even if clutch size may be similar across the altitudinal range, hatchability should decrease due to colder temperatures during incubation at higher compared to lower altitudes. Finally, I test the effects of lay date on fledging success and nestling mass to provide insights into phenological mismatch in this population, as such metrics of success should be modified by phenology-fecundity associations.

2.3 Materials and Methods

2.3.1 Study population and habitat

Climate and reproductive data were collected near the research Station for Theoretical and Experimental Ecology of Moulis (SETE, UMR 5321; 42°57’29” N, 1°05’12” E), in the French Pyrenees during the breeding seasons of 2012-2017 inclusive. In total, our 14 woodlots contain on average 634 Woodcrete Schwegler^{TM} 2M nest boxes (32 mm hole diameter) spaced at ca. 50 m intervals. The number of boxes per year ranged from 626 to 641. The mean distance between woodlots is 7.1 km (±5.1 SD; median = 5.2). The woodlots are connected by a contiguous mosaic of mixed deciduous woodland, primarily oak (*Quercus robur*), ash (*Fraxinus excelsior)* and hazel *(Corylus avellana)* and beech (*Fagus sylvatica*), with the latter tree species more common at higher elevations and the former at lower elevations. Temperature at three locations was obtained in three years: three loggers (Tinytag^{TM} types TGP-4500 and TGP-4505) were positioned before the breeding season of 2015 at 565, 847 and 1335 m elevation on tree trunks at 2 m high, set to 30 min interval readings. The loggers were programmed to record throughout the following years. Daily (24 h) averages were created to estimate variation in diurnal and nocturnal temperatures. Work was conducted under animal care permits to A. S. Chaine from the French bird ringing office (CRBPO; n°13619), the state of Ariège animal experimentation review (Préfecture de l’Ariège, Protection des Populations, n°A09-4) and the Région Midi-Pyrenées (DIREN, n°2012-07).

2.3.2 Phenology, investment and success

We recorded data on lay date, clutch size, hatching failure and fledging success (all years). Each of these parameters was known with precision owing to nest checks every 3-5 days, or every day before the onset of laying, from the sixth egg to clutch completion, at hatching and fledging, from day 11 of incubation and day 18 after chick hatching, respectively. Our blue tit population is single brooded, although pairs will have a second nesting attempt if the initial brood fails (personal observation). No differentiation between first and any second attempts was made, as these could not be clearly distinguished due to the blurred overlap in lay dates correlated with the altitudinal cline (see below). The total number of hatchlings was determined as the number of eggs that hatched successfully, and the total number of fledglings as the number of chicks at banding (around day 15), minus those found dead after the rest of the brood flew the nest, as predation is rare in the late period of rearing (personal observation). Starting in 2013, all chicks were weighed to the nearest 0.1 g (day 11-18 after hatching) using electronic scales. In addition, a unique metal ring was fitted to every chick.

Our full data set comprised 541 blue tit nests that laid a full clutch and for which I obtained the date of laying onset. However, the sample size is reduced in subsequent analyses, owing to rare cases of nest abandonment and the use of some nests in experiments for other purposes. For example, in 2013-14, 58 experimental nests were excluded from the clutch size analysis, as I modified egg laying in these nests (N = 483 remaining). However, this manipulation did not affect subsequent breeding parameters, since variation in the number of eggs incubated and hatchling numbers were controlled for through a cross-fostering approach (unpublished data). Nevertheless, 12 % of nests with zero hatchlings were excluded from the probability of hatch failure analysis (N = 479 remaining), since such cases appeared to be due to nest abandonment. Mass data were available from 2249 individual chicks from 374 broods.

2.3.3 Statistical analysis

Statistical analyses were performed in *R* 3.4.2 (R Core Team 2017). Distributions of dependent variables were visually inspected for normality. Normal response terms were analysed using linear models in the basic ‘stats’ package (R Core Team 2017). If the data were non-normal, generalised linear models (GLMs, package = MASS; Venables and Ripley 2002) were used adjusting variance structure accordingly, i.e. the error distribution family and log link function (see tables of each analysis; Thomas et al. 2013). Residuals were examined for normality and overdispersion and model distributions were again adjusted if these assumptions were not met (Zuur et al. 2009). Collinearity among explanatory terms was tested using a variance inflation factor (VIF) analysis which if above 5-10 degrees indicates large contribution of covariates to the standard error of a regression; i.e. high multicollinearity (Dormann et al. 2013; Naimi et al. 2014). However, the VIF between the main potential collinear term of ay date and altitude was low (1.22) and thus both could be included as continuous variables in the same models. Model selection was based on changes in deviance using the anova function in *R* (significance set at $\alpha < 0.05$), using a step-wise, backward deletion procedure (Zuur et al. 2009a).

Overall, I performed five basic models, pertaining to phenology (lay date), clutch size, hatching success, fledging success and brood mass. In all models, I fitted lay date, altitude and year as the primary fixed terms of interested, as well as two-way interactions including lay date and/or altitude. Although altitude was fitted as a linear predictor, to facilitate interpretation and graphical representation of significant interactions including altitude, I split the altitude into three altitudinal ranges (see Figures). The three categories (low, mid and high altitudes) were determined using the greatest gaps in altitude between successive nest boxes (see also Schöll et al. 2016), and correspond to the location of the thermometers (central in each altitudinal range).

First, I investigated how breeding phenology (lay date) changed with altitude and year (N = 536). Second, I analysed how clutch size was affected by lay date, altitude and year (N = 466). Linear models with normal error structure were applied in both cases. Following investigation of the separate effect of altitude and lay date on clutch size, the interaction between the two variables was tested. In 2013-14, 58 experimental nests were excluded from the clutch size analysis as I modified egg laying in these nests. However, this manipulation did not affect further breeding parameters, as variation in the number of eggs incubated and hatchling numbers were controlled for through a cross-fostering approach (unpublished data).

To investigate the probability of hatch failure, i.e. whether or not nests failed to hatch any eggs, I applied a GLM with binomial error structure. In this model, the number of eggs incubated was fitted as a covariate to test whether large clutches might be associated with increased hatching failure. Fledging success was investigated as a two-step process: first investigating the factors associated with the probability of fledging at least one nestling (N = 439 nests), and second, for those that did fledge at least one, the factors influencing the number fledged (N = 369 nests; 21 % of the 439 nests failed to fledge young). This two-step process was performed because alternative zero-inflated models failed to run when the interactions central to the question were included. Finally, because fitness returns from reproductive attempts might be influenced by offspring condition, I investigated factors affecting mean chick mass per brood in a linear model (N = 347). In addition to the primary predictors of interest (see above), linear and squared effects of brood age and size were added as covariates, as non-linear relationships may be expected with chick mass (Rytkönen et al. 1996; Parejo and Danchin 2006).

2.4 Results

2.4.1 Breeding phenology

Over the six years of study, lay date varied from the 27th March-11th June with a mean of the 16th April (±10.2 SD; Table 2.1a). The average daily (24 h) temperature during this period (1st April – 30th June 2015-2017) was 12.9 °C (±4.7 SD), but the average temperature at mid- (mean: 13.6 °C, ±4.1 SD) and high-altitudes (mean: 10.5 °C, ±5.1 SD) was 6 and 28 % lower than at low altitudes (mean: 14.5 °C: ±4.0 SD), respectively (Fig. 2.1a). Presumably as a consequence of temperature, lay date increased as a linear function of increasing altitude ($F_{1,529}$ = 185.35, P < 0.001; Table 2.1b; Fig. 2.1b): whereas the mean lay date was April 13th at low altitude (±7.3 SD), it averaged five days later at mid altitudes (±11.7 SD), and 11 days later at high altitude compared to low altitudes (±14.7 SD). Superimposing temperature data onto laying data across the altitudinal gradient suggested that the mean temperature on the mean lay date was 13.2 °C (±3.0 SD) at low altitudes, 12.0 °C (±2.6 SD) at mid altitudes and 8.2 °C (±3.9 SD) high altitudes. After controlling for variation in breeding phenology as a function of altitude, I found that lay date also varied significantly among years, for example 2017 was an average seven days earlier and 2013 five days later than the overall mean of our population ($F_{5,529}$ = 32.15, P < 0.001; Table 2.1b; Fig. 2.1b;).

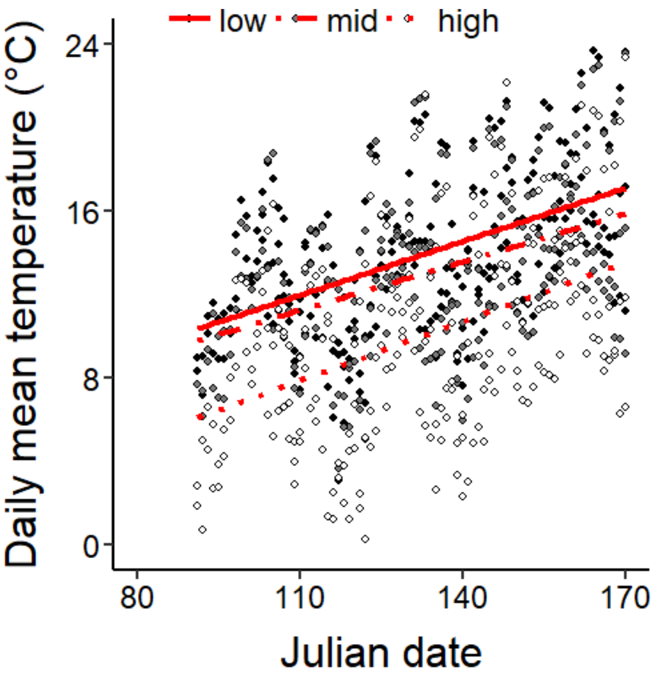

Figure 2.1a: Relationship between average daily temperature (°C) and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per altitudinal category (raw data).

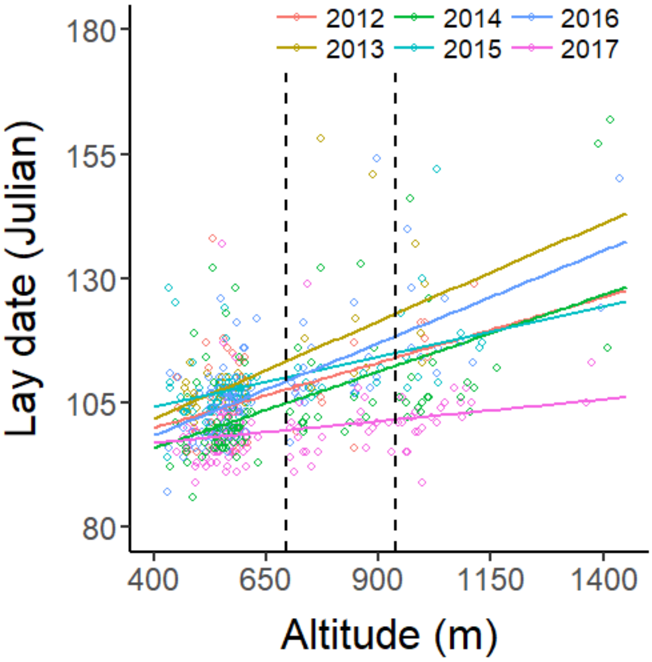

Figure 2.1b: Relationship between Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) and altitude (m; N = 536). Vertical, dashed lines indicate the cut-offs for the altitudinal categories. The best-fit lines are given per year.

Table 2.1a: Lay date characteristics per altitudinal category (low, mid and high) and per year (2012-2017).

Year	Mean	±SD	Min	Max	Mean °C ± SD	
Low						
2012	14th April	8.31	4th April	17th May	-	
2013	17th April	2.17	11th April	23rd April	-	
2014	11th April	8.50	27th March	12th May	-	
2015	17th April	5.32	4th April	8th May	12.30	2.80
2016	14th April	6.74	27th March	5th May	11.17	2.77
2017	8th April	6.16	30th March	17th May	11.17	3.23
Average	14th April	6.20	2nd April	9th May	11.55	2.93
Mid						
2012	13th April	6.86	5th April	26th April	-	
2013	29th April	18.09	17th April	7th June	-	
2014	17th April	10.55	9th April	13th May	-	
2015	19th April	2.31	16th April	22rd April	11.07	3.34
2016	22nd April	12.03	6th April	2nd June	9.62	3.06
2017	11th April	7.40	1st April	9th May	10.79	3.09
Average	19th April	9.54	9th April	14th May	10.49	3.16
High						
2012	30th April	5.50	23rd April	8th May	-	
2013	3rd May	9.65	23rd April	17th May	-	
2014	25th April	18.18	11th April	11th June	-	
2015	30th April	13.16	18th April	1st June	7.96	3.65
2016	2nd May	11.75	20th April	29th May	5.81	3.64
2017	11th April	5.15	30th March	23rd April	7.18	4.29
Average	27th April	10.57	16th April	21st May	6.98	3.86
Overall average	20th April	8.73	9th April	14th May	9.67	3.32

Table 2.1b: Model summaries predicting lay date. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		90.25	1.58	57.22	<0.001
Altitude		0.03	0.0018	185.35	<0.001
Year				32.15	<0.001
	2013	4.78	1.49		
	2014	-2.61	1.38		
	2015	2.81	1.39		
	2016	1.29	1.36		
	2017	-8.06	1.30		

2.4.2 Contributors to reproductive success: clutch size and hatching success

Average clutch size in our population was 8.2 eggs (±1.4 SD; range: 4-12) (Table 2.2). The greatest contributor to variation in clutch size was lay date, with clutch size declining by one egg for every two-week delay in the onset of laying ($F_{1,453} = 97.012$, $P < 0.001$; Fig. 2.2a). After controlling for this variation, I found that clutch size varied among years ($F_{5,453} = 4.53$, $P < 0.001$; Fig. 2.2b) and across the altitudinal gradient ($F_{1,453} = 15.22$, $P < 0.001$; Fig. 2.2a). For example, for a given lay date, clutches were on average 0.6 eggs (8 %) larger at high elevation compared with low elevation (Fig. 2.2a) and were 0.72 eggs greater (9 %) in 2013 than in 2015 (largest inter-annual difference; Fig. 2.2b). I found no evidence to suggest that clutch size was influenced by interactions between lay date and altitude ($F_{1,452} = 0.48$, $P = 0.49$, Fig. 2.2a), between altitude and year ($F_{5,448} = 0.84$, $P = 0.52$), or between lay date and year ($F_{5,448} = 1.46$, $P = 0.20$; see Table 2.2).

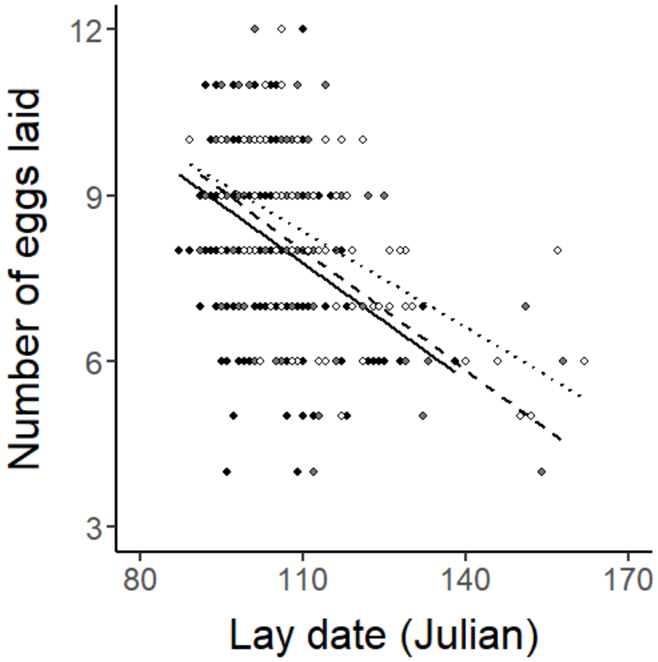

Figure 2.2a: Relationship between the number of eggs laid and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per altitudinal category – low: black, mid: grey, high: hollow points. Predictive lines controlling for year per altitudinal category - low: full, mid: dashed, high: dotted line (GLM with normal Gaussian error structure; N = 466).

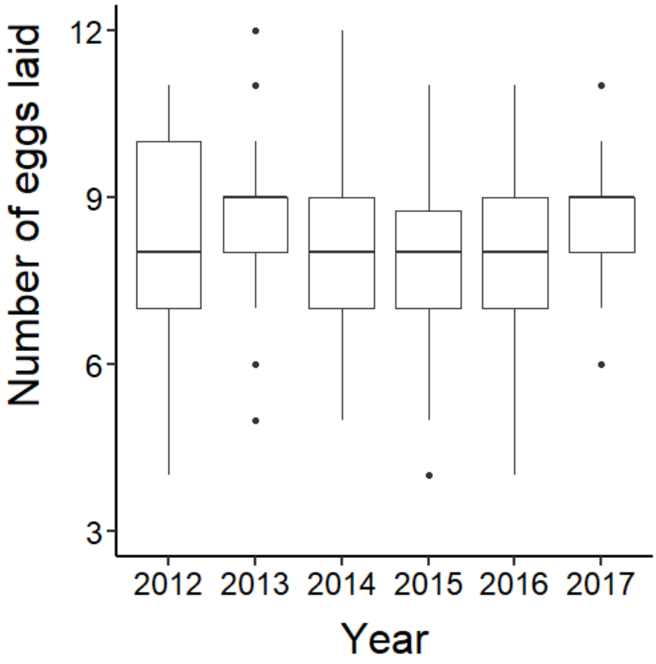

Figure 2.2b: Relationship between the number of eggs laid and year (2012-2017; N = 466). The raw boxplots represent the median, first and third quartiles, 1.5 * inter-quartile ranges as whiskers, and outliers as values outside these limits.

Table 2.2: Model summaries predicting clutch size. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		15.13	0.71	21.24	<0.001
Altitude		0.0014	0.00036	15.22	<0.001
Lay date		-0.073	0.0074	97.012	<0.001
Year				4.53	<0.001
	2013	0.75	0.28		
	2014	-0.043	0.25		
	2015	-0.29	0.22		
	2016	-0.27	0.22		
	2017	-0.39	0.22		
Altitude * lay date				0.48	0.49
Altitude * year				0.84	0.52
Lay date * year				1.46	0.20

Overall, in 70 % of nests, at least one egg failed to hatch (±46 SD), excluding entire clutches that failed to hatch presumably due to abandonment (12 %) (Table 2.3). The probability of hatch failure was unaffected by lay date on average ($\chi^2_{1,465}$ = -0.41, P = 0.52), but the effects of lay date on the probability that at least one egg failed to hatch varied among years (lay date * year interaction: $\chi^2_{5,460}$ = -11.65, P = 0.040; Fig. 2.2c). The probability of hatch failure also increased with altitude ($\chi^2_{1,469}$ = -4.61, P = 0.032; Fig. 2.2d), with an average of 6 % more nests failing to hatch some eggs at high versus low altitudes. Finally, hatching failure was more likely in large clutches, with an average 23 % increased chance of a nest experiencing some hatching failure for each increment of clutch size ($\chi^2_{1,469}$ = -8.18, P = 0.0042). All interactions between lay date and altitude ($\chi^2_{1,464}$ = -0.52, P = 0.47), lay date and clutch size ($\chi^2_{1,464}$ = -0.35, P = 0.55), and altitude and year ($\chi^2_{5,464}$ = -8.23, P = 0.14) were not significant.

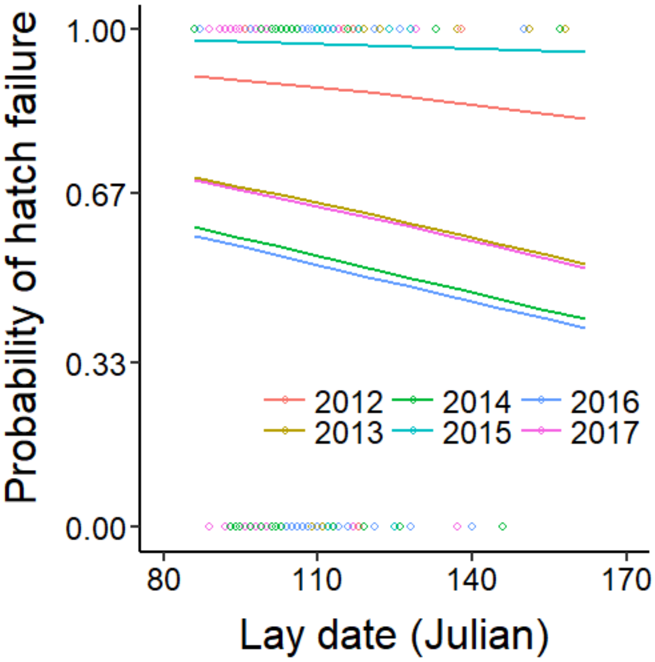

Figure 2.2c: Relationship between the probability of hatch failure and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per year: Predictive lines controlling for the number of eggs incubated and altitude (GLM with binomial error structure; N = 479).

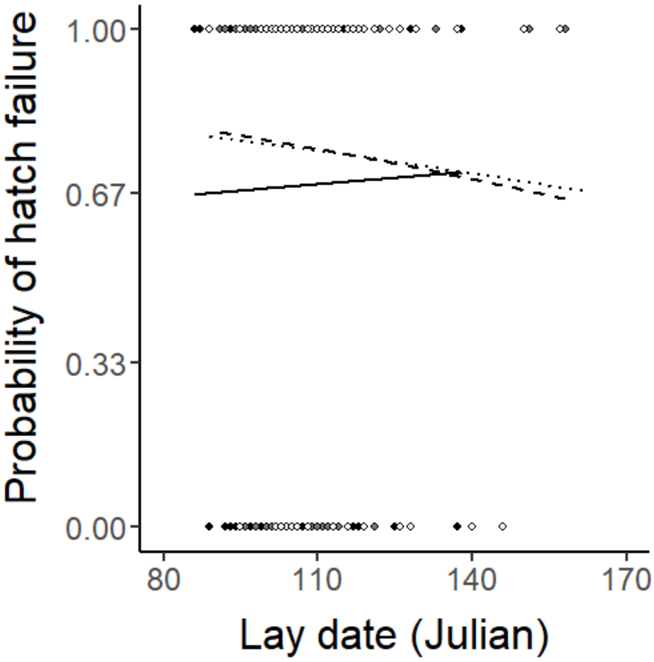

Figure 2.2d: Relationship between the probability of hatch failure and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per altitudinal category – low: black, mid: grey, high: hollow points. Predictive lines controlling for the number of eggs incubated and year per altitudinal category - low: full, mid: dashed, high: dotted line (GLM with binomial error structure; N = 479).

Table 2.3: Model summaries predicting the probability of hatch failure. Binomial GLM with logit link.

Predictors		Estimate	± SE	χ2 value	p-value
(Intercept)		-0.76	0.85	-0.89	0.37
Altitude		0.0012	0.00058	-4.61	0.032
No. eggs incubated		0.23	0.081	-8.18	0.0042
Year				-68.70	<0.001
	2013	-1.21	0.50		
	2014	-1.62	0.48		
	2015	1.70	0.73		
	2016	-1.74	0.47		
	2017	-1.17	0.46		
Lay date		-0.41	0.52	-0.41	0.52
Lay date * year				-11.65	0.040
Altitude * lay date				-0.52	0.47
Altitude * year				-8.23	0.14
Lay date * no. eggs incubated				-0.35	0.55

2.4.3 Reproductive Output

The mean probability that at least one nestling fledged from eggs that hatched was 84 % (±37 SD; Table 2.4). This probability was again influenced by an interaction between lay date and year ($\chi^2_{5,425}$ = -12.57, P = 0.028; Fig. 2.3a), and decreased with altitude ($\chi^2_{1,437}$ = -12.28, $P < 0.001$) – at high altitudes, nests were 15 % more likely to lose all nestlings than were those at low altitudes (Fig. 2.3b). The interactions between lay date and altitude ($\chi^2_{1,434}$ = -0.055, P = 0.81; Fig. 2.3b), lay date and clutch size ($\chi^2_{1,431}$ = -0.12, P = 0.73), and altitude and year were not significant ($\chi^2_{5,427}$ = -8.52, P = 0.13).

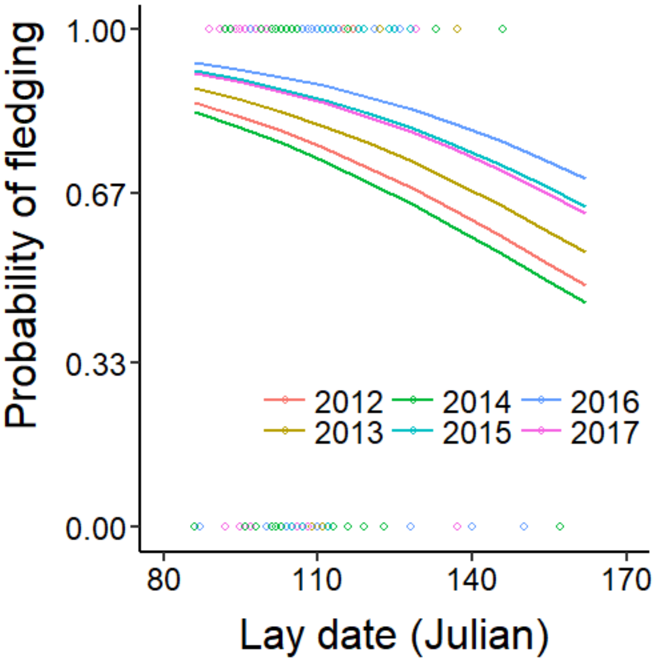

Figure 2.3a: Relationship between the probability of fledging and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per year: Predictive lines controlling for altitude (GLM with binomial error structure; N = 439).

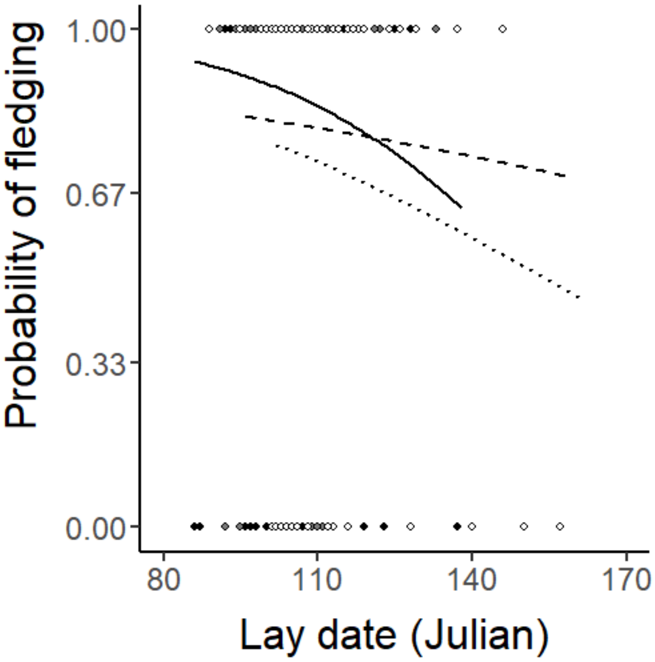

Figure 2.3b: Relationship between the probability of fledging and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per altitudinal category – low: black, mid: grey, high: hollow points. Presented are best-fit lines (minimal model only included altitude) per altitudinal category - low: full, mid: dashed, high: dotted line (GLM with binomial error structure; N = 439).

Table 2.4: Model summaries predicting the probability of fledging. Binomial GLM with logit link.

Predictors		Estimate	± SE	χ2 value	p-value
(Intercept)		3.12	0.44	7.049	<0.001
Altitude		-0.0021	0.00059	-12.28	<0.001
Lay date		-0.024	0.017	-2.28	0.13
Year				-8.81	0.17
	2013	0.26	0.77		
	2014	-0.14	0.74		
	2015	0.64	0.76		
	2016	0.90	0.76		
	2017	0.59	0.76		
Lay date * year				-12.57	0.028
Altitude * lay date				-0.055	0.81
Altitude * year				-8.52	0.13
Lay date * no. eggs incubated		-0.0034	0.0097	-0.12	0.73

An average of six nestlings fledged from nests wherein at least one did so (±1.89 SD; range: 1-11; Table 2.5). Later-breeding nests fledged less young than early nests, with the magnitude of this effect equating to a reduction of 0.08 nestlings fledging per day delay in laying ($F_{1,362}$ = 34.95, P < 0.001; Fig. 2.3c). There was also significant inter-annual variation in fledging numbers ($F_{1,362}$ = 6.71, P < 0.001); ranging from an average of five fledglings in 2015 to almost seven in 2013 (Fig. 2.3d). By contrast, there was no effect of altitude ($F_{1,361}$ = 0.004, P = 0.95) nor any interactions between lay date and altitude date ($F_{1,360}$ = 0.26, P = 0.61, Fig. 2.3c), lay date and year ($F_{5,357}$ = 0.45, P = 0.81), lay date and clutch size ($F_{1,359}$ = 0.96, P = 0.33) or altitude and year ($F_{5,356}$ = 0.53, P = 0.75).

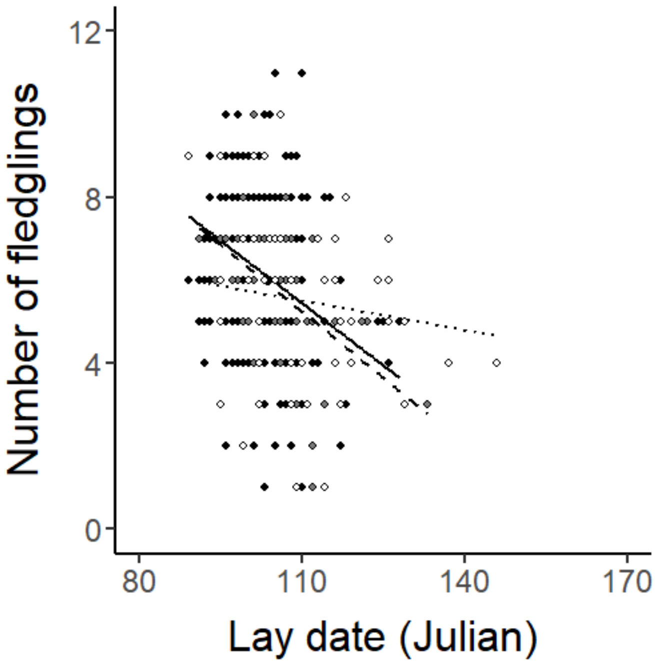

Figure 2.3c: Relationship between the total number of fledging and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per altitudinal category – low: black, mid: grey, high: hollow points. Predictive lines controlling for year per altitudinal category - low: full, mid: dashed, high: dotted line (GLM with normal error structure; N = 369).

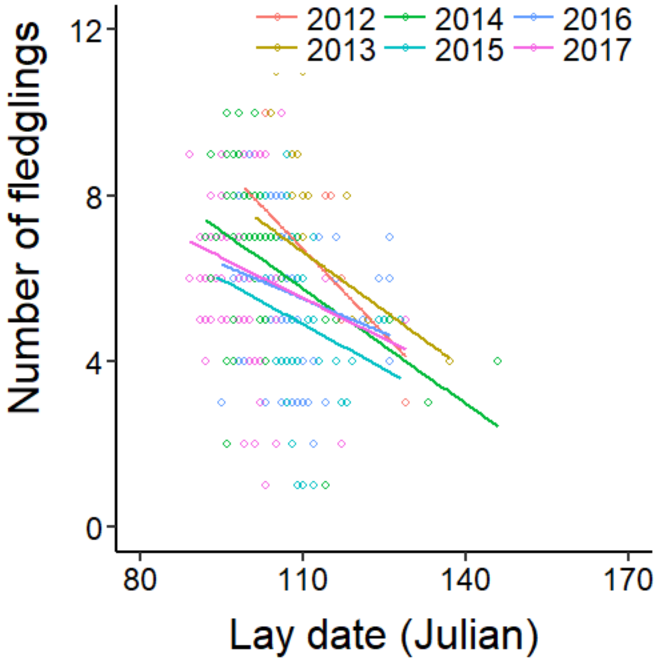

Figure 2.3d: Relationship between the number of fledging and Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) per year. Presented are best-fit lines (minimal model only included altitude; GLM with normal error structure; N = 369).

Table 2.5: Model summaries predicting the total number of fledging. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		15.13	1.56	9.71	<0.001
Lay date		-0.077	0.013	34.95	<0.001
Year				6.71	<0.001
	2013	0.031	0.61		
	2014	-0.75	0.60		
	2015	-1.73	0.59		
	2016	-1.19	0.58		
	2017	-1.23	0.59		
Altitude		-0.000037	0.00059	0.0040	0.95
Altitude * lay date				0.26	0.61
Altitude * year				0.53	0.75
Lay date * year				0.45	0.81
Lay date * no. eggs incubated				0.96	0.33

2.4.4 Nestling mass

Overall, average chick mass in broods was 10.40 g (±1.01 SD), ranging from 5.94-12.80 g. Older broods were heavier than younger broods (linear effect: $F_{1,341} = 11.29$, $P < 0.001$), with the squared effect of brood age being non-significant (squared effect: $F_{1,340} = 2.79$, $P = 0.096$). Linear and squared brood size did again not significantly affect chick mass, although the squared effect showed a more pronounced effect (linear effect: $F_{1,340} = 0.93$, $P = 0.34$ and squared effect: $F_{1,339} = 3.56$, $P = 0.060$, respectively). After controlling for the linear age effect, I found no evidence to suggest an effect of lay date on nestling mass ($F_{1,338} = 0.39$, $P = 0.54$), although I found a significant interaction between lay date and clutch size on nestling mass ($F_{1,335} = 4.80$, $P = 0.029$; Fig. 2.4). This interaction arose primarily because large broods declined in mass more steeply over the season than smaller broods. By contrast, altitude failed to explain variation in nestling mass ($F_{1,340} = 0.46$, $P = 0.49$), and all interactions were non-significant between lay date and year ($F_{5,337} = 0.082$, $P = 0.76$), as well as altitude and year ($F_{5,337} = 2.080$, $P = 0.15$; Table 2.6).

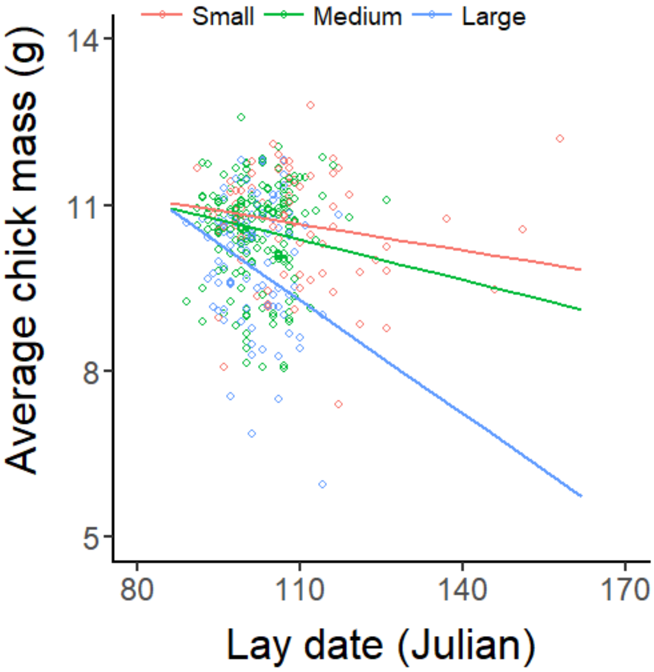

Figure 2.4: Relationship between Julian lay date (100 = 10th April in non-leap years/ = 9th April in leap years) and average chick mass per brood (g) per clutch size category (lay date * clutch size interaction: $F_{1,335} = 4.80$, $P = 0.029$) – small: white points, medium: grey points, large: small points. Predictive lines controlling for linear brood age, altitude and year per altitudinal category - small: full, medium: dashed, large: dotted line (GLM with normal error structure; N = 347).

Table 2.6: Model summaries predicting average chick mass per brood. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		10.55	0.16	64.418	< 0.001
Chick age		0.30	0.089	11.29	< 0.001
Chick age ^2		-1.72	1.027	2.79	0.10
Brood size		-0.029	0.030	0.93	0.34
Brood size ^2		-1.85	0.98	3.56	0.060
Year				3.64	< 0.002
	2014	0.30	0.20		
	2015	0.12	0.20		
	2016	-0.029	0.19		
	2017	-0.26	0.18		
Altitude		-0.00020	0.0003	0.46	0.50
Lay date		-0.0047	0.01	0.39	0.54
Altitude * year				3.95	0.0038
Lay date * year				2.36	0.053
Lay date * no. eggs laid		-0.010	0.0047	4.80	0.029
Altitude * lay date		0.00006	0.000033	2.88	0.091

2.5 Discussion

Our key aim was to use an altitudinal gradient within a population of birds to generate variation in temperature, and then test responses for breeding phenology (timing), associated investment and parameters of success. I found that breeding phenology varied significantly among years, but more profoundly across the altitudinal gradient, with those breeding at higher altitudes laying up to 11 days later on average than at lower altitudes. This delay means that females at low altitude are already incubating eggs when those at high altitude begin laying. Despite this, clutch size declined with lay date throughout the season similarly across the altitudinal gradient, and, if anything, for a given lay date, those at high altitude laid larger clutches. Further, clutch size also declined comparably with progression of the season across the altitudinal gradient and there were no obvious costs for laying large clutches early in the season generally, or at high altitude in particular. The lack of an obvious cost of laying large clutches early in the season or at high altitude arose despite the findings that both increasing clutch size and altitude had negative impacts on hatching success. By contrast, there was some suggestion that laying large clutches late in the season reduced nestling condition, although there was no impact on fledging success. Our evidence suggests that there is substantial plasticity in lay date in this population of blue tits, and so little evidence that given appropriate cues, pairs could not breed sufficiently early to match peaks in prey availability, but that there was a tight negative association between lay date and clutch size, with potential implications for understanding the ability of species to avoid phenological mismatches.

It is now clear that many species, particularly outside of equatorial regions, are advancing breeding phenology in response to warming springs (Parmesan and Yohe 2003; Root et al. 2003). However, higher trophic levels are typically responding with reduced magnitude compared with lower trophic levels, leading offspring of the former to miss the peak availability of their prey during growth (Buse et al. 1999). The typical explanation for this phenological mismatch is that higher trophic levels, with their commonly greater generation times, cannot respond as rapidly as their prey to changing climates (Thackeray et al. 2010). Further, a recent meta-analysis on more than 10,000 long-term phenological data sets showed that secondary consumers (predators) were less sensitive to climate than species lower down the food chain (Thackeray et al. 2016). Whether blue tits are missing the peak in their prey in our study is not known, since prey availability was not quantified. Nevertheless, that clutch size and metrics of reproductive success, and in particular nestling mass, all declined with progression of the breeding season is supportive. Assuming that phenological mismatch is operating in this study, at least for a significant number of pairs, the obvious question is why?

The primary hypothesis proposed to explain phenological mismatch is genetic: because predators have longer generation times, then cannot adapt as rapidly to changing climates as their prey (Parmesan 2006; Friman et al. 2008). For example, a long-term study in the Netherlands found that the advancement of budburst has been tracked by caterpillars, and to a lesser degree by insectivorous passerine birds, however their predators - sparrowhawks (*Accipiter nisus*) – failed to advance hatching date (Both et al. 2009b). However, this hypothesis ignores potential variation in the plasticity of predators and prey.

Quantifying the plastic scope of a population, the maximal range of responses shown by a population to environmental variation, is challenging because all individuals in a population typically experience the same environment in a given year. Further, while long-term studies can use annual variation in environmental conditions to quantify plastic responses (Gienapp et al. 2008; Charmantier et al. 2008; Charmantier and Gienapp 2014), such annual variation will commonly be limited. By generating an altitudinal gradient in a population of blue tits, I was able to gain important insights into potential levels of plasticity and whether or not constraints on plasticity are likely to generate phenological mismatch. The plasticity of breeding phenology in our population was substantial. For example, pairs at high elevation began laying 11 days later than those at low elevation, on average. Further, across the six years of study and three elevation categories, the first lay date varied by almost four weeks (i.e. earliest first lay date at low altitude over six years was the 27th March, while the latest first lay date at high elevation was the 23rd April; Table 2.1). While one might argue that at low elevation laying cannot advance further without genetic change, although two weeks still separated the earliest egg laid at low elevation across the six years, it is hard to use this rationale to explain any phenological mismatch at high altitude. This is because, at the level of the population, pairs breeding at high altitude would appear to be ‘genetically’ capable of advancing lay date by 11 days on average and 18 days in extreme cases (i.e. maximal within-year difference between mean lay date at low versus high altitude). Together these results strongly suggest limitations to plastic scope at the population level are not the only cause of phenological mismatch.

An additional possibility is that the trigger (cue) used to time reproduction offers a poor guide to adaptive breeding phenology under climate change. For example, if the breeding phenology of predators is more sensitive to day length and that of their prey more sensitive to temperature, then predators will struggle to advance their phenology to an equivalent extent as their prey. Although day length is thought to have a general impact on the onset of breeding in birds (Lack 1954; Lambrechts et al. 1996; Dawson et al. 2001), it is unlikely to impede advancement of lay date because of the dramatic variation in lay dates observed among years and altitudes in this study, despite unchanging day lengths. Another possibility is that the phenology of prey is more sensitive to temperature than predators (Visser et al. 2006). Although I was not able to test this possibility, again it seems unlikely that any variation in sensitivity to temperature explains phenological mismatch. This is because, while temperature did appear to play some role in explaining breeding phenology in this study, with earlier breeding occurring in warm years and at low altitudes, it did not explain all variation since laying was initiated at 3 °C lower temperature at high versus low elevations. Further, there was substantial variation in lay date within years, and altitudinal categories despite comparable temperatures, which would not be expected if temperature represented a primary cue to breeding phenology. If temperature does not account fully for breeding phenology, then early indicators of temperature, for example tree budding, is also unlikely to be the primary predictor either (Visser et al. 2002; Schaper et al. 2011). By elimination, this leaves the availability of prey and/or body condition as an additional trigger of phenology (Rowe et al. 1994; Nager and van Noordwijk 1995), with potentially detrimental consequences to the timing of breeding.

The problem for an endothermic predator using ectothermic prey availability to time reproduction is that under warming springs, prey will develop faster than usual. This is because the development of ectotherms, as well as the life cycle for insects, is far more sensitive to temperature than is the case for endotherms (Buckley et al. 2012). For example, it is known from temperature-controlled lab experiments that caterpillar development can be cut from 50 days to 20 days by rearing them at 15 °C instead of field temperatures (Buse et al. 1999). By contrast, in blue tits, as with most endotherms, development can rarely be hastened by more than just a few days. Thus, the only way for endothermic species such as blue tits to reduce the ensuing gap generated by hasted development of ectothermic prey under warming springs is to decrease their laying period and thus reduce fecundity. For example, in blue tits, halving clutch size could save five days in early breeders. On the contrary, as with other studies (e.g. Klomp 1970; Potti 2009), I found a strong, negative phenotypic correlation between lay date and clutch size, which is thought to be driven at least partly by an underlying negative genetic correlation (Sheldon et al. 2003). I conclude that for endothermic predators to match the peak of their ectothermic prey under warming springs, they will need to simultaneously evolve a new trigger to advance their breeding phenology and uncouple the negative association between phenology and fecundity.

Chapter Three

Testing the use of budburst as a reliable cue to breeding phenology in a population of blue tits breeding along an altitudinal gradient

3.1 Abstract

Environmental cues are crucial in fine-tuning reproductive timing in seasonally breeding birds. However, it remains debated to what extent environmental cues such as budburst are used in insectivorous birds. Some studies have found strong supporting evidence for the use of budburst; however other studies have found no signal. These studies mostly do not control for differing population origins and different habitat. Here I use a 1000 m altitudinal gradient in the French Pyrenees to generate representative 2-3 °C differences in temperature faced by breeders in a population of blue tits (*Cyanistes caeruleus*). Additionally, the diversity in vegetation, such as understory, tree species, particularly flowering trees decreases with altitude. I predict this will increase the reliability of budburst as a cue of general vegetation phenology at higher altitudes, as vegetation is more synchronous in development. During the five years of study, I found that budburst was delayed at high altitude, with breeding birds also delaying their laying after budburst more at high compared to low altitudes. As a consequence of this delay in laying at high altitudes, I found that reproductive phases such as incubation commence sooner in relation to laying. Similar relationships were observed for the lengths of the incubation and rearing periods. This is supportive of high altitude individuals being under larger time pressure to match optimum prey availability. I suggest that the use of budburst plays a larger role in comparison to other environmental cues such as temperature in more homogenous habitats.

3.2 Introduction

The fitness of organisms is influenced by prevailing environmental conditions and levels of reproductive investment. For all seasonal breeding organisms timing is crucial to ensure favourable climatic and food conditions. In accord, reproduction has been shown to be matched with food abundance (e.g. arthropods: Søreide et al. 2010); fish: Yoneda and Wright 2005, reptiles: Santos et al. 2005; mammals: Arlettaz et al. 2001). For example, in many plants flowering is timed to high levels of light to increase photosynthesis to produce energy (Hayama and Coupland 2003). Higher up the food chain; *Fejervarya limnocharis* is one of many tropical frog species that times its breeding cycle to the rainy season characterised by benign, food rich conditions (Othman et al. 2011). Some species have been shown to precisely time their breeding to maximise food availability using cues such as rising water levels, solar and lunar cycles (Ikegami et al. 2014; Barros et al. 2015; Juntti and Fernald 2016). Our understanding of reproductive timing is more limited in species such as birds, which however is heavily affected by the current changing climate (e.g. (Visser et al. 1998; Visser 2016).

In birds a variety of factors influence reproductive timing and investment. For example, in (sub) tropical habitats close to the equator rainfall plays a large role (Sharp 1984). Many bird species living in these regions time reproductive development to coincide with the monsoon. For instance, in the tropical spotted antbird (*Hylophylax n. naevioides*) gonadal growth starts ca. six weeks before the wet season (Wikelski et al. 2000). To time reproduction these antbirds use long-term cues such as photoperiod and more short-term occurrences of rain, which are linked to high insect abundance. In support, captivity experiments have revealed that male gonadal growth and song activity is stimulated shortly after supplying a cue of live crickets (Hau et al. 2000). More extremely, desert birds can start breeding and building nests almost immediately after heavy rains (Keast and Marshall 1954). In seasonal habitats, reproductive success decreases with the progression of the breeding season (Verhulst and Nilsson 2008), thus using environmental cues to breed early while accounting for annual climatic conditions is crucial. A variety of cues are known to fine-tune reproductive timing in these seasonal species, though the relative importance of each cue is still unclear.

Day length, temperature and direct or indirect measures of food availability have all been shown to act as environmental cues to kick-start reproduction in seasonal bird species (Marshall 1961; Perrins 1965; Jones 1972; Gwinner 1996; Visser et al. 2009). Lack (1954) suggested that increasing day length is used to trigger development of reproductive condition (activation of the neuroendocrine system; Sharp 1996). Herbivorous kakapo (*Strigops habroptila*) as most other seasonal bird species time breeding foremost to overall changes in day length (Cockrem 2006). Additionally, as these parrots feed on seeds of trees such as rimu (*Dacrydium cupressinum)*, breeding occurs only in mast years; every three to five years. Cues linked to flowering and the development of these conifer seeds and fruit likely prompt breeding. Further, seasonal, insectivorous bird species have to align breeding with peaks in prey abundance (Kluyver 1951; Perrins 1970; Blondel et al. 1993). To achieve tight alignment, these insectivorous birds may use appropriate environmental cues to predict prey peaks and hence start reproduction (Blondel et al. 1993). For example, in long-distance migratory species such as Garden Warbler (*Sylvia borin*) day length, as part of the circannual rhythm, has been suggested to control duration and distance of migration from their overwintering grounds (Gwinner 1996). However, day length may be insufficient to align migrants’ arrival time and advanced phenological development at spring breeding grounds due to climate warming (Both and Visser 2001).

In non-migrant, insectivorous birds, other cues such as temperature and budburst, underlying annual variation in prey peaks, may play a large role in refining reproductive timing (Wingfield et al. 1992). Budburst, “*defined as the day when […] buds on half of the trees reach the stage when green () first break free from tree*” (Cannell and Smith 1983), has been shown to be closely linked to temperature and may be thus used as a cue for annual climatic conditions (Lack 1966; Van Balen 1973; Perrins and McCleery 1989). Classic studies have demonstrated that budburst enables the caterpillars of winter moths (*Operophtera brumata*) to start feeding on the young leaves of trees, mainly oaks (Kluyver 1951; Varley and Gradwell 1960; Perrins 1973; Van Noordwijk et al. 1995). These preferred, high energy prey items of birds such as Paridae species then become available for parents to feed themselves and their offspring (García-Navas and Sanz 2010a). Thus, individuals must accurately predict maximum offspring requirements and environmental productivity of these prey items. The start of the reproductive cycle – lay date – has been shown to correlate with budburst (Blondel et al. 1993). However, it is still disputed to what extent budburst is used as a cue to start reproduction (Lyon et al. 2008; Schaper et al. 2011). Disentangling use of budburst from other possible cues has been hard to achieve as most studies have either been performed over large geographical scales leading to latitudinal confounders of day length, or on relatively small scales in fairly homogenous landscapes requiring long-term sampling effort.

Here I investigate the importance of budburst in governing reproductive timing in a population of a small (12 g) European passerine - the blue tit - inhabiting nest boxes along a 1000 m altitudinal gradient in the French Pyrenees. Such an altitudinal cline provides a rare opportunity to generate significant variation in some parameters but holding others constant to shed light on the specific cues used. Compared to latitudinal studies day length does not vary between different altitudes, enabling the study of a gradual shift in budburst and its effect as a cue on reproductive timing. A gradual delay in budburst should be produced by the decrease in temperature previously shown with altitude (Körner 2007). This is of advantage, as it remains ambiguous to what extent budburst acts as a fine-tuning cue in blue and great tits. The occurrence of leaves and caterpillars has been shown to be correlated with the lay date of both species in natural settings (Nager and van Noordwijk 1995). For example in Corsican blue tits the correlation has been estimated as $r^2 = 0.87$, which is independent of forest type (Bourgault et al. 2010). In contrast, path-analyses have revealed that vegetation phenology versus temperature is a more important cue in deciduous than evergreen habitats on Corsica (Thomas et al. 2010; also see Swedish study - Nilsson and Källander 2006). Another European-wide study revealed that most captive populations supplied with budding oak and birch branches did not change lay date or concentrations of reproductive hormones compared to controls (Visser et al. 2002; Schaper et al. 2011). However, Corsican blue tits advanced their lay date when exposed to oak branches. These studies suggest differing levels of importance of budding in determining reproductive timing.

By use of an altitudinal gradient and the predicted delay in budburst, I aim to crystallise out the importance of budburst by controlling for day length, different population origins and habitat types. To this end, I will investigate: (1) the effect of altitude on the timing of tree budding; (2) the relationship between budburst and breeding phenology across the altitudinal gradient; (3) strategies employed by breeding birds to improve the association between budburst and hatching; and (4) how such strategies affect the duration of key life-history stages and metrics of reproductive success. Previous studies have given first egg lay date a mostly fixed role in aligning avian reproductive events with peak prey availability (Visser et al. 1998, 2015; Phillimore et al. 2016). However, mechanisms, such as shortening the lengths of the different reproductive periods such as lay gaps (Nilsson and Svensson 1993a; Lessells et al. 2002; Cresswell and McCleery 2003; Matthysen et al. 2011), the start of incubation (Van Balen 1973; Haftorn 1981), and the duration of incubation and rearing (Haftorn 1988; Nilsson and Smith 1988), may exist to realign breeding after laying with phenological events. These mechanisms may thus provide a means of decoupling lay date from the time window when nestlings require most food, though have not received much attention previously. Specifically, I predict that our blue tits can ‘catch up’ and realign reproductive events with maximum environmental productivity after laying late by implementing such temporal mechanisms.

3.3 Materials and Methods

Climatic, phenological and reproductive data were collected near the Research Station for Theoretical and Experimental Ecology of Moulis (SETE, UMR 5321, 42°57’29” N, 1°05’12” E), in the French Pyrenees. Overall, our 14 woodlots contain on average 634 Woodcrete Schwegler^{TM} 2M nest boxes (32 mm hole diameter) spaced at ca. 50 m intervals. A more detailed description of the field site, including tree species and their basic distribution, can be found in Chapter Two. To record temperature changes with altitude, three loggers (Tinytag^{TM} types TGP-4500 and TGP-4505) were positioned before the breeding season of 2015 at 565, 847 and 1335 m elevation on tree trunks at 2 m high, set to 30 min interval readings. The loggers were programmed to record throughout the following years. Daily (24 h) averages were created to account for variation in diurnal and nocturnal temperatures. These altitudes represent central points pertaining to low, mid and high altitudes.

During the breeding seasons of 2013-2017, all nest boxes were visited every 1-3 days to quantify ambient plant phenology of the nest box tree. Trees were classified into three different stages of budding: no development, budburst or full leaves (similar method to (Blondel et al. 1993). Daily visits were also paid to nest boxes during these six breeding seasons to determine the number of laying gaps and date of clutch completion, i.e. when egg numbers stabilised, and incubation had started. Equivalently, I deemed incubation to have started when eggs were warm to the touch, and retrospectively I recorded clutch size from the maximum numbers of eggs seen in a nest. During incubation I visited the nest just once or twice to reduce disturbing the female. Visitation frequency was daily around the estimated hatch and fledge date, i.e. from day 11 of incubation and day 18 after chicks hatching, respectively. Hatch date was recorded as the date of first chick eclosion and fledge date when all chicks had left the nest. These records enabled me to calculate the lengths of the incubation (from the first day the eggs were warm to the touch over two consecutive days until the first chick hatched) and rearing periods (from the first day chicks hatched to the day the last chicks fledged).

3.3.1 Statistical analysis

Statistical analyses were performed in *R* 3.4.2 (R Core Team 2017). All data sets were examined for outliers, though none were found to significantly impact the results. Distributions of dependent variables were visually inspected for normality. If the data were non-normal, generalised linear models (GLMs, package = MASS; Venables and Ripley 2002) were used adjusting variance structure correspondingly, i.e. the error distribution family and log link function (Thomas et al. 2013). Residuals were examined for normality and overdispersion and model distributions were again adjusted if these assumptions were not met (Zuur et al. 2009). A step-wise, top-down model selection procedure was applied using changes in deviance of the minimal model to evaluate the contribution of each variable to the model (significance set at $\alpha < 0.05$). I included year as a fixed factor in *all* models to account for inter-annual variation due to changes in our sampling regime, which albeit also precluded me from a detailed examination of year to year variation in life history characteristics.

(a) Vegetation phenology

Firstly, I investigated if budburst was delayed with increasing altitude (due to a decrease in temperature - Chapter 2). In line with this, I fitted the normally distributed response variable of first budburst date into a linear model including altitude and year as covariates. In total 450 data points were available from individual nest box trees over the five breeding seasons of when trees started leaving (budburst – see above). Specifically, I collected data from 53 trees of different nest boxes with blue tit nests in 2013, 89 in 2014, 86 in 2015, 96 in 2016 and 126 in 2017.

Next the delay of laying after budburst was analysed. I already know that lay date is on average one day later per 40 altitudinal meters (Chapter 2), however it is unclear whether later laying is linearly related to budburst with increasing altitude. I had information on this delay period in 445 nests. Broken down, these data consisted of 51 nests in 2013, 89 nests in 2014, 86 nests in 2015, 93 nests in 2016 and 126 nests in 2017. This normally distributed delay was fitted into a linear model including altitude and year as covariates.

(b) Timing parameters post-laying

While it is generally assumed that blue tit females lay one egg per day, in our population 23 % of 396 intensively tracked nests skipped a minimum of one laying day. Specifically, I had data from 66 nests in 2013, 81 in 2014, 64 in 2015, 86 in 2016 and 127 in 2017. Nests that had experienced a minimum of one lay gap were binned into to one group and zero gap nests into another group. Thus, the probability of lay gaps was fitted to a binomial GLM. Full model variables included the delay to laying after budburst, altitude and then their interaction, year, and clutch size. Clutch size was included as it may be negatively correlated with the probability of lay gaps occurring (Nilsson and Svensson 1993b).

Alignment, i.e. the gap between clutch completion and the start of incubation was measured in days. In total, the sample size consisted of 437 nests. Specifically, data were available from 44 nests in 2013, 87 in 2014, 81 in 2015, 94 in 2016 and 131 in 2017. In some nests incubation started before clutch completion thus those days were assigned negative values, however in some cases incubation started some days after clutch completion and were thus assigned a positive value. The normally distributed response variable of this incubation alignment was fitted into a linear model including the standard fixed factors of the delay to laying after budburst, altitude and year, and then the interaction between budburst and altitude. In addition, the laying period was added (period from first to last egg laid) as a possible negative influence was expected (Cresswell and McCleery 2003).

The incubation period measured in days was known in 414 nests. I had data points from 41 nests in 2013, 82 in 2014, 76 in 2015, 88 in 2016 and 127 in 2017. This variable was normally distributed and hence fitted into a linear model including the same standard variables as for the above analysis. Previous breeding phases may also influence the length of incubation, thus instead of only the laying period the entire pre-incubation period from the first egg laid was included in the model.

The rearing period measured in days was known in 331 nests. I had data points from 27 nests in 2013, 56 in 2014, 62 in 2015, 78 in 2016 and 108 in 2017. This variable was normally distributed and hence fitted into a full, linear model including the covariates of the delay to laying after budburst, the pre-hatching period, the number of hatchlings, altitude and year. After analysing the individual effects of these fixed factors, I again included the interaction between the delay to laying after budburst and altitude.

(c) Reproductive success

We estimated how the delay in laying after budburst affected reproductive success, by looking at the number of chicks successfully fledging the nest. Fledging success was modelled in a zero-inflated count model with Poisson error structure, as 27 % of blue tit nests had no chicks fledge. The covariates of the delay to laying after budburst, altitude and year were included. After analysing the individual effects of these fixed factors, I again included the interaction between the delay to laying after budburst and altitude.

3.4 Results

3.4.1 Phenology of budburst and lay date

Budburst occurred on average on the 13th April (±8.12 SD; range: 15th March – 17th May). Budburst was delayed by 1.5 days per 100 altitudinal meters ($F_{1,444}$ = 72.49, $P < 0.0001$), and varied with year ($F_{4,444}$ = 21.28, $P < 0.0001$). Budburst date was negatively related with temperature at low and middle altitudes, however at high altitudes this relationship was positive (Fig. 3.1; Table 3.1). Lay date occurred on average 2.7 days after budburst (±10.54 SD; range: -25 – 53). Annual variation existed in delay to laying following budburst ($F_{4,439}$ = 6.082, $P < 0.001$). The delay to laying after budburst was extended by a mean of 0.78 days per 100 altitudinal meters ($F_{1,439}$ = 8.97, $P = 0.0029$; Fig. 3.2).

Table 3.1: Characteristics of budburst date and lay date and temperature over the study period (2013-2017) with altitude.

		Budburst date					Lay date					Temperature across budburst period				
Altitude	Year	Average	±SD	Min	Max	Variance	Average	±SD	Min	Max	Variance	Average	±SD	Min	Max	Variance
Low	2013	16 April	5.87	04 April	25 April	34.44	18 April	2.16	12 April	24 April	4.68	-	-	-	-	-
	2014	08 April	4.84	29 March	18 April	23.38	12 April	8.50	28 March	13 May	72.21	-	-	-	-	-
	2015	13 April	7.59	31 March	01 May	57.58	18 April	5.32	05 April	09 May	28.32	13.52	3.40	8.88	20.56	11.55
	2016	15 April	6.63	15 March	26 April	43.93	14 April	6.74	28 March	06 May	45.47	11.54	2.67	6.80	16.12	7.10
	2017	09 April	7.20	25 March	26 April	51.79	08 April	4.50	31 March	28 April	20.28	12.17	3.37	3.08	19.25	11.37
Mid	2013	17 April	3.40	13 April	24 April	11.57	30 April	18.09	18 April	08 June	327.42	-	-	-	-	-
	2014	10 April	5.79	04 April	19 April	33.52	18 April	10.55	10 April	14 May	111.32	-	-	-	-	-
	2015	13 April	5.04	04 April	19 April	25.41	20 April	2.31	17 April	23 April	5.34	12.44	4.15	6.65	21.40	17.20
	2016	16 April	7.79	03 April	03 May	60.74	22 April	12.03	07 April	03 June	144.73	10.75	3.25	5.05	16.32	10.54
	2017	08 April	6.43	29 March	26 April	41.36	11 April	7.40	02 April	10 May	54.82	11.80	3.28	3.65	18.74	10.74
High	2013	05 May	17.68	23 April	18 May	312.50	04 May	9.65	24 April	18 May	93.20	-	-	-	-	-
	2014	18 April	11.36	31 March	13 May	129.00	26 April	18.18	13 April	12 June	330.51	-	-	-	-	-
	2015	24 April	6.97	16 April	04 May	48.53	30 April	13.16	19 April	02 June	173.29	9.67	4.90	2.63	21.57	23.99
	2016	19 April	6.11	13 April	05 May	37.36	03 May	11.75	21 April	30 May	138.08	6.70	3.83	0.27	15.26	14.64
	2017	17 April	10.85	30 March	11 May	117.64	12 April	5.15	31 March	24 April	26.51	8.36	4.46	-1.58	17.14	19.90

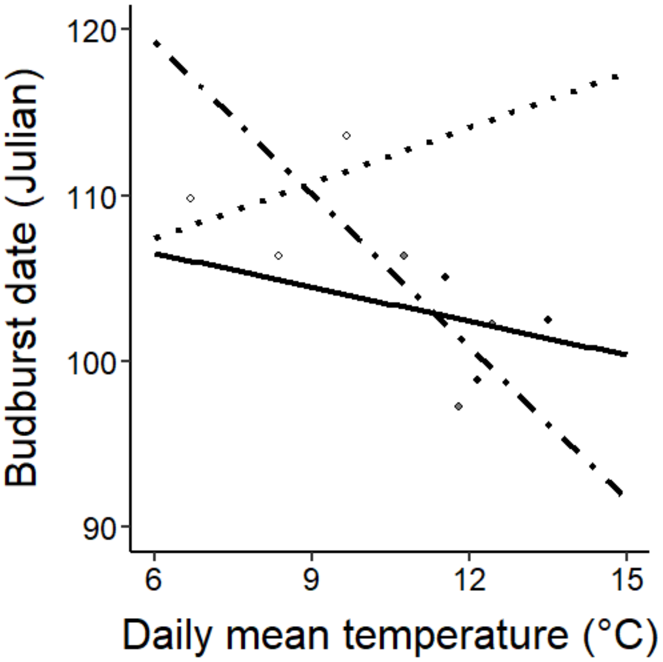

Figure 3.1: Budburst date (Julian: 100 = 10th April in non-leap years/ = 9th April in leap years) with average daily temperature (°C) over the budburst period per altitude category – low: black, mid: grey, high: hollow points. Predictive lines controlling for year per altitudinal category - low: full, mid: dashed, high: dotted line. The points are annual measures.

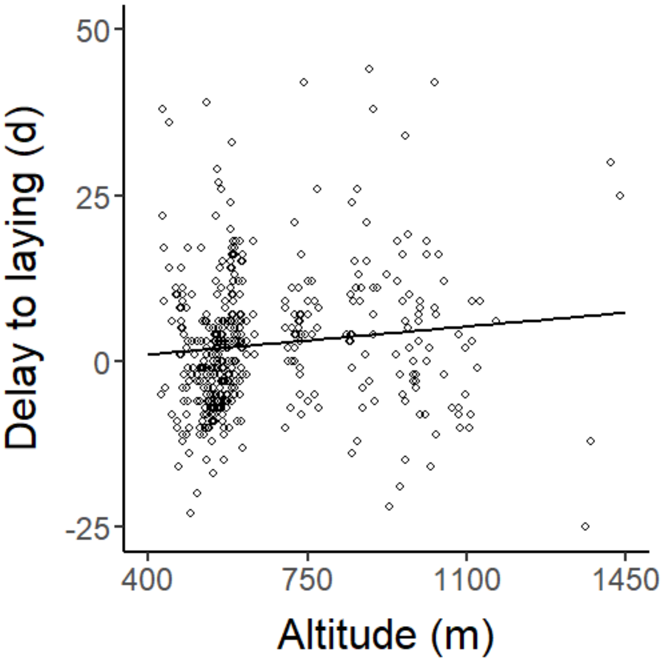

Figure 3.2: The delay to laying after budburst (d) in relation to altitude (m). Raw data points and predictive line controlling for year.

3.4.2 Timing parameters post-laying

The mean number of laying gaps was 0.39 days (±0.93 SD; maximum: 6 days). The delay to laying after budburst did not significantly affect the probability of skipping days during egg laying ($\chi^2_{1,364}$ = 2.91, P = 0.088; Table 3.2). Year explained variation in the probability of laying gaps occurring ($\chi^2_{4,391}$ = -17.23, P = 0.0017). There was no effect of clutch size or altitude on laying gaps (respectively: $\chi^2_{1,361}$ = 0.16, P = 0.70 and $\chi^2_{1,363}$ = 2.26, P = 0.13). The interaction between the delay to laying after budburst and altitude was not significant ($\chi^2_{1,362}$ = 1.86, P = 0.17; Fig. 3.3).

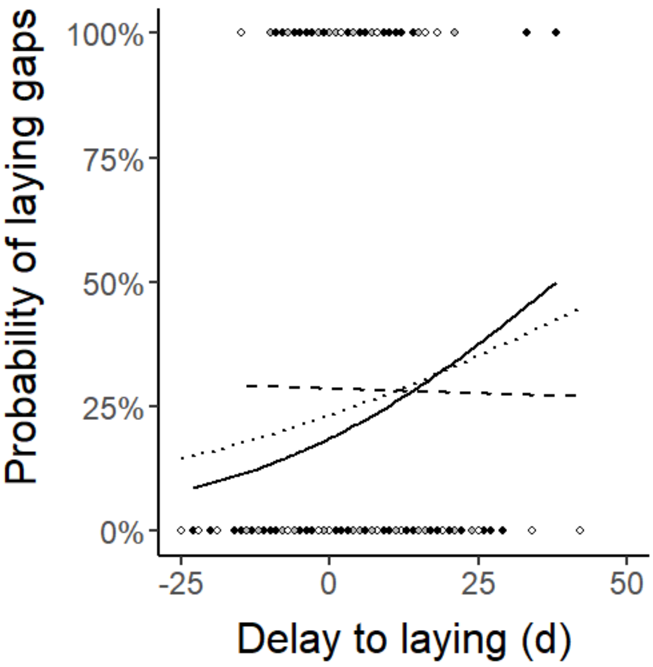

Figure 3.3: The probability of laying gaps occurring in relation to the delay in laying after budburst (d) – low: black, mid: grey, high: hollow points. Predictive lines controlling for year, per altitudinal category - low: full, mid: dashed, high: dotted line.

Table 3.2: Model summaries predicting the probability of laying gaps. Binomial GLM with logit link.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		-1.48	0.46	-3.24	<0.001
Delay to laying		0.022	0.013	-2.91	0.09
Year					
	2014	0.37	0.53	-16.13	<0.003
	2015	0.38	0.54		
	2016	0.70	0.52		
	2017	-0.66	0.54		
Altitude		0.0010	0.00068	-2.26	0.13
Clutch size		-0.029	0.094	-0.15	0.70
Delay to laying * altitude				-1.86	0.17

Changing the timing between clutch completion and the start of incubation may be another mechanism to realign breeding after laying. In our system, blue tits started to incubate on average 0.59 days after clutch completion (±2.00 SD; range: -5 - 8). This delay in incubation was negatively affected by the delay to laying after budburst; it was reduced by 0.030 days with each additional day it took blue tits to lay after budburst ($F_{1,351}$ = 8.32, P = 0.0042; Table 3.3). Additionally, blue tits reduced the delay to incubation by 0.22 days per extra day of laying ($F_{1,351}$ = 12.25, P < 0.001). Year explained variation in this incubation delay ($F_{4,351}$ = 15.29, P < 0.001). Per 100 altitudinal meters this incubation delay was extended by 0.16 days ($F_{1,351}$ = 9.92, P < 0.002). Further, the interaction between the delay to laying after budburst, and altitude was significant ($F_{1,350}$ = 8.25, P = 0.0043); females started incubating sooner in relation to the delay to laying after budburst with increasing altitude (Fig. 3.4).

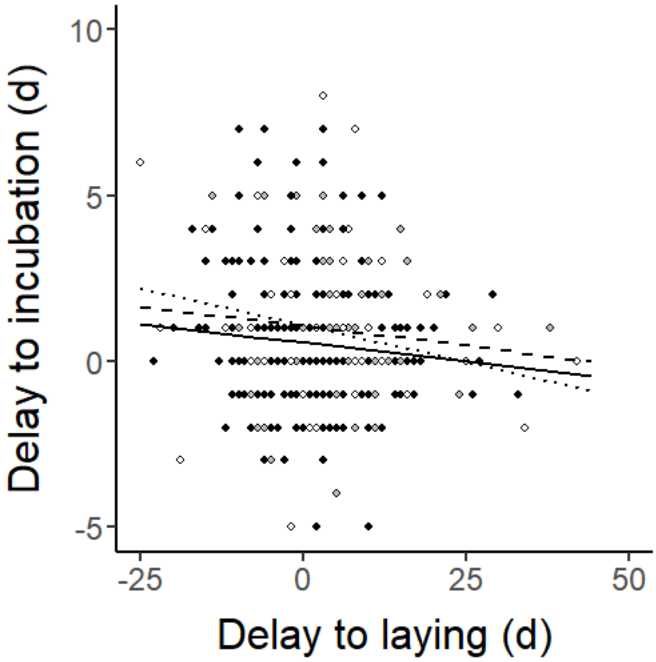

Figure 3.4: The delay to incubation after clutch completion (d) in relation to the delay in laying after budburst (d) – low: black, mid: grey, high: hollow points. Predictive lines controlling for the length of the laying period and year, per altitudinal category - low: full, mid: dashed, high: dotted line.

Table 3.3: Model summaries predicting the delay to incubation. Linear model with normal error structure.

Predictors		Estimate	± SE	χ2 value	p-value
(Intercept)		2.16	0.75	2.90	<0.004
Delay to laying		-0.030	0.010	8.32	0.0042
Laying period		-0.22	0.06	12.25	<0.001
Altitude		0.0016	0.00052	9.92	<0.002
Year				15.29	<0.001
	2014	-1.23	0.41		
	2015	-0.39	0.43		
	2016	0.57	0.41		
	2017	-1.28	0.40		
Delay to laying * altitude				8.25	0.0043

If parents can also alter incubation duration, the link between first egg date and hatching may shift to better accommodate variation in the emergence of insect prey. The incubation period ranged from 10-20 days with a mean of 13.83 days (±1.39 SD). The delay to laying after budburst had no effect on the length of the incubation period ($F_{1,379}$ = 0.52, P = 0.47; Table 3.4). Birds that took longer to start incubation from the time of first egg laid had a shorter total incubation period ($F_{1,404}$ =119.75, $P < 0.001$); on average 0.31 days less per additional pre-incubation day. Females increased the incubation period by 0.20 days per additional egg incubated ($F_{1,404}$ =16.52, $P < 0.001$). The incubation period was also extended by 0.15 days per 100 altitudinal meters ($F_{1,404}$ =21.78, $P < 0.001$). Year also explained variation in the incubation duration ($F_{4,404}$ = 5.50, $P <$ 0.001). In addition, the interaction between the delay between budburst and laying and altitude was near significant ($F_{1,378}$ = 3.72, P = 0.054); the length of the incubation period was less positively related to the delay to laying after budburst with increasing altitude (Fig. 3.5).

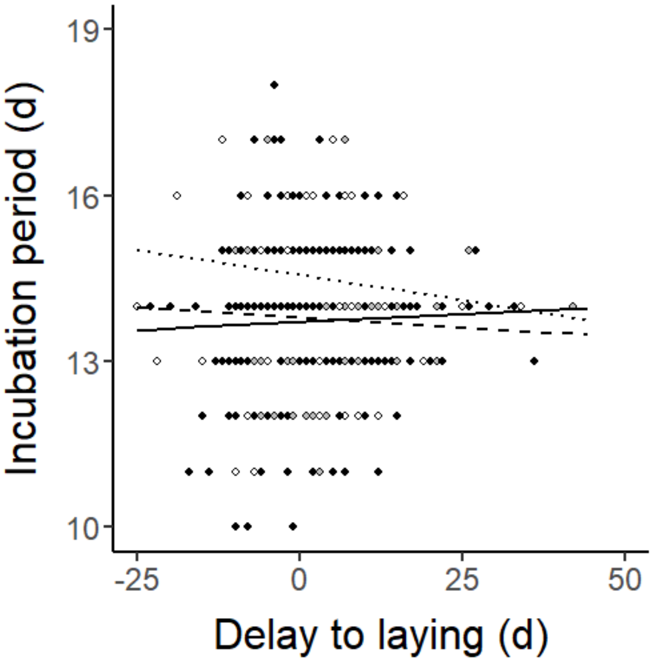

Figure 3.5: The length of the incubation period (d) in relation to the delay in laying after budburst (d) – low: black, mid: grey, high: hollow points. Predictive lines controlling for the length of the pre-incubation period, the number of eggs incubated and year, per altitudinal category - low: full, mid: dashed, high: dotted line.

Table 3.4: Model summaries predicting the length of the incubation period. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		14.57	0.48	0.48	30.45
Pre-incubation period		-0.31	0.028	119.75	<0.001
No. eggs incubated		0.20	0.050	16.52	<0.001
Altitude		0.0015	0.00032	21.78	<0.001
Year				5.50	<0.001
	2014	-0.22	0.23		
	2015	-0.33	0.25		
	2016	-0.44	0.23		
	2017	-0.86	0.22		
Delay to laying		-0.0048	0.0067	0.52	0.47
Delay to laying * altitude				3.72	0.054

The rearing period is the crucial time for chicks to growth, however an extended time in the nest may risk predation and reduce the time young can forage to accumulate fat reserves before the winter. In our population, the rearing period was on average 21.21 days long (±1.21 SD; range: 17 - 26). The delay to laying after budburst had no effect on the length of the incubation period ($F_{1,312} = 0.54$, $P = 0.46$; Table 3.5). Year explained again variation in the length of the rearing period ($F_{4,326} = 4.69$, $P = 0.0013$). In addition, the rearing period was not affected by the length of the pre-hatching period ($F_{1,325} = 2.22$, $P = 0.14$), nor the number of hatchlings ($F_{1,325} = 0.31$, $P = 0.58$) and altitude ($F_{1,325} = 0.053$, $P = 0.82$). However, the interaction between the delay between budburst and laying and altitude was significant ($F_{1,310} = 4.30$, $P = 0.039$); the rearing period was shorter in relation to the delay to laying after budburst with increasing altitude (Fig. 3.6).

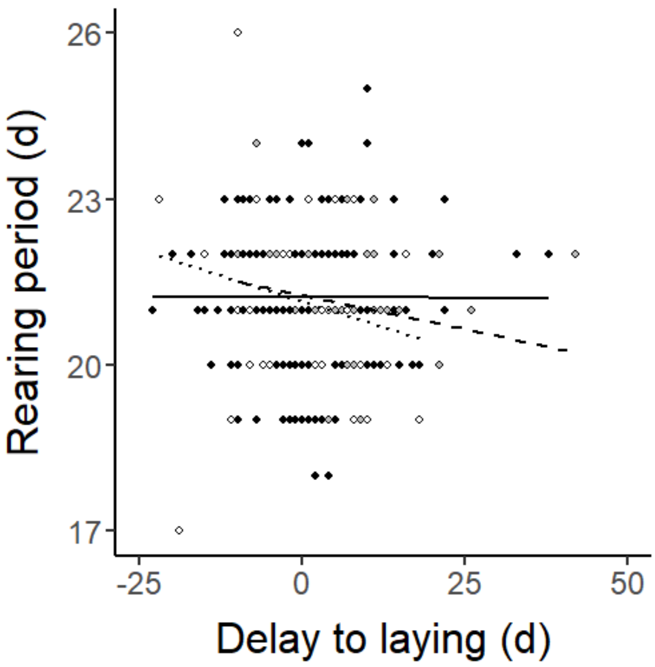

Figure 3.6: The length of the rearing period (d) in relation to the delay in laying after budburst (d) – low: black, mid: grey, high: hollow points. Predictive lines controlling for year, per altitudinal category - low: full, mid: dashed, high: dotted line.

Table 3.5: Model summaries predicting the length of the rearing period. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		21.56	0.23	94.50	<0.001
Year				4.69	0.0013
	2014	-0.14	0.28		
	2015	-0.80	0.27		
	2016	-0.53	0.26		
	2017	-0.13	0.26		
Delay to laying		-0.0057	0.0078	0.54	0.46
Altitude		-0.00013	0.00041	0.053	0.82
No. hatching		-0.027	0.043	0.31	0.58
Pre-hatching period		-0.047	0.034	2.22	0.14
Delay to laying * altitude				4.30	0.039

3.4.3 Reproductive success

An average of 5.99 chicks successfully fledged the nest (±1.87 SD; range: 1 - 11), excluding complete nest failures. The delay to laying after budburst had a significant negative effect on the number of fledglings ($\chi^2_1 = 9.50$, $P = 0.0021$; Table 3.6); per additional delay day 0.008 less chicks fledged per nest and a nest was 4.5 % more likely to fail the entire brood. Year explained variation in fledging success ($\chi^2_4 = 13.84$, $P = 0.0078$). Altitude tended to affect fledging success ($\chi^2_1 = 3.46$, $P = 0.063$); 0.023 less chicks fledged per nest per 100 altitudinal meters and a nest was 21 % more likely to completely fail. The interaction between the delay to laying after budburst and altitude did not influence the number of chicks successfully fledging the nest ($\chi^2_1 = 2.65$, $P = 0.27$; Fig. 3.7).

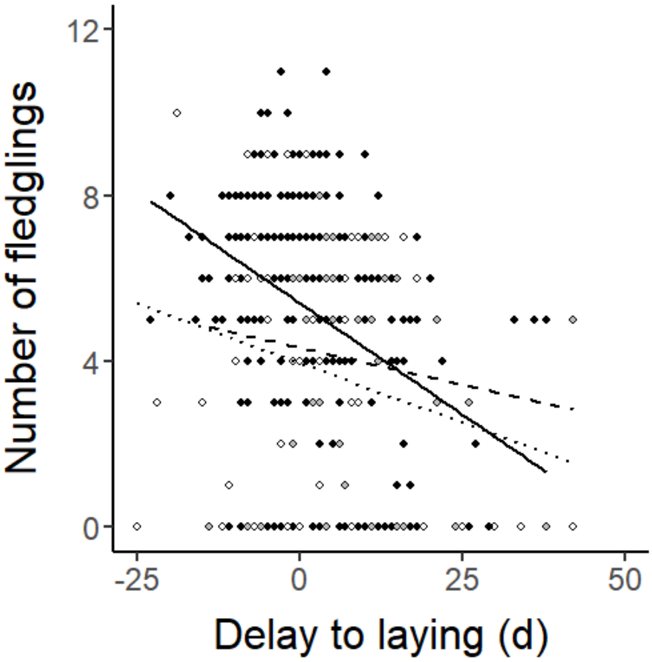

Figure 3.7: The number of fledglings in relation to the delay in laying after budburst (d; delay * altitude interaction: χ^2_1 = 2.65, *P* = 0.27) – low: black, mid: grey, high: hollow points. Predictive lines controlling for year, per altitudinal category - low: full, mid: dashed, high: dotted line.

Table 3.6: Model summaries predicting the number of fledglings. Zero-inflated model with Poisson error structure.

Predictors		Estimate	± SE	χ2 value	p-value
Count model coefficients					
(Intercept)		2.05	0.10	21.51	<0.001
Delay to laying		-0.0082	0.0027	9.50	0.0021
Altitude		-0.00023	0.00012	3.46	0.063
Year				13.84	0.0078
	2014	-0.03	0.082		
	2015	-0.26	0.085		
	2016	-0.16	0.080		
	2017	-0.073	0.075		
Delay to laying * altitude				2.65	0.27
Zero-inflation model coefficients					
(Intercept)		-3.00	0.58		
Delay to laying		0.046	0.013		
Altitude		0.0022	0.00069		
Year					
	2014	0.44	0.47		
	2015	-0.10	0.50		
	2016	-0.36	0.51		
	2017	-0.38	0.49		

3.5 Discussion

Various taxa have adapted their timing of breeding to changing climatic conditions (e.g. Bellot et al., 1991; Dunn, 2004). Environmental cues may help adjust reproductive timing with advancing prey phenology. A variety of cues such as daylength, temperature and direct or indirect measures of food availability have been suggested to be used by animals such as birds to prompt reproduction (Marshall 1961; Perrins 1965; Jones 1972; Gwinner 1996; Visser et al. 2009). One such indirect measure of food availability may be budburst. At budburst the associated fresh, young leaves provide food for prey of insectivorous birds (Blondel et al. 1993; Buse et al. 1999). Such budburst cues can fine-tune reproductive alignment with annual shifts in prey peaks (Lack 1954). However, it remains controversial to what extent budburst is directly used as a reproductive cue (Lyon et al. 2008; Schaper et al. 2011). Altitudinal gradients lend themselves as a useful model to investigate the reliability of budburst in timing reproduction due to the expected shift in budburst associated with a gradual decline in temperature. In support, I found that budburst date was delayed with altitude (Table 3.1). Budburst date was negatively related with temperature at low and middle altitudes, however at high altitudes this relation was positive (Fig. 3.1). Pilot studies have revealed that heterogeneity in tree species decreases with altitude and also shifts to more hardy, cold tolerant species such as beech (*Fagus sylvatica;* Broadribb 2017), which is characteristic of the Northern Pyrenees (Ninot et al. 2017). Thus, the negative relationship at low altitude may be due to species being more temperature sensitive and in turn waiting for warmer conditions to spring into budburst. Although budburst is delayed with altitude, it is suggestive that high altitudes tree species have a greater tolerance to lower temperatures. This budburst-altitude relationship with temperature enables insight into the importance of budburst in cuing reproduction. On average lay date occurred three days after budburst. In turn, birds laid later in relation to budburst with increasing altitude; on average one day per 100 meters upwards (Fig. 3.2). This delay in laying after budburst affected consequent breeding parameters.

Even though previous studies have suggested breeding phenology to be fixed by lay date (Visser et al. 1998, 2015; Phillimore et al. 2016), my results suggest that blue tits can additionally adjust timing after the first egg has been laid. Particularly females seemed to flexibly adjust consecutive breeding phases in relation to each other, to better time chick requirements and prey availability. Those birds with longer laying periods started incubation closer to clutch completion and also shortened the duration of incubation. These adjustment mechanisms seemed to be related to budburst. After egg laying was complete, the start of incubation was advanced in relation to a longer delay to laying after budburst. In contrast, the start of incubation after clutch completion was delayed with altitude. Further, at higher altitudes the incubation period was significantly extended. This increase was likely due to overall lower temperatures at higher elevations. Similar results have been found in ground-nesting mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*), which increase their incubation intensity during cold periods (Zerba and Morton 1983). Further, the delay to incubation was more negatively related to the delay in laying after budburst with increasing altitude; suggesting that individuals may shorten pre-hatching periods more to catch up with the budburst at high than low altitudes (Fig. 3.4). Similar results were found for the length of the incubation and rearing period (Fig. 3.5 & 3.6).

Due to the lower heterogeneity of vegetation at high compared to low altitude (pilot study: Broadribb 2017), budburst cues may be a more reliable estimate of general vegetation development and thus prey phenology. Pilot studies on our system have revealed, that for instance, at low elevation there is a higher percentage of understory, which springs into leaf before the budburst of larger vegetation. In addition, as the species of trees are also more diverse at low compared to high altitudes, the timing of budburst of trees is more varied (personal observation). Finally, the higher proportion of flowering trees such as wild cherries and plum may supply more diverse sensory cues to birds feeding on them in early spring. In contrast, a more homogenous environment at high elevation should lead birds to use of budburst as a more reliable cue in fine-tuning reproductive schedules. In line with this hypothesis, my results seem to generally suggest that reproductive timing at high altitude is more highly correlated with budburst.

This observational study sheds light on the mechanisms used by birds to fine-tune reproduction. It highlights the importance of distinguishing different habitat types, specifically homo- versus heterogenous habitats, in which the use of budburst differs for the timing of avian reproduction. These discrepancies might explain the variation in importance ascribed to budburst in previous studies (Visser et al. 2002; Nilsson and Källander 2006; Thomas et al. 2010). Further, our study system demonstrated the power of altitudinal gradients in generating varying levels of environmental variability, though by also controlling for confounding variables such as daylength and differences in population origins. In the future, it is recommended that more emphasis is placed on these confounding variables in evaluating the importance of specific cues for the timing of reproduction.

Chapter Four

Inducing females to lay more eggs leads to increased *per capita* provisioning rates of nestlings in blue tits

4.1 Abstract

Theory on the evolution of bi-parental care typically predicts that male and female provisioning rates are determined by brood demand, and that increases in investment by one member of the pair should be met by partial reductions by the other. While empirical tests are largely supportive, significant unexplained variation remains. A recent model suggested that some of this variation could be accounted for if female provisioning rates are impacted by the costs of egg-laying, leading females investing heavily in eggs to provision the ensuing nestlings less than expected and their partner in turn more. Here I tested this hypothesis in a population of blue tits (*Cyanistes caeruleus*) breeding in the French Pyrenees, by inducing females to lay an average of 1.9 eggs more than females of unmanipulated clutches, though experimentally equalising the number of eggs incubated and hatched between treatment groups. Contrary to expectation, I found that females in experimental groups provisioned nestlings 30 % more than those in controls. Experimental males also showed a trend for increasing their provisioning rates, leading to 18 % increased provisioning rates overall in experimental nests. Additionally, I show that female feeding rates are not only related to current brood demand, but also to the initial number of eggs laid. My results suggest that: (a) female provisioning rates are tuned to their egg investment, and (b) males do not respond negatively to elevations in female provisioning rates, supporting recent theory on behavioural matching. I recommend greater consideration of female investment at the egg stage in attempts to understand the evolutionary dynamics of bi-parental care.

4.2 Introduction

Bi-parental care, where both mother and putative father cooperate to rear joint offspring occurs in a few species of invertebrates, reptiles and fish, up to 5 % of mammals, including humans, and is the norm for birds (Cockburn 2006; Royle et al. 2012). Notably in this care system, each member of a pair is usually unrelated, and as contributions are costly, each will benefit from the other contributing more than its 'fair' share (Williams 1966; Trivers 1972). Due to this point of tension between diverging fitness interests, contributions of each parent are key to understanding the evolution and maintenance of bi-parental care. A considerable body of theoretical work suggests that bi-parental care is stabilised when increases or decreases in contributions by one member of the pair are met with only partial compensatory changes in the opposite direction by the other (Houston and Davies 1985; Winkler 1987; McNamara et al. 1999, 2003). This incomplete compensation leads to fitness costs for cheating, thus placing a limitation on how far parents can drop investment before their offspring receive insufficient care. On the whole, empirical work is supportive; in a recent meta-analysis of 54 experimental studies, Harrison et al. (2009) found that on average, the predicted patterns of incomplete compensation were met. However, this study also acknowledged that significant variation existed in whether it was the male or female that contributed more and that outcomes other than incomplete compensation were common. Only a few alternative models have been created to understand systems that do not show partial compensation.

Johnstone & Hinde (2006) developed one of the first game-theoretical models to support matching response by parental birds. This model provides an explanation for why the sex with more information of brood demand should contribute more overall and, why the individual with less information should respond positively to changes in any contributions made by the more informed member of the pair. Supporting evidence for each prediction comes from experimental studies of great tits (Hinde 2006) and long-tailed tits (*Aegithalos caudatus*, Meade et al. 2011). More recently, factors additional to current conditions have been highlighted that may explain deviating parental responses. In another game-theoretic model, Savage et al. (2013a) showed that the costs of investment during earlier stages of a reproductive attempt might impact later contribution and subsequent responses of partners during provisioning. In this case, if females invest heavily in egg investment, for example, they might be expected to contribute less during nestling provisioning, with the male fully compensating for this reduction to reduce his fitness losses. This negative correlation in investment by the female over consecutive breeding stages might be explained by a strategy to balance current versus future reproductive costs. It has been demonstrated previously that combining investment at both egg and nestling stages can shape our understanding of relative investment levels of males and females to a reproductive event, and that full compensation can be explained by incorporating egg stage investment (Russell et al. 2007, 2008). However, to date, no study to our knowledge has explicitly manipulated early investment, and then measured the resulting levels of provisioning by each member of the pair during subsequent nestling rearing.

Here I directly test the effects of early investment on subsequent provisioning rates of male and female blue tits by inducing females to lay additional eggs in a nest box population in the French Pyrenees. This species lends itself well to clutch manipulations as it is an indeterminate egg-layer, adjusting laying to the number of eggs currently present (Kennedy 1991). Importantly, through our experimental technique of removing the first four eggs on the day they were laid, I induced females to lay more eggs, but not to incubate or provision more nestlings. Consequently, any treatment effects on female and male provisioning rates would stem directly from our inducement of extra egg laying, and not through changes to incubation costs or brood demand. First, I detail the consequences of the experiment for the number of eggs laid. In conjunction, I test whether experimental and control nests have comparable investment in: egg volume; the numbers of eggs incubated and hatching; and the degree of hatching synchrony, which may alter feeding rates. I predict (a) that an augmentation in egg laying will cue parental provisioning rates at the rearing stage; and (b) that compared to long-lived species our blue tits will not experience such a sharp, negative trade-off between laying and rearing in parental investment. Thus, in a second step I examine the effect of the experiment on male and female provisioning rates, as well as on the proportion of caterpillars versus other prey items delivered. Finally, I investigate whether differences exist between experimental and control nests in nestling mass, a proxy for offspring quality, which may arise due to differences in feeding rates between the two treatment groups.

4.3 Methods

We performed our study over two consecutive reproductive seasons in 2013-2014. Our colour-banded nest box population is located near to the Station for Theoretical and Experimental Ecology in Moulis (SETE, UMR 5321; 42°57’29” N, 1°05’12” E) in the French Pyrenees. Our nest box populations span four sites along a 1000 m altitudinal gradient (within 15 km of the research station), though the vast majority of nests (87 %) used in this study were from two sites at relatively low elevation (mean elevation of nest boxes used in this study = 617.84 m, ±156.45 SD, range: 461-1105 m). Our Woodcrete boxes are placed at 50 m intervals in the mixed deciduous woodland habitat. Work was conducted under animal care permits to A. S. Chaine from the French bird ringing office (CRBPO; n°13619), the state of Ariège animal experimentation review (Préfecture de l’Ariège, Protection des Populations, n°A09-4) and the Région Midi-Pyrenées (DIREN, n°2012-07).

4.3.1. Experimental design

Blue tit nests were identified using observations or video recordings (Sony HDR-CX220E Handycam® Camcorders) during nest building. First egg lay date was known with precision owing to daily nest box checks from when nests neared completion. Experimental and control nests were assigned when at least two nests, within 300 m distance, overlapped in laying (mode: 3 days). Doing so ensured that there were no systematic differences in lay date between experimental (mean: 13th April, ±4.64 SD) and control nests (mean: 13th April, ±8.35 SD; GLM with Poisson error structure including the covariates altitude and year: $\chi^2_{1,46} = -0.016$, $P = 0.90$), and that inevitable heterogeneity in habitat was minimised between the two treatment groups.

In experimental nests, the first four eggs were removed on the day each was laid, with the first frozen in the lab as part of a separate study and the latter three being placed under the nest in a padded plastic container 1.8cm high and 5cm in diameter, with a replaceable cardboard lid to prevent moisture transfer. Our use of Woodcrete boxes with removable front doors allowed nests to be raised slightly without damage. Following clutch completion, two of the three eggs under the nest were re-inserted into their nest cup, and another was transferred to control nests (see control nests below for rationale). The motivation to remove two eggs permanently from experimental nests was to ensure that females did not incubate more eggs or provision more nestlings in experimental nests compared with controls. Previous studies of similarly reproducing great tits using the same protocol found that females laid two additional eggs (Oppliger et al. 1996; Visser and Lessells 2001; Gill et al. 2005). Across the two breeding seasons, I attempted to manipulate the number of eggs laid at 34 nests.

In control nests, the first egg was also removed and frozen as part of another study, but this time it was replaced by a decoy egg, which was accepted without exception. This decoy was then replaced by an egg from under an experimental nest at clutch completion, before incubation onset, to ensure no reduction in natural clutch size. Further, by introducing an egg from under the nest of experimental nests ensured that all nests contained eggs that had been subjected to under-nest conditions, equating to an average of 25 % of eggs in experimental nests and 16 % of eggs in control nests. Finally, experimental and control nests were visited with similar regularity to monitor egg-laying and obtain the precise date of clutch completion, and all eggs were handled at least once to estimate their volumes. Overall, I compared the 34 experimental nests with 16 control nests, with the reduced number of controls owing to the use of most other nests in a concurrently running experiment.

4.3.2 Treatment effects pre-provisioning

To ensure that investment was not experimentally changed for reproductive traits other than clutch size, I compared the following among treatment groups; the size of eggs laid, the number of eggs incubated, hatchling number and hatching synchrony. The overall number of eggs laid and the numbers incubated were known with precision in all cases through repeated nests visits towards the end of laying and early in incubation. In 2014 I also calculated egg volumes using the following method: at the end of egg laying or within the first few days of incubation, eggs were removed from the nest and photographed from above (minimum 50 cm distance), to minimise parallax and thus maximise measurement consistency, on a 1x1 cm gridded black background. Photographs were then analysed using the programme ImageJ and a script developed by Enrico Sorato on *R*. All nests were checked daily for hatching from 11 days following final lay date. The number of hatchlings was determined by the number of eggs that hatched successfully. Finally, hatching synchrony was estimated by weighing all hatchlings in each nest, as weight in the first couple of days is mainly related to days since hatching. I weighed chicks on the first day that all eggs had hatched or at the latest on day three after the first chick hatched in very asynchronous broods. The maximum hatching asynchrony in our population rarely exceeds three days. Thus, the greater the variance in hatchling mass within broods, the greater the spread in ages. This estimate will not be confounded by eggs that failed to hatch unless hatching failure differed significantly between treatment groups, which would in turn artificially reduce asynchrony estimates in one treatment (see Results).

4.3.3 Provisioning behaviour

Differences in parental provisioning behaviour at the chick rearing stage between the treatment groups were determined using video footage. At least two hours per nest were recorded (Sony HDR-CX220E Handycam® Camcorder), though the first and last ten minutes were not analysed to minimise any disturbance effects. Videos were analysed blind to treatment group. Broods were nine to 17 days old at recording to ensure both parents were feeding at peak rates (fledging occurs from day 18-22, unpublished data). In particular, females feed less in the first week after chick hatching, as they are occupied with brooding (Sasvari 1986). From each video, I then extracted female and male provisioning events, and the proportions of different prey types delivered to the nest. Prey types were either classified as caterpillars (Lepidoptera larvae) or other, small arthropods (García-Navas et al. 2012). The female and male were identified through their unique colour-ring combinations. Previously unringed adults were caught on the nest and ringed at a minimum of 11 days of chick age to avoid desertion. Overall, I analysed 85 hours of video footage at 50 nests (34 experimental and 16 control nests). Finally, all chicks were weighed (±0.05g) on day 10-16 post hatching.

4.3.4 Statistical analysis

Statistics were performed in *R* 3.4.2 (R Core Team 2017). All data sets were examined for outliers and distributions of dependent variables were visually inspected for normality. Full models underwent checks for overdispersion and heteroscedasticity in model residuals, which were controlled for by changing the model error distribution as necessary (Zuur et al. 2009b). Normal response terms were analysed using linear models in the basic ‘stats’ package (R Core Team 2017). If the data were non-normal, generalised linear models (GLMs, package = MASS; Venables and Ripley 2002) were used adjusting variance structure accordingly, i.e. the error distribution family and log link function (see tables of each analysis; Thomas et al. 2013). Model selection was based on changes in deviance using the anova function in *R* (significance set at $\alpha < 0.05$) using a step-wise, backward deletion procedure (Zuur et al. 2009a). The focal variable - categorical treatment - was retained in all models.

The effects of our experiment on the number of eggs laid, average egg volume per clutch, the number of eggs incubated, hatchling number and synchrony were analysed using a series of separate, linear models. Generalised instead of general linear models were used for the number of eggs incubated with a Poisson error structure (GLM, package = MASS; Venables and Ripley 2002). Hatching synchrony was normalised using Tukey’s Ladder of Powers, which determines the power transformation that most closely fits the data to a normal distribution (Mangiafico 2016). In the analysis of hatchling number I controlled for lay date, altitude, year and the number of eggs incubated as continuous covariates. The same covariates were added to the egg volume analysis, except for year as data was only available from 2014. In the hatching synchrony analysis, I additionally controlled for age at first weighing and brood size (replacing the number of eggs incubated).

Next at the chick rearing stage, I investigated the effect of the increased egg laying treatment on separate female and male hourly provisioning rates. For these analyses, linear models with normal error structure were applied. In the full models, additionally to treatment, lay date, altitude and year, I also included both brood size and age, and their squared effects due to possible non-linear effects as fixed covariates (Rytkönen et al. 1996; Parejo and Danchin 2006). Second, I analysed the effect of a sex * treatment interaction on provisioning rates to see whether males shifted their feeding in response to any mean change in female response due to the additional eggs laid. A mixed model controlling for non-independent measures of females and males from the same nest was applied by adding nest box identity as a random effect (nlme package, (Pinheiro et al. 2017). Third, I examined total feeding rates (combined female and male rates), to ascertain any overall provisioning differences between the treatment groups, using a general linear model with normal error structure. In the latter two analyses, I fitted the same covariates as in the first provisioning analysis.

The proportion of caterpillars delivered to the nest may negatively confound the intensity of provisioning, as they are larger and thus richer in protein than other prey items (Royama 1966; Van Balen 1973). Thus, possible differences in the proportion of caterpillars due to treatment were analysed. Firstly, a mixed model was applied to investigate sex-differences in caterpillar delivery rates, controlling for pairs from the same nest by including box identity as a random factor (lme4 package, Bates et al. 2015). Due to the response variable being proportional, the error structure was changed to binomial. Secondarily, a non-mixed GLM with binomial error structure was run to analyse differences between treatment groups in the overall proportions of caterpillars of both parents combined (package = MASS; Venables and Ripley 2002). The same fixed factors as in the hourly feeding rate per sex analyses (i.e. treatment, lay date, altitude and year, brood size and age, and their squared effects), were used throughout. Further, I also included the fixed covariate of hourly feeding rates, as a limitation can be expected on the maximum number of caterpillars collected by the number of foraging trips (Grieco 2002; Navalpotro et al. 2016).

Finally, differential investment in the brood due to the additional egg laying in experimental groups was tested by looking at variation in chick mass between the treatment groups. This variable should support any parental provisioning strategies found, as chick mass is directly affected by any changes in feeding rates. To this end, I fitted individual chick mass in a linear mixed model (nlme package, (Pinheiro et al. 2017). Individual chick mass was normalised using Tukey’s Ladder of Powers, which determines the power transformation that most closely fits the data to a normal distribution (Mangiafico 2016). In addition to the standard fixed covariates of lay date, altitude and year, I included brood size and age plus the variance of mass at hatching, and nest of origin as a random factor to control for repeated measures of chicks from the same nest.

4.4 Results

4.4.1 Treatment effects pre-provisioning

Experimental females laid on average 10.38 eggs (±1.16 SD), which was a mean of two eggs more than control females (controlling for the following covariates of lay date, year and altitude - mean: 8.44 eggs, ±0.96 SD; $F_{1,45}$ = 32.75, $P < 0.001$; Fig. 4.1). Clutch size decreased on average by 0.12 eggs per day in the season ($F_{1,45}$ = 5.69, $P = 0.021$). In 2014 females laid 1.38 eggs less than in 2013 ($F_{1,45}$ = 4.42, $P = 0.024$). There was a trend for a positive effect of altitude on the number of eggs laid ($F_{1,45}$ = 3.64, $P = 0.063$).

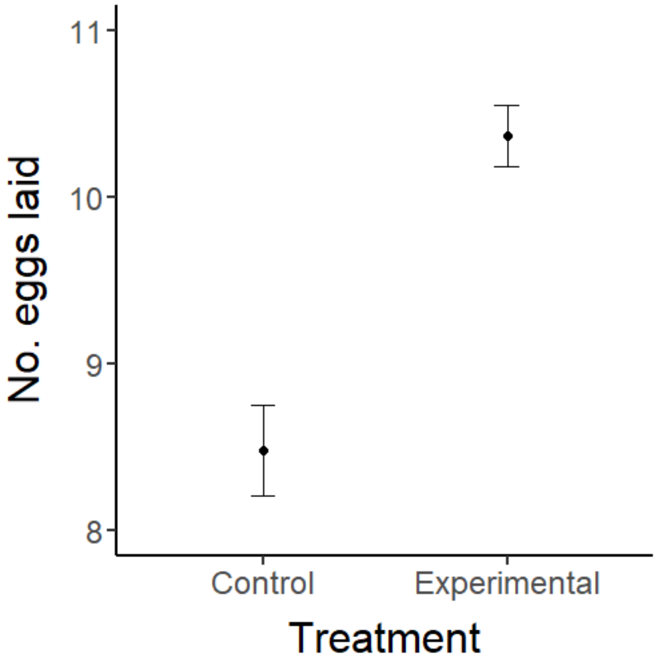

Figure 4.1: The average number of eggs laid in control versus experimental treatments after controlling for lay date, altitude and year. Shown are the predicted mean ± SE.

Table 4.1: Model summaries predicting the number of eggs laid. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		22.19	5.015	4.42	<0.001
Treatment	Control	-1.88	0.33	32.75	<0.001
Lay date		-0.12	0.052	5.69	0.021
Altitude		0.0028	0.0015	3.64	0.063
Year	2014	-1.38	0.59	5.42	0.024

We did not find further differences between controls and experimental groups in variables that could influence female investment (after controlling for lay date, altitude and year, and the number of eggs incubated or hatched where necessary). On average, females laid the same sized eggs across the treatment groups (experimental mean: 1.11 cm^3, ±0.13 SD; control mean: 1.14 cm^3, ±0.053 SD; $F_{1,27} = 0.77$, $P = 0.39$; Table 4.2a; Fig. 4.2a), and incubated comparable clutch sizes due to our cross-fostering approach (experimental mean: 8.41 eggs, ±1.18 SD; control mean: 8.31 eggs, ±1.14 SD*;* $\chi^2_{1,45} = -0.0016$, $P = 0.97$; Table 4.2b; Fig. 4.2b). Further, there was no treatment difference in the number of hatchlings (experimental mean: 7.44 chicks, ±1.71 SD; control mean: 7.50 chicks, ±1.71 SD; $F_{1,47} = 0.23$, $P = 0.64$; Table 4.2c; Fig. 4.2c). Finally, I found little evidence to suggest that levels of hatching synchrony (i.e. variance in chick mass due to differences in hatch date; see Methods), differed due to treatment (experimental mean: 0.49, ±0.31 SD; control mean: 0.55, ±0.27 SD; $F_{1,22} < -0.36$, $P = 0.56$, Table 4.2d; Fig. 4.2d).

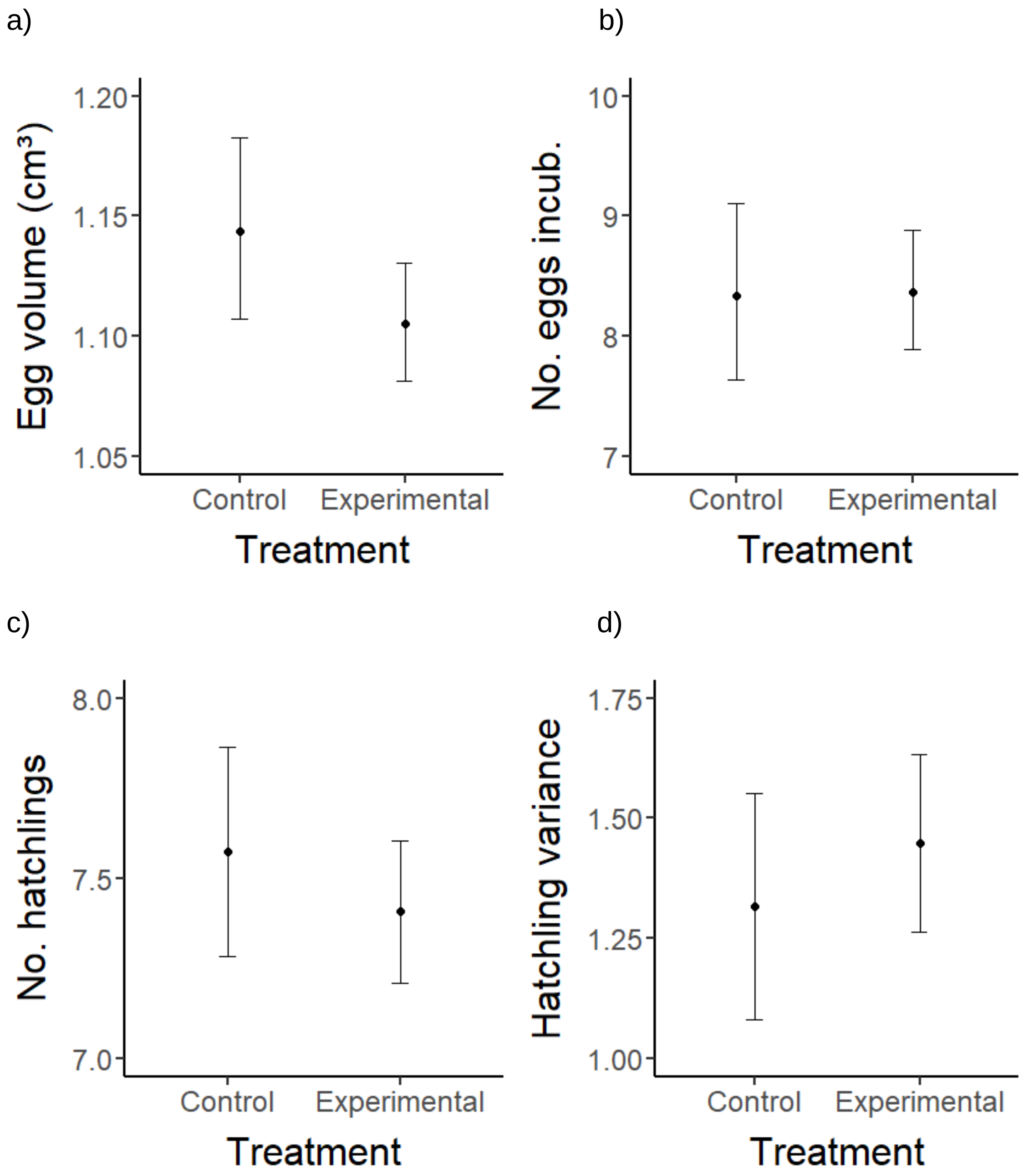

Figure 4.2: The relationship between control and experimental treatment group and a) average egg volume per clutch, b) the number of eggs incubated, c) the number of hatchlings and d) hatching synchrony, after controlling for lay date, altitude and year where relevant, plus the number of eggs laid for the average egg volume per clutch and the number of eggs incubated for the number of hatchlings. Shown are the predicted mean ± SE.

Table 4.2a: Model summaries predicting average egg volume per clutch. Gamma GLM.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		1.11	0.024	45.38	<0.001
Treatment	Control	0.038	0.044	0.77	0.39
Lay date		-0.0037	0.0063	0.35	0.56
Altitude		0.00011	0.00020	0.31	0.58
No. eggs laid		0.0039	0.025	0.025	0.88

Table 4.2b: Model summaries predicting the number of eggs incubated. Poisson GLM.

Predictors		Estimate	± SE	χ2 value	p-value
(Intercept)		4.47	1.71	2.61	0.0092
Treatment	Control	-0.0042	0.11	-0.0016	0.97
Lay date		-0.024	0.018	-1.94	0.16
Altitude		0.00046	0.00047	-0.92	0.34
Year	2014	-0.23	0.19	-1.44	0.23

Table 4.2c: Model summaries predicting the number of hatchlings. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		-1.69	1.22	-1.39	0.17
Treatment	Control	0.17	0.35	0.23	0.64
No. eggs incubated		1.086	0.14	57.86	<0.001
Lay date		-0.00070	0.0018	0.27	0.61
Altitude		0.034	0.066	0.16	0.69
Year	2014	0.23	0.74	0.10	0.76

Table 4.2d: Model summaries predicting hatching synchrony. Linear model with normal error structure.

Predictors		Estimate	± SE	F value	p-value
(Intercept)		0.74	0.043	17.27	<0.001
Treatment	Control	0.045	0.074	0.36	0.56
Brood age		0.078	0.075	1.087	0.31
Brood size		0.039	0.028	1.94	0.18
Lay date		0.021	0.019	1.28	0.27
Altitude		-0.00014	0.00021	0.45	0.51
Year	2014	0.073	0.095	0.60	0.45

4.4.2 Provisioning behaviour

Overall provisioning rates were not different due to variation between treatment groups in brood age (experimental mean: 13.15 days, ±1.21 SD; control mean: 13.31 days, ±1.70 SD; $F_{1,48} = 0.16$, $P = 0.69$), nor in the size of broods (experimental mean: 7 chicks, ±1.87 SD; control mean: 7 chicks, ±1.78 SD; $F_{1,48} = 0.11$, $P = 0.74$). However, across treatment groups there was a tendency of brood age to positively influence variation in provisioning with an average increase of 0.96 hourly visits per day post-hatching ($\chi^2_{1,45} = 3.51$, $P = 0.061$), though no squared effect was observed ($\chi^2_{2,45} = 3.70$, $P = 0.16$). Brood size had a squared effect ($\chi^2_{2,47} = 43.72$, $P < 0.001$; Fig. 4.3), so that provisioning rate increased to and then diminished again over a brood size of around seven chicks. Provisioning rate decreased throughout the season with 0.54 less hourly visits per day ($\chi^2_{1,45} = 10.04$, $P < 0.002$). There was no change in feeding rate with altitude ($\chi^2_{1,44} = 1.28$, $P = 0.26$). Parents provisioned young on average five times less per hour in 2014 than 2013 ($\chi^2_{1,45} = 5.98$, $P = 0.014$), with females contributing on average 3.75 times fewer feeds per hour than males ($\chi^2_{1,46} = 7.79$, $P = 0.005$).

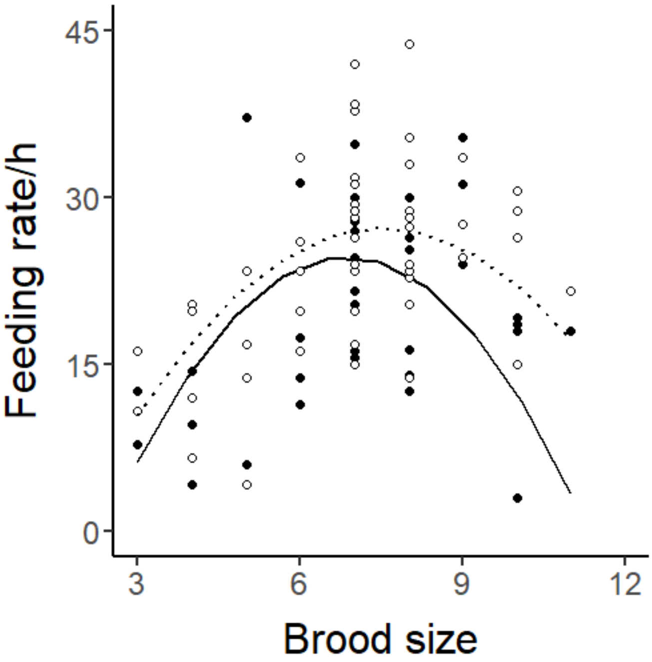

Figure 4.3: Hourly feeding rates of females (full line) and males (dotted), in relation to brood size, controlling for the number of eggs laid, linear brood age, lay date and year. Shown are the predicted lines of best fit and raw data points for females (filled circles) and males (hollow circles) from a mixed model including sex as a covariate and nest ID as a random factor.

After controlling for this natural variation (i.e. brood age and size, season, altitude and year), I found that experimental females had a 30 % higher hourly provisioning rate relative to control females ($F_{1,43}$ = 9.13, P = 0.004; Fig. 4.4), with the latter group feeding an average of 15.91 times per hour (±8.21 SD; range: 3.00-30.00). In males there was a similarly positive but marginal difference (8 % more hourly visits in experimental than control groups; control mean: 23.025 ±10.00 SD; range: 4.20-42.00; $\mathrm{F}_{1,43}$ = 2.87, P = 0.098; Fig. 4.4). There was an indication for the treatment to affect the sex contributions, with provisioning being shared more similarly between the sexes in experimental compared to control groups (sex * treatment interaction: $\chi^2_{1,46}$ = 3.01, P = 0.083). These sex-specific differences impacted overall provisioning (combined female and male rates) positively, leading to experimental nests being visited 18 % times more often per hour than control nests ($F_{1,41}$ = 9.92, P = 0.003).

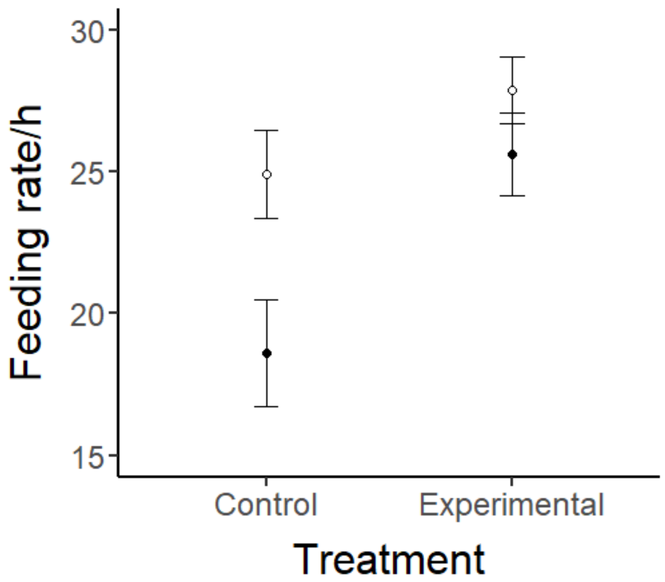

Figure 4.4: Hourly feeding rates of females (filled circles) and males (hollow circles), in relation to control and experimental treatment, controlling for the squared effect of brood size, linear brood age, lay date and year. Shown are the predicted mean ± SE from separate sex-specific models.

Further, to investigate the relative importance of the number of eggs laid versus current brood size in governing parental feeding behaviour, I substituted treatment with the number of eggs laid in a linear mixed model (including the same fixed factors as above; linear and squared brood size, linear brood age, lay date and year). Nest identity was kept as a random effect to control for collinearity of parents from the same nest box. This analysis confirmed that parents fed chicks significantly more per egg laid (estimate: 1.86, $\chi^2_{1,45} = 8.15$, $P = 0.004$), and that there was a trend for females to respond more strongly to the number of eggs they had laid than males (estimate: 1.70; $\chi^2_{1,46} = 3.13$, $P = 0.077$; Fig. 4.5). To examine this sex difference in more detail, I also compared the results of two pairwise partial correlation tests performed on females and males separately. First, partial correlation between feeding rate and the number of eggs laid was tested, controlling for linear and squared brood size. Second, I tested partial correlation between feeding rate and linear brood size, controlling for the number of eggs laid and squared brood size. For females, the number of eggs laid were significantly partially correlated with feeding rate (Pearson's coefficient r = 0.48, $P < 0.001$), which was nearly as important as current brood size in determining provisioning (Pearson's coefficient r = 0.62, $P < 0.001$). The pattern observed in females did not hold for males. Male feeding rate was determined by brood size (Pearson's coefficient r = 0.56, $P < 0.001$), but not the number of eggs the female had laid (Pearson's coefficient r = 0.059, $P = 0.70$).

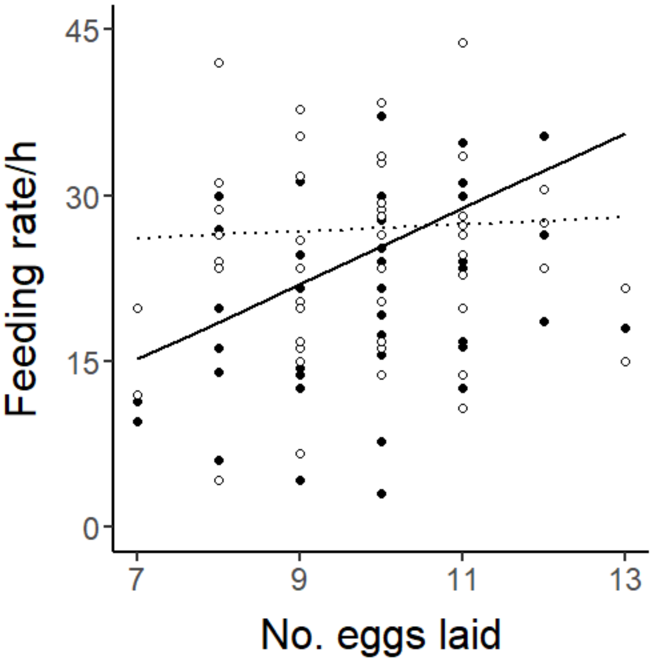

Figure 4.5: Hourly feeding rates of females (full line) and males (dotted), in relation to the number of eggs laid (controlling for the squared effect of brood size, linear brood age, lay date and year). Shown are the predicted lines of best fit and raw data points for females (filled circles) and males (hollow circles) from a mixed model including sex as a covariate and nest ID as a random factor.

Changes in the number of visits may be confounded by prey quality with caterpillars being more profitable in comparison to smaller arthropods. Overall, caterpillars comprised 9 % of all prey items brought to the nest. I never observed more than one prey item per visit being brought to the nest. There was a non-significant trend for general provisioning rates to be negatively correlated with the proportion of caterpillars brought to the nest (estimate: -0.011; $\chi^2_1 = 2.99$, $P = 0.084$). After controlling for this effect, I found no evidence of females and males contributing different amounts of caterpillars between treatment groups (estimate: 0.014; $\chi^2_1 < 0.007$, $P = 0.94$). Additionally, the overall proportional delivery rate of caterpillars did not differ between treatment groups (estimate: 0.17; $\chi^2_1 = 0.81$, $P = 0.38$).

4.4.3 Nestling mass

At weighing, there was no significant difference in the age of chicks due to treatment (experimental mean: 14.91 days, ±0.69 SD; control mean: 15.29 days, ±0.60 SD; GLM: $F_{1,43} = 3.14$, $P = 0.084$). Brood size did also vary between experimental nests (mean: 7.03, ±1.91 SD) and control nests (mean: 6.81, ±1.94 SD; GLM: $F_{1,45} = 0.18$, $P = 0.67$). Chick mass was not influenced by either variance in hatching mass ($\chi^2_1 = 1.21$, $P = 0.27$), brood age ($\chi^2_1 = 2.52$, $P = 0.11$) nor brood size ($\chi^2_1 = 1.42$, $P = 0.23$). Additionally, no effects of altitude ($\chi^2_1 = 2.77$, $P = 0.10$), nor year were found ($\chi^2_2 = 0.20$, $P = 0.66$). However, there was a negative effect of hatch date on chick mass, with chicks being on average 0.1 % lighter per day in the season ($\chi^2_1 = 12.51$, $P < 0.001$). After taking account of these natural factors influencing brood characteristics (i.e. variance in hatching, brood size and age, altitude, year and hatch date), experimental chicks tended to be 8 % heavier than chicks from control nests ($\chi^2_1 = 3.81$, $P = 0.051$, Table 4.3, Fig. 4.6).

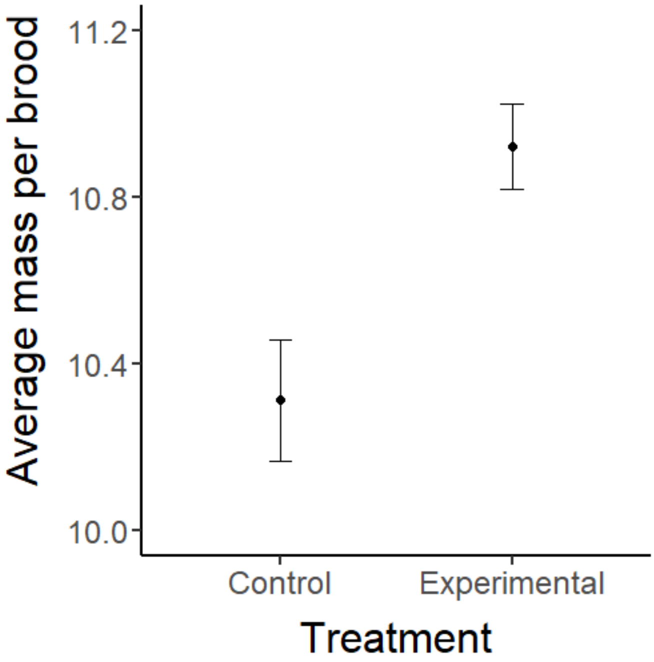

Figure 4.6: The relationship between control and experimental treatment group and average mass per brood, after controlling for brood age, lay date and altitude as fixed effects and nest ID as the random factor. Shown are the predicted mean ± SE.

Table 4.3: Model summaries predicting average chick mass per brood. Mixed model with nest ID as the random factor.

Predictors		Estimate	± SE	χ2 value	p-value
(Intercept)		26.77	4.91	5.46	<0.001
Treatment	Control	-0.76	0.43	3.81	0.051
Brood age		-0.54	0.33	2.52	0.11
Brood size		0.12	0.084	1.42	0.23
Variance in hatching mass		0.71	0.62	1.21	0.27
Hatch date		-0.13	0.04	12.51	<0.001
Altitude		-0.0020	0.0012	2.77	0.10
Year	2014	0.14	0.86	0.20	0.66

4.5 Discussion

My results strongly suggest that laying costs *per se* affect later nestling provisioning behaviour. In this study, I artificially increased egg production, with an average augmentation of 1.9 eggs more compared to controls. Due to the experimental design, I detected no further differences in the number of eggs incubated and other associated parental investment. In the successive rearing phase, experimental parents provisioned young five times more often per hour than control parents. When I looked at each sex separately, I found that experimental females provisioned young 30 % more frequently than control females, with males showing a similar, but weaker increase (8 %, Fig. 4.3). In turn my results suggest, that this increase in parental care had a positive effect on offspring mass, with chicks from experimental nests weighing on average 8 % more than chicks from control nests.

It is surprising to me that females who laid more eggs and thus likely incurred higher egg laying costs, also invested more in nestling feeding. In the following, I cover various hypotheses for why this positive link may be found in the natural world, and how this finding compares to previous studies. Before delving into the various explanations, it is important to highlight that I do not think that this positive relationship can be explained by unintended differences in egg or chick development induced by my experiment. In support of this, the size of eggs, the number of hatchlings and hatching asynchrony did not differ between treatment groups (Fig. 4.2). The increase in experimental feeding rates should not have occurred at the cost of food quality: previous studies, for example in blue tits, have found that increased provisioning rates resulted in a shift towards more easily available, though less preferred prey items (Sasvari 1986). This process does not hold in the current experiment, as no significant difference in the proportion of caterpillars brought to the nest was distinguished between treatment groups. Additionally, the fact that chicks tended to be heavier in experimental nests compared to controls does not support heightened feeding rates compensating for a decrease in other, undetected parental care behaviours such as brooding. Overall, I have no reason to believe that the experimental protocol confounded the existing results. Thus, what is the rationale behind our blue tit females adopting a positive investment response in provisioning to increased egg laying?

General theory predicts that animals should balance the costs and benefits of reproduction to maximise life-time fitness (Williams 1966). Previous studies have demonstrated a trade-off between current versus future reproductive investment, mainly by manipulating brood size post hatching, i.e. post birth (Tinbergen and Both 1999, and references within). For example, blue tit parents with experimentally enlarged brood sizes had lower probabilities of a second brood in the same season (Parejo and Danchin 2006). Such short-lived species should invest higher in a current breeding attempt than long-lived species, as their survival expectancy and thus future reproductive chances are lower (Stearns 1992). In addition, key resources used for reproduction may be depleted faster in shorter-lived than longer-lived species. For example, in blue tits egg laying is protein limited, and future clutches may be less certain as specific amino acids required for laying cannot be sufficiently produced (Ramsay and Houston 1998). The same may also be true for calcium, carotenoid or antioxidants (Williams 2005). Parents may thus allocate a set amount of resources to each breeding attempt to be able to optimise investment in reproduction across their lifetime (congruent with the individual optimization hypothesis; Morris 1987; Pettifor et al. 1988). Further, a set amount may be partitioned across the different breeding stages. Thus, if future egg laying and reproductive success is compromised by increased early investment, I might expect females of short-lived species to provision higher in line with this previous investment (similar to the terminal allocation hypothesis; Snow 1954; Perrins and Moss 1975).

Previous research has mainly focused on trade-offs between reproductive attempts, though individuals should also optimise their investment across different time points in one breeding attempt. A brood manipulation in the red-flanked bluetail (*Tarsiger cyanurus)* demonstrated that parental birds do not respond to artificially increased brood sizes, though increase responsiveness to decreased broods compared to controls (Tanaka et al. 2016). This study is suggestive of a division of a set resource allocation into different components of a breeding attempt; however, manipulating brood size fails to manipulate initial investment in the current breeding attempt. In a rare experiment investigating intra breeding attempt trade-offs (though also see Oppliger et al. 1996), lesser black-backed gull (*Larus fuscus*) females were induced to lay an egg additional to the modal (and maximum) clutch size of three (Monaghan et al. 1998). Those females had reduced offspring rearing capabilities and body condition. In common terns (*Sterna hirundo*, invariant clutch size of three eggs), induced egg laying also led to reduced chick provisioning and growth (Heaney and Monaghan 1995). Conversely, in my study the same experimental procedure led parents to feed chicks at higher rates. Why do blue tits increase rather than decrease provisioning? One reason might be that in comparison to these seabirds, blue tits are not geared for a fixed clutch size and thus are more flexible in adjusting investment to raise more offspring. Regardless, this still begs the questions why our parental birds increased feeding after additional egg laying, even though they did not actually have a larger brood.

The balance between current and future reproductive investment can vary between the sexes due to differential reproductive costs (Kokko and Jennions 2008). For example in birds, females are mostly responsible for egg laying and incubation, though in the majority of species chick rearing is shared bi-parentally (Cockburn 2006). Each parent has an advantage of their partner contributing more than themselves to lower investment costs, though to evade cheating strategies partial compensation has been found to be the evolutionary stable strategy (Houston and Davies 1985; Winkler 1987; McNamara et al. 1999, 2003; Royle et al. 2012). The main pitfall of these theoretical models is that they largely ignore costs outside of the rearing window. Savage and colleagues (2013a) more recently developed a revised parental conflict model, incorporating offspring production costs for females. This model predicts that early costs will lead to drops in female investment during subsequent nestling provisioning, and in turn full compensation by males. Exemplifying the drawback of older models, my results confirm that investment during laying affects later offspring provisioning, in particular for females. However, my results also highlight a novel contradiction with Savage et al.'s model by showing a positive, not negative response of females across stages of one breeding attempt.

How do my results line up with previous empirical and theoretical findings of partner responses in bi-parental care? In comparison to older theoretical models (Houston and Davies 1985; Winkler 1987; McNamara et al. 1999, 2003), I did not find any negative compensatory responses of males to the increase in experimental female feeding rates compared to those of controls. This may be an indication of positive matching of males with the increase in experimental female provisioning (Fig. 4.4). Even though I am aware that my experiment does not directly test for the underlying mechanisms driving male responses, the augmentation in feeding may be congruent with recent empirical and theoretical findings - the less-informed parent should match the care behaviour of the better-informed parent (Hinde 2006; Johnstone and Hinde 2006; Meade et al. 2011). In my case study, females who have greater “knowledge” on the initial number of eggs laid, through the potential mechanisms discussed below, seem to lead provisioning at higher rates, with less-well informed males paralleling these provisioning rates. Additionally, my results highlight that males do not respond to initial clutch size (Fig. 4.5), only to current brood demand which they may have more accurate information on (Fig. 4.3). Even though I removed the eggs on the day laid, and thus it was near impossible for males to know the true clutch size laid, I believe my results reflect realistic levels of information. In blue tits, males have much less contact with the offspring before the peak in feeding than females, as they are not directly involved in laying, incubating and brooding (Gooders 1987). Further, as our nest boxes are dark (similar to natural nest cavities), visual cues are probably less useful in detecting offspring number than tactile ones, which should mainly be exploited by females (Heeb et al. 2003; Wesołowski and Maziarz 2012). Auditory cues such as begging, which may be used by both parents, may not be so pertinent to determine offspring numbers early on, as begging is not as developed. These different pieces of evidence are consistent with a positive matching of the less-informed males with the feeding rates of greater-informed females, though other hypotheses may exist to explain the observed pattern.

In the following part, I highlight possible mechanisms underlying the positive link between original clutch size and the increase in provisioning rates observed. My post-hoc analyses revealed that particularly for females, the initial number of eggs laid was nearly as important in determining chick provisioning rates as current brood size (Fig. 4.3 & 4.5). An ultimate explanation for why parents might tune their investment to the initial offspring quantity produced, may be that since chicks don’t beg much early on, clutch size might be a reasonably good predictor of early nestling demand, particularly if hatching failure is low. In support, it has been shown that less than 10 % of eggs laid by blue tits, fail to hatch (Deeming and Feu 2011). However, direct tests of this hypothesis are still lacking. From a more proximate perspective, counting and memorising the number of eggs laid may be another mechanism to retain information on the initial offspring output. This mechanism most probably requires excessive cognitive abilities and has been hard to demonstrate unambiguously in avian species (Lyon 2003). Hormones may be a more proximate mechanism, playing an important role as part of a positive feedback loop between original clutch size and post-hatching care. In dark-eyed juncos (*Junco hyemalis*), females injected with gonadotropin-releasing hormone - which acted as a physical “challenge” - released more testosterone (Cain and Ketterson 2013). Consequently, this led to higher provisioning rates. Gonadotropin-releasing hormones are indirectly responsible for the initiation of egg laying (Sockman et al. 2006). Thus, one could infer that artificially increasing clutch production, and extending the total time a female is in laying mode, may pose a similar “challenge”. Furthermore, it has been demonstrated that larger clutch sizes are directly linked to a later disruption of ovarian follicular growth in blue tits, and this flexibility in clutch termination has been hypothesised to be partly governed by gonadotropin hormones (Haywood 1993a, b). Thereby, it seems reasonable that hormonal cascades as seen in the dark-eyed juncos might result in similarly positive feeding rates found in my study. From an evolutionary point of view, such simple hormonal mechanisms might be easily selected in females to sufficiently adjust early feeding rates.

The aim of this study was to look at the effects of increased investment in offspring production on later patterns of parental provisioning behaviours. I found that inducing females to lay more eggs augmented parental provisioning rates in comparison to controls. Though, no differences in average brood size and other investment between treatment groups were detected. In comparison to males, initial clutch size, in addition to current brood size, plays an almost equally large part in determining female feeding rate. I suspect that simple mechanisms, such as hormonal cascades, may play a crucial role in driving the observed increase in provisioning. Further research, particularly into hormones, is needed to shine light on the underlying mechanisms governing carry-over effects from one breeding stage to another. In future, I deem it worthwhile for more studies to experimentally decouple the costs of different rearing phases of single breeding attempts, and to investigate how initial offspring investment manifests itself on later offspring care strategies and consequent cooperative dilemmas between caregivers. In particular, it would be beneficial to undertake experimental studies in a variety of bi-parental species - short and long lived - to tease apart subtle differences in life history settings which should greatly influence the costs and benefits of providing at a set level for current offspring. Only after gathering these sorts of data can a general framework of the current versus future investment pressures, including intra and inter breeding attempt trade-offs shaping bi-parental care, be established. My findings support recent models predicting the importance of investment in egg production for parental care strategies post-hatching. To understand the evolutionary dynamics of bi-parental care, I believe there is a need to focus more on parental reproductive costs before the rearing period.

Chapter Five

Brood size manipulations across an altitudinal gradient shed new light on investment strategies in a bi-parental care system

5.1 Abstract

Existing theory aimed at understanding the maintenance and dynamics of bi-parental care systems typically predicts that individual variation in the levels of care provided and responses to circumstance are the outcome of a negotiation between each member of the pair. As such, existing theory makes no predictions about sex differences in either contributions to shared investments or response rules to variation in circumstance. Here I tested sex differences by performing a temporary brood size manipulation in a population of blue tits (*Cyanistes caeruleus*) breeding along a 1000 m altitudinal gradient in the French Pyrenees. I found that, on average, males provisioned nestlings at a significantly higher rate than females, and particularly following brood reduction. While both sexes partially reduced their contribution to nestling provisioning in broods of reduced size, females did so to a greater extent than males, suggesting additionally that males might partially compensate for the greater reductions by females. Further, while both sexes also increased their provisioning rate partially for enlarged broods, in this case males and females were similarly responsive, suggesting that they do not respond to each other's contribution. These results were not obviously confounded by variation in prey load size, with the proportion of food deliveries involving caterpillars being controlled in all analyses and not varying as a consequence of the treatment. Nor were they modified by altitude, suggesting that they were not influenced by the environment or breeding density. Instead, I suggest that the results arise because females benefit more than males from reducing investment when current fitness returns are expected to be low, while both sexes benefit from investing heavily in current reproduction when the expected returns are high. Neither suggestion is currently incorporated in formal bi-parental care models,

but I suggest that doing so would improve our understanding of selection acting to stabilise such systems.

5.2 Introduction

Parental investment in offspring care is integral to reproductive success of many animal species (Trivers 1972; Clutton-Brock 1991). While investment in uni-parental care systems can be largely understood through understanding fitness returns from investment in current versus future offspring, in bi-parental systems, the behaviour of partners is expected to complicate optimal investment strategies (Williams 1966; Trivers 1972). Most notably, conflict is expected over the relative contributions of each member of the pair to offspring care, because each benefits from the other contributing more than its ‘fair’ share. The resolution of this conflict is usually explained through partial compensation, with a reduction by one pair member being met with a partial increase by the other (Houston and Davies 1985; Winkler 1987; McNamara et al. 1999). In this way, the ‘defector’ suffers fitness costs through reduced overall sustenance to the brood; hence stabilising the interaction. While empirical tests are broadly supportive, significant variation in both the contributions of each sex, as well as their response rules, has been demonstrated (Harrison et al. 2009). An obvious explanation for such discrepancies between observation and theoretical expectation is that one or more of the underlying assumptions is violated, but tests of such possibilities remain scant (e.g. Johnstone and Hinde 2006).

Classic bi-parental care models make at least three related underlying assumptions (e.g. Houston and Davies 1985; McNamara et al. 1999). First, both members of the pair suffer comparable costs and accrue comparable benefits from investing currently in post-natal provisioning. Violation of this assumption has shown that both expected contributions and response rules can deviate from general expectation. For example, game-theoretic modelling shows that by increasing the number of offspring produced, females can increase the relative fitness currently on offer to their male partners (Smith and Härdling 2000), which, coupled with the increased costs of egg-investment, generate greater male contributions and more positive response rules than expected (Savage et al. 2013b). Second, it is assumed that contributions to other forms of care, for instance brooding or nest defence, have negligible impacts on the relative contributions of each sex to offspring provisioning or their response rules. On the contrary, a comparative study on orange-crowned warblers (*Oreothlypis celata*) showed that in a northern population where females brooded more, males provisioned more, while in a warmer southern population, provisioning contributions of the two sexes were comparable (Yoon et al. 2017). Finally, it is assumed that increasing contributions to a given breeding event is associated with accelerating costs and diminishing benefits, and that both members of a pair invest in comparable parameter space where the distance between the two functions maximally differs. However, there are at least two ways in which this assumption can be violated (McAuliffe et al. 2015). If the two sexes differ in the proportion of lifetime fitness gained from a current circumstance, then their cost and benefit functions will also differ, leading the sex with less to gain currently operating lower in parameter space (i.e. reducing current costs at the expense of current benefits) than the sex with more to gain (i.e. increasing current benefits at the potential expense of future benefits). Alternatively, if the opportunities for current fitness are relatively high, both sexes might favour current benefits at the expense of future benefits and invest higher in parameter space than expected. In either case, predictions other than partial compensation might be expected (McAuliffe et al. 2015).

Most previous studies testing individual contributions to offspring provisioning, and response rules, have compared natural contributions with those observed following handicapping (e.g. tail-weighting, plucking a flight feather or administration of testosterone; Harrison et al. 2009). A potential problem with such methods is that they are long-lasting, and might generate changes to perception of mate quality, which are known to influence provisioning behaviour (Harrison et al. 2009). An alternative has been to supplement begging, and so perception of offspring hunger, using begging playbacks (e.g. Wright et al. 2002; Hinde 2006). Whilst this method circumvents issues with the above methods, a drawback here is that only the consequences of simulated increases in brood demand are feasible to test parental responses. Instead, I use a brood size manipulation approach in a nest box population of blue tits to test sex differences in provisioning contributions and response rules, as well as the validity of common underlying assumptions. While such an approach is more commonly adopted in cooperative breeders (e.g. Russell et al. 2008; Liebl et al. 2016) than in bi-parental care systems (Neuenschwander et al. 2003; García-Navas and Sanz 2010b), a general advantage is that it permits assessment of responses to both increases and decreases in brood demand or size (see Discussion). Further, by conducting this experiment across a 1000 m altitudinal gradient, I am further able to provide the first assessment of the impact of ecology on contributions and response rules in a bi-parental care system.

Blue tits are a small (12 g) European passerine, which at our field site in the French Pyrenees, is single brooded and lays clutches of 4-12 eggs (mean = 8.2) from late March to early June. Females alone build the nest and incubate the eggs (Perrins 1979), and presumably suffer greater potential for pre-nestling stage costs (Blondel et al. 1992; Visser and Lessells 2001; Székely et al. 2014), although males perform more territory defence and also are known to seek extra-pair copulations (Föger 1991; Kempenaers et al. 1997; Sheldon et al. 1999). Finally, annual adult mortality is high, and most individuals only breed once in their lifetimes, meaning that selection to capitalise on high potential current fitness returns is likely to be strong (Snow 1954; Hilden 1982; Blondel et al. 1992). Against this backdrop, I first use natural observations of provisioning rates across four years and a 1000 m altitudinal gradient to investigate sex differences in overall contributions to nestling provisioning. Second, I then use a brood size manipulation to test response rules and associating impacts of the ecological gradient. Finally, I assess the functional significance of the changes to provisioning rates observed during manipulation using measures of nestling mass. Classic models of bi-parental care predict: (a) comparable provisioning contributions of males and females independently of ecology; (b) partial compensation response rules by both sexes; and (c) these partial response rules to be manifest as overall increases in nestling mass compared to controls. Specifically, I predict that artificially increasing brood size will increase fitness benefits for both sexes, resulting in similar augmented levels of provisioning. However, when brood sizes are decreased, females should benefit more than males from reduced investment, as their resources have been depleted more heavily by prior investment in egg laying, incubation etc.

5.3 Materials and Methods

This study was carried out during four consecutive breeding seasons (2013-2016), near the research Station for Theoretical and Experimental Ecology of Moulis (SETE, UMR 5321) in the French Pyrenees (42°57’29” N, 1°05’12” E). Our field sites comprise mixed deciduous woodlots separated by small fields for livestock situated along an altitudinal gradient ranging from 430-1530 m a.s.l. (see Chapter Two for more detail). The focal blue tit population breeds in Woodcrete Schwegler^{TM} 2M Bird Boxes (32 mm diameter entrance holes), which are placed at ca 50 m intervals in each woodlot (N= ~650 nest boxes). Nest boxes are represented across the altitudinal gradient, ranging from 430-630 m at low altitude; 700-920 m at mid altitudes; and 940-1530 m at high altitude. A greater number are employed at high altitudes in order to maximise sample size: the occupancy of nest boxes with blue tits averages 42 % but declines significantly with altitude. Work was conducted under animal care permits to A. S. Chaine from the French bird ringing office (CRBPO; n°13619), the state of Ariège animal experimentation review (Préfecture de l’Ariège, Protection des Populations, n°A09-4) and the Région Midi-Pyrenées (DIREN, n°2012-07).

We conducted basic breeding surveys over the spring seasons of 2013-2016, leading to precise data on lay date, clutch size, hatch date, brood size, and fledging success. Each breeding parameter was known due to nest checks every 3-5 days, or daily before the onset of laying, at clutch completion (from the sixth egg), at hatching (from day 11 of incubation) and fledging (from day 18 after chick hatching). The sex of each pair member was identified using unique colour-ring combinations: at least one member of each pair represented in this study was identifiable in this way before provisioning observations were conducted. Females were identified using the presence of a brood patch. To quantify parental provisioning rates, blue tit nests were filmed with a camouflaged video camera (Sony HDR-CX220E Handycam® Camcorders) at a distance of ca. 10 m. During observations on natural brood sizes, recordings were made when broods were 14 days old on average (±1.31 SD; range: 8-18 days), with fledging occurring on day 18-24 post hatching. The age range observed coincides with a general decrease in female brooding and asymptote of brood provisioning rates; so, I will generally not expect brood age to be significant in my analyses. Overall, I obtained 96 control videos over the four-year period across most of the altitudinal range (Mean = 666 m, ±190.70 SD, range: 430-1130 m), including the pre-manipulation control video in brood manipulated nests (see more detail below). Hourly visitation rate and prey type brought to the nest by each parent were recorded over a two-hour period. Blue tits are single prey loaders, and prey was either classified as large items (Lepidoptera caterpillars) or less well-definable, small arthropods (e.g. spiders; (García-Navas et al. 2012).

To test response rules, a brood size manipulation was performed over two consecutive breeding seasons (2015-2016). Manipulated nests spanned most of the altitudinal range occupied by our blue tit population (mean: 673 m; ±193 SD; range: 461-1130 m). Nests of similar age (0-2 d difference) and altitude were paired up for reciprocal brood swaps. The brood size manipulation was conducted over four consecutive days, with observations conducted on two days of natural brood sizes acting as controls (day one: pre-manipulation control and day four: post-manipulation control). This allowed any brood age effects to be controlled. The experiment began on day 12 (mean: 12.2 days, ±0.5 SD; range: 11-14 days) with a 3-h pre-manipulation control video, after which the nestlings were weighed (±0.1 g), colour-ringed and two nestlings from one of the nests were fostered to a paired, neighbouring nest. The next day, the increased and decreased broods were video-recorded simultaneously for 3-h, before being weighed, and four nestlings from the increased brood being fostered to the previously reduced brood. Then on the next day (d 14), again the two nests were video-recorded for 3-h, and the nestlings weighed, before the original number and composition of broods were reinstated. Finally, on the 4th day (d 15), a final control video of 3-h was obtained for each nest. Cross-fostering rarely took more than 10 min, and during this time nestlings were protected in cotton bags and placed close to our body to retain warmth. Videos started at an average of 11 am (± 1:56 SD, range: 8:45 am – 16:30 pm), and parents generally had 24 h to become accustomed to the new brood size before recording. Overall, I obtained 47-51 videos for each treatment and control group across the two years, with slight variation owing to video or background nest failure (N = 15) during the experiment. I analysed the latter 2-h of each 3-h video in order to reduce any impacts of disturbance induced by setting up the video camera.

Statistics were performed in *R* 3.4.2 (R Core Team 2017). All data sets were examined for outliers and distributions of dependent variables were visually inspected for normality (Zuur et al. 2009a). Full models were checked for overdispersion and heteroscedasticity in model residuals, which were controlled for by changing the model error distribution as necessary. Model selection was performed using the anova algorithm in *R*, based on changes in deviance relative to the minimal model to evaluate the contribution of each variable to the model (significance set at $\alpha < 0.05$).

5.3.1 Sex differences in provisioning natural brood sizes

Sex differences in provisioning rates and potential underlying causes were investigated in natural brood sizes using a linear mixed effects model in the package lme4 (Bolker et al. 2009; Bates et al. 2015). In this case, the provisioning rate of each individual was fitted as the response term, and nest box identity was fitted as a random term to account for the fact that partner provisioning rates might not be independent. Explanatory terms included parental sex, linear and squared brood size and age (Rytkönen et al. 1996; Parejo and Danchin 2006), the proportion of deliveries including caterpillars, altitude and year. Further, I tested the interactions between sex and brood size, as well as sex and altitude to elucidate whether sex differences in contributions are influenced by opportunities for current fitness and ecology.

5.3.2 Responses to brood size manipulations

To analyse the effect of my brood size manipulation on parental provisioning rates, I ran a series of mixed effects models in the lme4 package (Bolker et al. 2009; Bates et al. 2015). First, I investigated the effects of the experiment on the provisioning rate of males and females. In this case, provisioning rate was fitted as the response term and both nest identity and individual identity fitted as random intercepts. Sex, treatment and altitude were fitted as the main terms, along with their interactions, while brood size, age and prey type (see above), were fitted as covariates. Second, to investigate the effects of the treatment for offspring provisioning rates, the provisioning rates of both parents were combined and divided by brood size to generate a *per capita* nestling feeding rate. Further, the *per capita* rate of food acquisition on the two experimental days was standardised by subtracting rates on control days away from enlargement days, and on reduction days away from control days. The resulting variable was fitted as the response term in a mixed model, with the random term set as brood identity to account for the fact that each brood is represented twice. Here the explanatory terms were treatment, original brood size and altitude, along with their interactions. Finally, I then reran the above analysis, but wherein the response term was split by sex, such that I obtained sex-specific changes in *per capita* provision rates. The motivation here was to test sex-specific response rules. The random term in this case was brood identity nested within individual identity, while the explanatory terms were as for the previous analysis, except that sex was also included.

5.3.3 Treatment effects on brood mass

To investigate the direct effect of the brood size manipulation on chick condition, I analysed chick weight data collected after each video recording. I fitted average chick mass per brood as the response term into a linear mixed model with nest box identity as the random effect to control for non-independence of repeated measures on the same nest box (Bolker et al. 2009). In addition to experimental treatment type, I included the linear and squared effects of brood size and brood age, altitude and year as fixed covariates. Lastly, I investigated the direct consequences on offspring of parental responses to the brood. To this end, I fitted the changes in mean nestling mass between control days and the two experimental days (experimental minus a combined control mass variable). As in previous analyses, I tested for the main effect of treatment, followed by the interactions between treatment and brood size, and treatment and altitude.

5.4 Results

5.4.1 Sex differences in provisioning natural brood sizes

Observations of provisioning rates at nests with natural brood sizes indicated an average parental provisioning rate during the latter half of the nestling period of 21 feeds / h (±9 SD; range: 2-49). With two parents and an average brood size of seven nestlings, this rate equates to each nestling receiving an average six feeds per hour. Natural variation in provisioning rates was explained by brood size, the proportion of prey loads comprising caterpillars and parent sex, but not brood age, altitude or year (Table 5.1). Provisioning rates increased as a decelerating function with increasing brood size (linear effect - $\chi^2_1 = 7.29$, $P = 0.007$; squared effect - $\chi^2_1 = 4.084$, $P = 0.043$) and declined as the proportion of feeds comprising caterpillars increased ($\chi^2_1 = 5.47$, $P = 0.019$). After controlling for these effects, I found that, on average, males provisioned four more times per hour than females, equating to a 20 % greater contribution by males than females ($\chi^2_1 = 11.61$, $P < 0.001$). Finally, there was no firm evidence for an interaction between sex and brood size or sex and altitude (Table 5.1), but in both cases, there were possible trends (Fig. 5.1a, b).

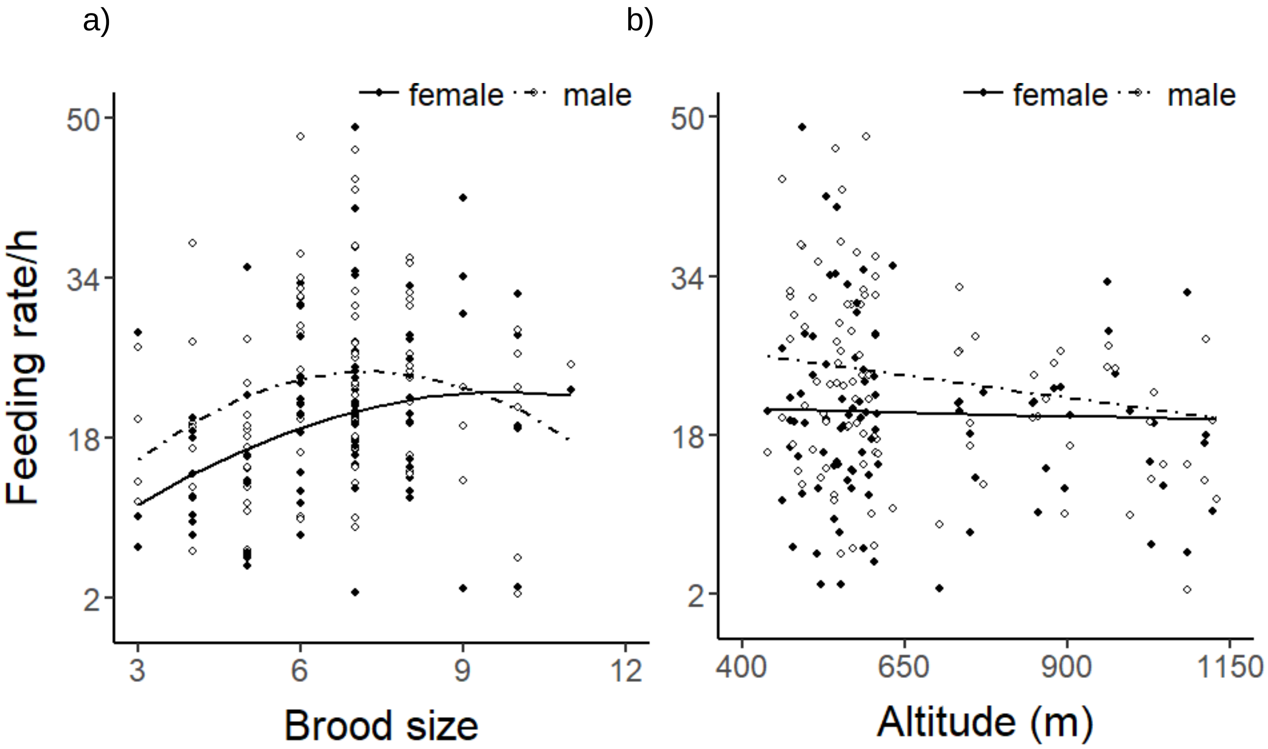

Figure 5.1: Hourly feeding rates of females (full line) and males (dotdashed), in relation to (a) natural brood size (controlling for the proportion of caterpillars delivered and the random factor of nest box identity); and (b) altitude (m; controlling for linear and squared brood size, the proportion of caterpillars delivered and the random factor of nest box identity). Shown are the predicted lines of best fit and raw data points for females (filled circles) and males (hollow circles).

Table 5.1: Model summaries predicting provisioning rate / h, from linear mixed models including nest box ID as random effect.

Predictors		Estimate	± SE	χ2 value	p-value
(Intercept)		20.637	1.00	20.628	<0.001
Brood size		26.87	10.02	7.29	0.007
Brood size ^2		-20.26	10.11	4.084	0.043
Proportion of caterpillars		-8.31	3.57	5.47	0.019
Sex		3.73	1.066	11.61	0.0007
Altitude		-0.0055	0.0039	2.072	0.15
Brood age		-0.18	0.50	0.14	0.70
Brood age ^2		-8.54	11.22	0.81	0.67
Year				0.86	0.84
	2014	-1.55	3.12		
	2015	-2.50	2.90		
	2016	-2.22	2.75		
Sex * brood size		-0.84	0.61	1.90	0.17
Sex * brood size ^2		-11.67	14.66	2.55	0.28
Sex * altitude		-0.0091	0.0054	2.85	0.091

In order to clarify these possible interactions, I conducted sex-specific analyses of the effects of brood size and altitude on provisioning rate, controlling for significant effects of caterpillar delivery (see above). These ‘post-hoc’ analyses suggested that females (but not males) varied their provisioning rates as a function of brood size, whereas males (but not females) varied their provisioning rates according to altitude. For example, while females increased their provisioning rate by an average of 1.3 feeds / h for incremental increases in brood size ($F_{1,93} = 7.34$, $P = 0.008$), males showed no obvious association ($F_{1,93} = 0.96$, $P = 0.33$). Further, while males reduced their provisioning rate by 1.5 feeds / h for every 100 m increase in elevation ($F_{1,94} = 10.29$, $P < 0.002$), there was no obvious association between altitude and provisioning rate in females ($F_{1,92} = 0.040$, $P = 0.84$). Finally, to clarify whether these possible sex-differences represent among- versus within-pair effects, I investigated the effects of brood size and altitude on the differences in provisioning rates by pair members in a linear model (again controlling for delivery rates of caterpillars). In this case, although there was no obvious effect of brood size (linear effect - $F_{1,93} = 2.035$, $P = 0.16$; squared effect - $F_{1,92} < 0.001$, $P = 0.98$; Fig. 5.2a), there was a near significant influence of altitude on the difference in provisioning rates within pairs: the greater contribution by males at low altitude declined by ca. 1 feed / h / 100 m increase in elevation ($F_{1,94} = 3.72$, $P = 0.057$; Fig. 5.2b). This effect led males to provision 19 % more than females at low altitude, but comparably to females at high altitude (4 % less).

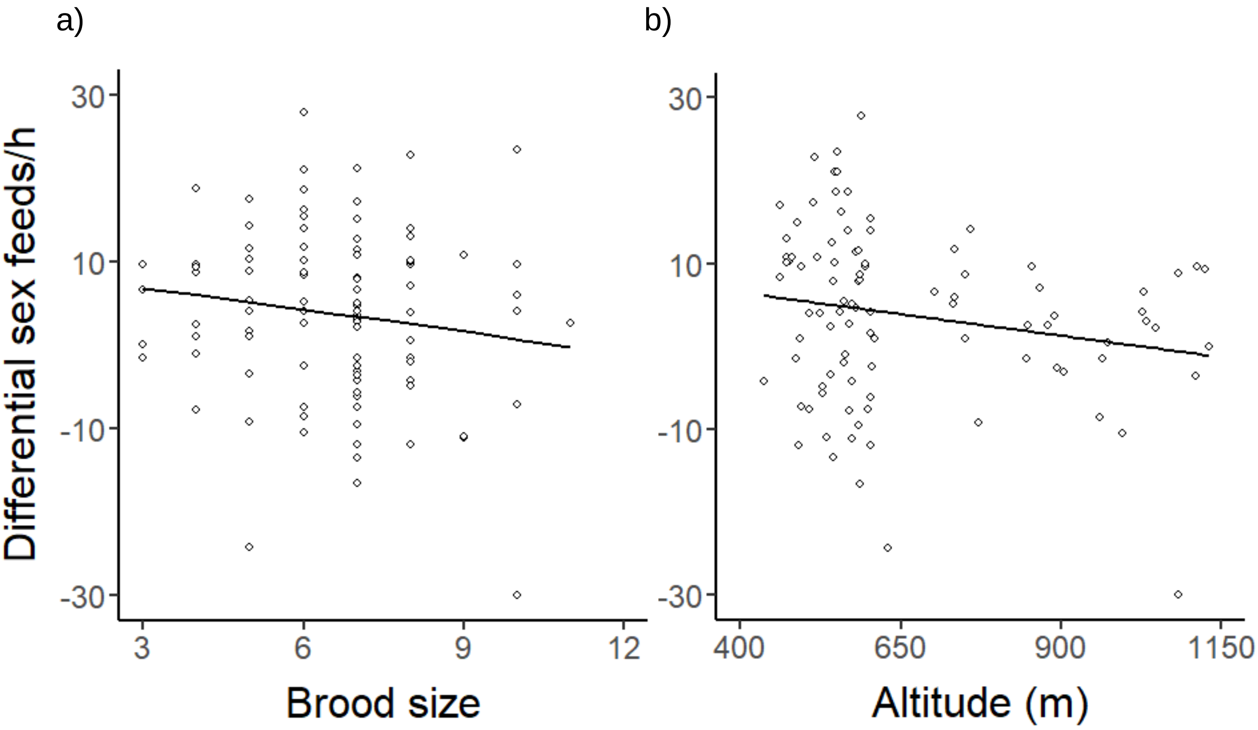

Figure 5.2: Differences in hourly feeding rates of males minus females, in relation to: (a) brood size (controlling for altitude); and (b) altitude (m; not controlling for other covariates). Shown are the predicted lines of best fit and raw data points.

5.4.2 Responses to brood size manipulations

While natural observations can help clarify overall variation in contributions to care, and underlying predictors, they cannot elucidate response rules. The brood size manipulation had a significant impact on parental provisioning rates (χ^2_2 = 81.78, $P < 0.001$): relative to controls, on average broods were provisioned four times more / h following enlargement (±7 SD; range: -14 to +22) and five times less / h following reduction (±6.14 SD; range: +7 to -19; Fig. 5.3; Table 5.2). These results were found after controlling for significant effects of the proportion of deliveries comprising caterpillars (χ^2_1 = 13.59, $P < 0.001$) and original brood size (χ^2_1 = 15.12, $P < 0.001$). Again, males were found to feed at a significantly higher rate than females (χ^2_1 = 11.13, $P < 0.001$). Nevertheless, I found little evidence of a sex by treatment interaction (χ^2_2 = 1.15, $P = 0.56$), suggesting that, on average, males contributed more across the treatments. Nor were responses apparently modified by altitude, since I failed to find evidence of a three-way interaction between sex, treatment and altitude (χ^2_2 = 1.15, $P = 0.56$). These results suggest that males and females have broadly comparable response rules, but identifying these response rules and testing whether or not they are comparable between members of a pair requires more targeted analyses.

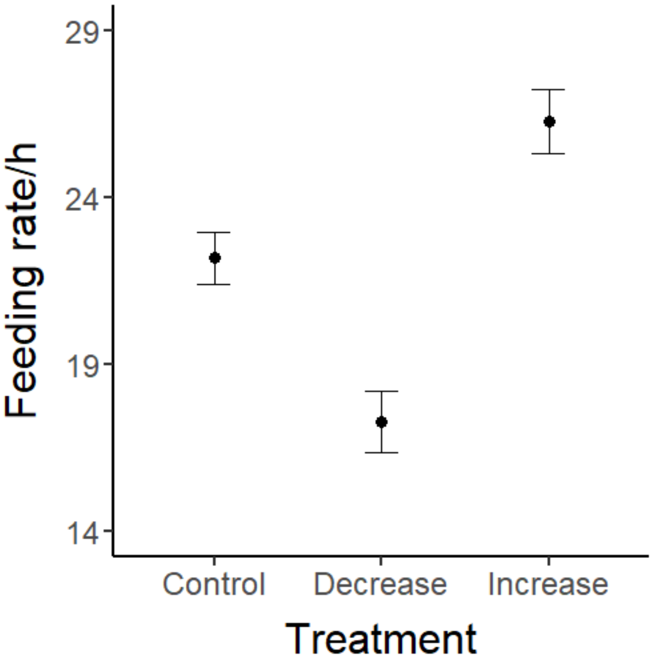

Figure 5.3: Hourly feeding rates in relation to treatment groups. The fixed covariates altitude, original linear brood size and proportion of caterpillars delivered are controlled for, in addition to the random factor of nest box identity. These covariates were taken from the minimal models. Shown are the predicted mean ± SE.

Table 5.2: Model summaries predicting provisioning rate / h, from linear mixed models including nest box ID as random effect. I = increased broods, D = decreased broods – both are compared to controls.

Predictors		Estimate	± SE	χ2 value	p-value
(Intercept)		11.5128	2.7407	4.201	<0.000
Treatment				73.20	<0.001
	I	-4.92	0.87		
	D	4.064	0.88		
Brood size		1.72	0.42	15.12	<0.001
Proportion of caterpillars		-10.88	2.89	13.59	<0.001
Sex		2.36	0.71	11.13	<0.001
Altitude		-0.0060	0.0033	3.42	0.065
Brood size ^2		-10.17	12.39	0.71	0.40
Brood age		0.31	0.31	0.95	0.33
Brood age ^2		-6.67	9.40	1.85	0.40
Year		-0.33	1.28	0.067	0.80
Treatment * original brood size				20.54	<0.001
	I	0.80	0.58		
	D	-2.092	0.59		
Treatment * altitude				4.41	0.11
	I	0.00038	0.0046		
	D	0.0090	0.0045		
Treatment * sex				1.18	0.55
	I	1.84	1.71		
	D	0.60	1.73		
Treatment * sex * altitude				0.57	0.75
	I	0.0049	0.0091		
	D	-0.0028	0.0089		
Treatment * sex * original brood size				0.38	0.83
	I	-0.60	1.13		
	D	-0.55	1.16		

First, to clarify the response rules, I investigated the effects of the brood size manipulation on the changes in *per capita* food acquisition rates between control days and the two manipulation days. Doing so revealed that parental responses to changes in brood size, although significant, were incomplete in both directions - meaning that nestlings received proportionally less food in enlarged broods and proportionally more food in reduced broods ($\chi^2_1 = 19.65$, $P < 0.001$; Fig. 5.4). For example, while each nestling received an average 7.5 deliveries / h in control broods (mean control brood size = 6), in enlarged broods each nestling received food at an estimated 12 % lower rate, while in reduced broods, they received an estimated 15 % higher rate. Further, I found no evidence to suggest that these changes in response to brood manipulation were further influenced by original brood size (treatment * original brood size interaction: $\chi^2_1 = 0.044$, $P = 0.83$) or altitude (treatment * altitude interaction: $\chi^2_1 = 0.24$, $P = 0.62$).

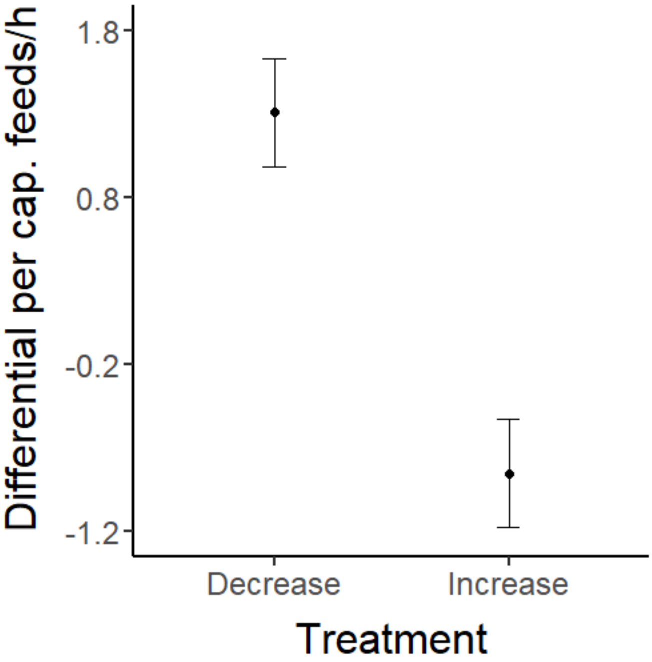

Figure 5.4: Differences in total hourly feeding rates of treatments minus combined controls. The fixed covariate altitude is controlled for, in addition to the random factor of nest box identity. This covariate was taken from the minimal models. Shown are the predicted mean ± SE.

Second, to test whether pairs follow comparable response rules, I analysed the change in *per capita* provisioning rates between control and experimental days for each parent separately. This analysis revealed a significant interaction between sex and treatment on changes in *per capita* provisioning responses (interaction: $\chi^2_1 = 7.00$, $P = 0.0082$; main effect of sex: $\chi^2_1 = 3.11$, $P = 0.078$). This interaction was generated because males were generally less responsive than females to changes in brood size within pairs; in particular were less responsive than females to reductions in brood size (Fig. 5.5a). Parental responses to changes in brood size were uninfluenced by their contributions on control days (control provisioning rates * treatment interaction: $\chi^2_1 = 1.15$, $P = 0.28$), and there was no evidence for a treatment by altitude interaction ($\chi^2_1 = 0.38$, $P = 0.54$; Fig. 5.5b).

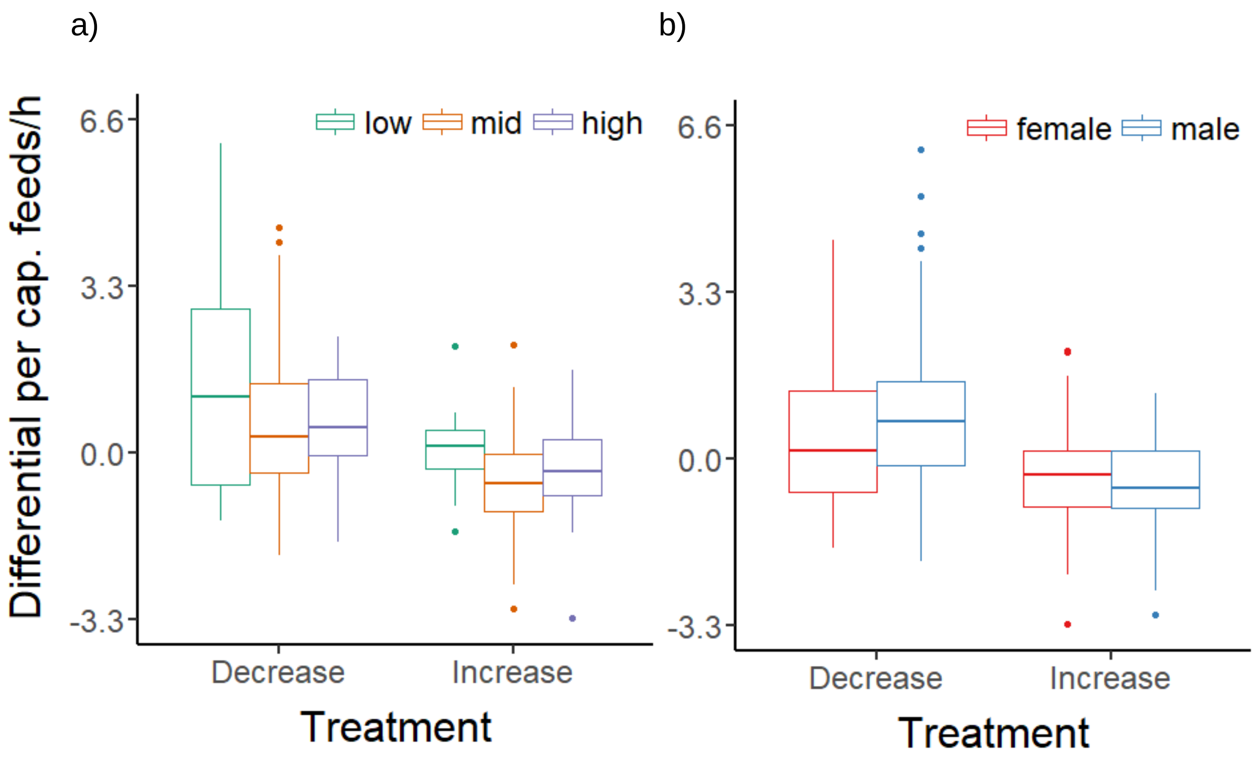

Figure 5.5: Differences in hourly feeding rates of treatments minus combined controls, a) per sex, b) per altitudinal category. The raw boxplots represent the median, first and third quartiles, 1.5 * inter-quartile ranges as whiskers, and outliers as values outside these limits.

5.4.3 Treatment effects on brood mass

To assess the potential functional consequences of the above results, I measured mean nestling mass during all control and manipulation days. Across all days, nestlings averaged 10.50 g (±0.84 SD, range: 8.20-12.45). Mean chick mass increased 0.1 g per day of age ($\chi^2_1 = 16.95$, $P < 0.001$). Mass was not significantly influenced by altitude ($\chi^2_1 = 3.26$, $P = 0.071$). There was no significant among-year effect in average chick mass ($\chi^2_1 = 1.054$, $P = 0.32$). On control days, average nestling mass was determined by brood size: for every incremental increase in brood size, average nestling mass decline by 0.15 g, leading to a 16 % decrease in mass between the largest and smallest broods ($\chi^2_1 = 6.21$, $P = 0.013$). Given these results, it is unsurprising that the brood size manipulation caused a significant change to average nestling mass overall ($\chi^2_2 = 29.93$, $P < 0.0001$; Fig. 5.6). However, this difference was largely due to broods being significantly heavier on reduced days ($\chi^2_1 = 12.71$, $P < 0.001$), rather than their being significantly lighter on enlargement days ($\chi^2_1 = 2.53$, $P = 0.11$). These results corroborate the suggestion that pairs only partially compensate for changes in brood size. To investigate the possibility of partial compensation further, I measured changes in mean nestling mass between control days and the two manipulation days. Doing so revealed that parental responses to changes in brood size, although significant, were incomplete in both directions - meaning that nestlings weighed less in enlarged broods and more in reduced broods ($\chi^2_1 = 15.95$, $P < 0.001$; Fig. 5.7). I found no evidence to suggest that these changes in response to brood manipulation were either influenced by original brood size (treatment * original brood size interaction: $\chi^2_1 = 0.080$, $P = 0.78$) or altitude (treatment * altitude interaction: $\chi^2_1 = 0.058$, $P = 0.81$).

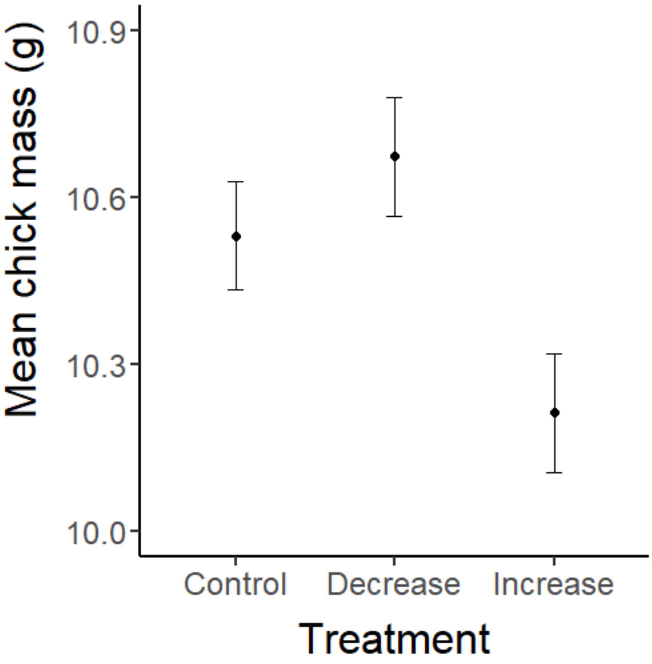

Figure 5.6: Mean chick mass per brood (g) in relation to treatment groups. The fixed covariates altitude, original linear brood size, squared brood age and date are controlled for, in addition to the random factor of nest box identity. Shown are the predicted mean ± SE.

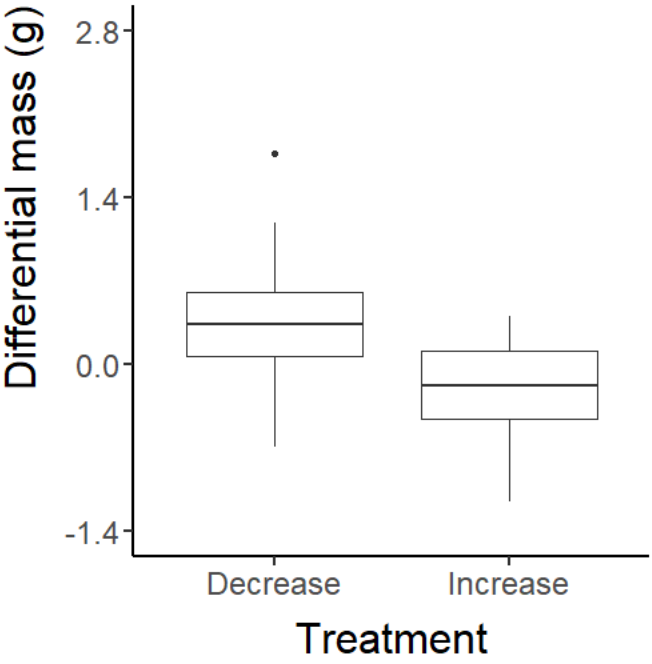

Figure 5.7: Differences in mean brood mass (g) of treatments minus combined controls. The raw boxplots represent the median, first and third quartiles, 1.5 * inter-quartile ranges as whiskers, and outliers as values outside these limits.

5.5. Discussion

The overall objective of this study was to elucidate variation in provisioning contributions and response rules, as well as the underlying predictors of each, in order to provide new insights into the dynamics of bi-parental care. Using natural observations, I found that males provisioned more than females on average, although there was some suggestion that males (but not females) contributed less as altitude increased, leading to similar contributions by both members of the pair at high altitude (Fig. 5.2b). Further, both members of the pair increased their provisioning rates with increasing brood size up to a point. This is in line with previous studies, showing that natural brood size positively influences parental provisioning (Gibb 1955; Royama 1966; Barba et al. 2009; García-Navas et al. 2012). However, there was also some suggestion that brood size had a greater influence on provisioning rates in females than males. Responses to brood size manipulations not only clarified the likely robustness and causal bases of the above results collected for natural brood sizes, but elucidated the response rules and any sex differences in these rules. Most notably, while both sexes were partially responsive to changes in brood size, females decreased their contribution following brood reduction more than males, while both sexes increased their contributions to an equivalent degree following brood enlargement (Fig. 5.5a). Together these results suggest that the sexes respond partially to changes in their partner’s contribution following brood reduction, but show no response following brood enlargement. These findings suggest that response rules can vary in the same pair as a function of the costs and benefits of care, and are likely to be meaningful, because the magnitude of responses were largely manifest in variation in nestling mass.

Quantifying sex differences in contributions to a given reproductive phase provides invaluable insights into potential trade-offs among versus within phases in accounting for variation in investment, as well as their potential underlying predictors. In blue tits females alone perform nest building, egg-laying and incubation, whereas males perform most territorial defence and commonly seek extra-pair options (Perrins 1979; Föger 1991; Kempenaers et al. 1997). If such activities are costly, I might expect variation in them to predict relative contributions to the joint endeavour of nestling provisioning. For example, given that egg-laying and incubation have been shown to be costly (Visser and Lessells 2001), I might expect sole contributions by females to each of these activities to negatively impact her contribution to nestling provisioning. While females did provision nestlings at lower rates than males, there was no effect of brood size on her relative contributions, which might be expected since large broods are associated with large clutches and higher incubation costs (Erikstad and Tveraa 1995; Deeming and Reynolds 2015). Indeed, observations on natural brood sizes showed that females, if anything, contributed relatively more than males when brood sizes were large. Further, female increases in response to the brood size manipulation were not influenced by original brood size, and so presumably clutch size, again suggesting that the level of investment in prior activities does not negatively impact her ability to invest in nestling provisioning. Similarly, there was little obvious evidence in our system to suggest that male investment in territorial defence or extra-pair copulations impacted his contributions to nestling rearing. For example, despite the costs of each being expected to decline with increasing altitude, due to reduced densities, males at higher altitude, if anything, reduced their contribution to nestling provisioning relative to females. Thus, in neither sex are nestling provisioning rates obviously associated with costs of prior investment.

This leaves two obvious alternatives: (a) females contribute more to other forms of parental care during provisioning than males, and suffer greater trade-offs with provisioning as a consequence; and/or (b) females are more sensitive to current fitness returns than males. Regarding the former, females alone brood nestlings during provisioning which not only might reduce their time available for provisioning, but reduce the potential to recoup costs of provisioning through lost foraging time (e.g. Russell et al. 2003). Although observations were conducted when the need for brooding is reduced, females spent longer in the nest box than males, presumably tending for the nestlings in some way, including brooding (mean control times for females: 6:07 (±3:14 SD) min / h and males: 2:59 (±1:07 SD) min / h). Nevertheless, the total amount of time females spent in the nest box was of debatable biological significance and did not vary in response to treatment (mean time spent in nest box = 7 min 43 s / h (decreased treatment) vs. 6 min 48 s / h (increased treatment)). Thus, investment in brooding is unlikely to explain either sex differences generally, or reductions in female contributions following brood reduction in particular. A related, but more complex alternative hypothesis, is that the contribution of current fitness to lifetime fitness differs as a function of current brood size (Nur 1986). For example, if current costs are impacted by provisioning effort, and such costs have a greater impact on future benefits for females over males, then when brood sizes are small, females might be expected to favour future investment over current investment more so than males. This is in line with theory of life history predicting that reproductive investment is decreased when the fitness returns are lower (Trivers 1972; Stearns 1992; Harris and Uller 2009). Such an effect could be generated, for example, if current investment influences future breeding phenology and fecundity, both of which are under female control. By contrast, when current brood size is large, females and males might be more aligned in favouring high current investment at the expense of future success, especially in this short-lived species.

Experimental manipulation of brood size revealed that both sexes were strongly, but only partially, responsive. In other words, while parents were clearly highly responsive to manipulation of brood sizes, the average prey acquisition rates of each nestling deviated from control levels: had parents been fully responsive, there would be no change in *per capita* nestling provisioning rates. On average, each nestling received food at a higher rate in reduced broods and at a lower rate in enlarged broods (for similar results see Barba et al. 2009). The differences are likely to be meaningful: nestlings were relatively heavier following brood reduction and lighter following brood enlargements, compared with on control days. The degree to which such changes in provisioning responses are generated by changes in begging intensity versus perception of changes in current fitness returns is not known, and would require testing whether responses vary as a function of brood size controlling for variation in metrics of brood demand. Nevertheless, if brood demand were the only mechanism through which changes in provisioning were observed, I would not expect partial reductions in response to reduced brood sizes, since in such cases, offspring would be presumably less hungry and so show reduced begging intensity (Leonard et al. 2000). This suggests that parents are able to ‘perceive’ potential fitness returns through cues other than hunger and allocate investment accordingly. If this is the case, it suggests that the shape of benefit functions vary as a function of potential current benefits on offer; with implications for understanding bi-parental care dynamics (see below).

Finally, the results reported in this study help elucidate how individuals respond to each other's contributions. For example, given that both individuals show partial reductions in contributions to reductions in brood size, but that females reduce their contributions more than males, suggests that individuals respond partially to each other's contributions. Had one or other sex shown no compensation, that sex would not have shown a reduction in contribution from control days. By contrast, had either parent fully compensated, then the overall rate of food acquisition by the brood would have remained unchanged, because that individual would have fully compensated for the reduction by the other. On the other hand, partial compensation does not easily explain partner responses to increasing brood sizes, otherwise there should be a significant difference between the contributions of the sexes. Nor do the results suggest full compensation, otherwise there should be no increase in overall provisioning rates following brood enlargement. Thus, the response rules to brood enlargement appear to suggest no compensation, with each parent responding to brood demand, but not to each other. Together these results suggest that response rules can vary as a function of variation in brood size, and presumably the net benefits currently on offer.

In conclusion, on average males provisioned at a higher rate than females and did so particularly when brood size was reduced. As a consequence of this latter effect, although both members of the pair reduced their contribution partially in response to reduced brood sizes, males did so to a lower extent than females, suggesting that they partially compensate for the greater reduction by females. By contrast, although both members of the pair also partially increased their contribution following brood enlargement, they did so to equivalent degrees, suggesting that the sexes do not compensate for each other's contributions in such circumstances. These effects suggest that modifications to the underlying assumptions of classic bi-parental care models are required to provide a more complete theoretical framework for understanding sex difference in contributions and responses rules in such bi-parental breeding systems (McAuliffe et al. 2015). For example, that males contributed more than females and particularly during brood reductions, suggests that the shape of cost-benefit functions are sex-specific, and that females suffer greater costs to lifetime fitness by contributing heavily to reduced fitness returns in the present (Perrins and Moss 1975; Nur 1986; Stearns 1992). While the expected partial compensation rule was found during brood reduction, it is debatable whether this was driven by negotiation (McNamara et al. 1999), since negotiation should not lead to systematic sex differences in response. Further, surprisingly, both sexes similarly increased their contribution during brood enlargement, albeit to a partial degree. This suggests that, at least in short-lived blue tits, both members of the pair are under selection to operate in parameter space that favours current fitness over future fitness when the circumstances arise. While further empirical work is required to test assumptions regarding sex-differences in future costs as a function of current investment in provisioning, it would also appear that game-theoretic modelling aimed at understanding sex-differences in contributions and response rules are required to help understand the evolutionary maintenance and dynamics of bi-parental care system.

Chapter Six

General discussion

6.1 PhD findings

The findings from this PhD constitute a combination of observational and experimental results. The first two data chapters focus on how the environment influences specific life history decisions such as when to breed using cues and the cascading impacts on fitness. I found that temperature decreased up to 0.5 °C per 100 altitudinal meters. Altitude differences such as this temperature decrease affected breeders; blue tits delayed breeding at high compared to low altitudes. Large variation in lay date with altitude and between years was revealed suggestive of high plasticity in our population. Early breeders were generally associated with larger clutches and consequently more fledglings. Even though hatching success was lower at high altitudes, clutch size and brood mortality did not differ significantly across the altitudinal gradient. Further, budburst was delayed by 1.5 days per 100 altitudinal meters. Birds laid later in relation to budburst with increasing altitude. There was indication that higher altitude breeders shortened reproductive phases to advance hatch date to “catch up” with optimal environmental productivity. I suggest that budburst is a more reliable and thus more frequently used cue in adjusting breeding with peak food availability in homogenous environments such as at higher elevations.

The latter two data chapters focus on parental investment in offspring care and make use of both observational and experimental approaches. Over two consecutive breeding seasons, I manipulated early breeding phases by increasing egg production in females and then investigated how later investment levels at the rearing stage were affected by previous investment. My results suggest that: (a) female provisioning rates are tuned positively to their egg investment, and (b) males do not respond negatively to elevations in female provisioning rates. Finally, I recorded natural provisioning during rearing and temporarily manipulated reproductive costs (brood size) across the altitudinal gradient to investigate changes in parental provisioning by both sexes. In natural provisioned nests females seemed to respond more strongly to brood size, while males responded more strongly to altitude. My results suggest that: when the costs of rearing are changed, through the altitudinal gradient or the temporarily alterations in brood size, (a) overall parents respond incompletely, (b) while both sexes partially reduced their contribution to nestling provisioning in broods of reduced size, females did so to a greater extent than males, suggesting additionally that males might partially compensate for the greater reductions by females, and (c) additionally, both sexes benefit from investing heavily in current reproduction when the expected returns are high. These experimental results were largely independent of altitude.

6.2 General conclusions

6.2.1 Altitudinal effects on parental investment

Overall, I found that our blue tits shifted investment with altitude more in the pre-hatching versus the rearing stage. For example, with altitude lay date is delayed and fewer chicks hatch in higher compared to lower nest. In general, life history predicts that reproductive investment levels should match expected fitness returns (Williams 1966; Trivers 1972; Stearns 1992). Thus, environmental factors changing potential fitness returns should also affect parental investment in reproduction (Harris and Uller 2009). Abiotic factors such as the increase in environmental "harshness" with altitude may thus affect reproductive decisions of parents (Clutton-Brock 1991). Such changes in reproductive decisions may function as maternal (or paternal) effects and inform following generations of prevailing environmental conditions (Cheverud and Moore 1994; Mousseau and Fox 1998). Early investment "choices" such as a delay in laying observed in my study may be classified as maternal effects. However, only a few examples have shown whether or not the interplay between maternal investment in offspring plasticity and success is adaptive. For instance, seed beetles (*Stator limbatus*) mothers plastically adapt their egg size to the type of host plant, depending on juvenile mortality risk of boring through different seed coats (Fox et al. 1997). A similar process has been illustrated in great tits, which when exposed to increased predation risk lay eggs containing lower testosterone concentrations, and resulted in offspring being smaller with accelerated wing growth, aiding predator escape (Coslovsky et al. 2012). These few studies provide examples of heightened offspring fitness due to maternal strategies, which in turn may affect evolutionary responses on the species level (Räsänen and Kruuk 2007; Mousseau et al. 2009). In our system such maternal effects may have buffered against differences in chick mortality with altitude. Thus, a general lack in response during the rearing stage to altitude may be due to life history decisions, e.g. maternal effects, before the rearing stage buffering against environmental “harshness”. Parents of high nests may thus invest at similar care levels during the rearing stage compared to low nests. Thus, I suggest that selection at high altitude acts before the rearing stage. More fine-scale analyses of incubation schedules of females and egg content may further highlight differences across the gradient.

Reproductive investment choices may be part of a larger “pace of life” strategy characterizing species’ life histories (Gaillard et al. 1989; Ricklefs and Wikelski 2002; Réale et al. 2010). A “pace of life” strategy may be dependent on environmental conditions. For example, individuals may flexibly invest higher in quantity rather than quality of offspring in less predictable environments, where future reproduction is less certain. In support, in harsher and more heterogenous conditions life history theory predicts that parents should invest more into each offspring to increase their survival chances, rather than into producing more offspring (Smith and Fretwell 1974; Lloyd 1987; Stearns 1992). To ascertain whether the shift to fewer though similar quality offspring at higher altitudes is part of a slower “pace of life” in our blue tits, general adult and offspring survival data is required. So far, data on recapture rates in our population are low. In part this may be due to the large unfragmented habitats enabling high movement in comparison to other study systems (e.g. Wytham Wood near Oxford, England (Perrins 1965), or Hoge Veluwe, Netherlands (Van Balen 1973)). In the future, such recapture data will complete our insight into overall life history strategies pursued by bird species such as blue tits. As part of a “pace of life” strategy, I predict that individuals breeding at high altitude may shift to a slower pace including a lower number of higher quality offspring produced, longer maturation time and higher survival chances (Gaillard et al. 1989; Ricklefs and Wikelski 2002; Réale et al. 2010; Hille and Cooper 2015).

6.2.2 Parental care during rearing

In Chapter Four and Five parents seemed to act largely in parallel to rear offspring. Traditional models have established that any change in one partner should be matched by partial compensation in the other partner as part of an evolutionary stable strategy (Chase 1980; Houston and Davies 1985; McNamara et al. 1999). Partial compensation should limit the spread of cheating in bi-parental care at the population level. However, empirical data demonstrates much variation from this theoretical prediction in avian species (Harrison et al. 2009). My findings support rules other than partial compensation governing stabilisation of bi-parental care. Recent theories on matching may explain the parallel responses to changes in investment costs observed between the sexes (Johnstone and Hinde 2006). To clearly attribute these parental responses to matching, experimental manipulation of one partner’s information levels during rearing, for example through directed playback experiments, and investigation of the care response of the less-informed partner are required.

Bi-parental care during the rearing phase may not be representative of overall parental investment in a breeding attempt. I recommend larger consideration of differences in underlying costs for both parents over the entire reproductive attempt in future models of bi-parental care. In addition, direct manipulation of costs at different time points of a breeding attempts should help identify investment links in a variety of species and should shed light on the fine-tuning of life history trade-offs during reproduction. In general, this PhD thesis highlights the complexity of reproductive investment choices.

6.3 Global vision

How do these PhD results add to the bigger picture of reproductive investment in avian species? This PhD project constitutes a first, extensive investigation of blue tits breeding in the French Pyrenees. This study system adds a contrasting South European landmark compared to the more Northern classic populations studies. In addition, the altitudinal gradient is another facet to help shed light on reproductive investment. As aforementioned, reproductive investment is integral to species‘ life histories (Stearns 1992). Animals must balance the cost and benefit of reproduction to maximise life-time fitness (Williams 1966). Such studies of parental investment have the potential to greatly enhance our understanding of underlying mechanisms driving evolutionary processes. Additional years of data are required to fully understand the different facets of breeding strategies adopted by blue tits in this novel study system, particularly in the face of climate warming. This study already reveals that even though the blue tit is one of the most thoroughly studied model organism, large gaps remain in our understanding of their life history.

So far, changes in reproductive investment and fitness indices across the altitudinal gradient are purely observational. A cross-fostering approach may help clarify the role of plasticity versus genetic fixture in shaping individuals for the different environments inhabited. In addition, we still lack estimates of connectivity in our population at different altitudes. Future landscape genetics should ascertain levels of connectivity between the population and should help make inferences of my PhD results on the population scale. These studies are very useful to unveil population-ecology dynamics and further our overall understanding of how a model organism responds to changes in environmental “harshness”.

Further, from model organisms such as blue tits we are able to draw parallel conclusions on life history responses in more endangered species. This is of particular importance in the ever-growing pressure of climate change. The long-term consequences of climate change are potentially extremely serious, with an estimated five degree warming by 2100 (Intergovernmental Panel on Climate Change 2014). As a results of this warming, major losses of biodiversity have already been documented, resulting in a destabilisation of crucial ecosystem functions (Cardinale et al. 2012). Climate change is expected to have impacts on species‘ fitness, though its current and future threats are still poorly understood (Pacifici et al. 2017). It has been highlighted that birds are one of the most important indicators of how species are coping with climate change, as changes in life history functions such as reproduction have been well documented (Parmesan 2006; Zuckerberg 2017). It is already known that avian species living at high altitude have experienced negative consequences of climate change (Freeman and Class Freeman 2014; Boyle et al. 2016). Hence, such detailed studies as presented in this thesis are important for understanding potential consequences of climate change. In particular, using environmental gradients, such as altitudinal ones, to generate gradual clines in environmental “harshness” can clarify general constraints on reproduction and can help make prediction on species’ adaptation to climate change. Such findings should be considered and contribute to improving policies on species management in relation to climate change.

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