Faecal sac removal and parental coordination in relation to parental predation risk in the blue tit (*Cyanistes caeruleus*)

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1.0 Introduction

The study of parental care is an important aspect of behavioural ecology due to the vast diversity of care strategies present throughout the animal kingdom (Smiseth *et al*. 2012). Intentions are to understand the evolutionary forces shaping parental care, and why care strategies differ between species and between individuals of the same species, in particular males and females, which are under different pressures from sexual selection (Trivers 1972; Clutton-Brock 1991). In addition to the worthy study of parental care in its own right, the field has ties with many other focusses of behavioural ecology, due to the coevolution of parental care, sexual selection, kin selection, mating systems, and social systems (Smiseth *et al*. 2012).

Parental care has been defined as "any form of parental behaviour that appears likely to improve the fitness of a parent's offspring" (Clutton-Brock 1991). The benefits of parental care to the parent performing the behaviour, increasing the fitness of their own genes present in the offspring they are caring for, are offset against the costs to that parent's ability to successfully produce other offspring. This idea was first outlined by Trivers in his 1972 framework on parental investment and sexual selection, where he defined parental investment 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).

Parental care exists on a spectrum, where the form and extent is determined by environmental, physiological, and social constrains, and ultimately the balance between costs and benefits associated with parental investment. Under the above definitions of parental care, any species which produces eggs can be considered to exhibit a form of parental care (Klug *et al*. 2012). Indeed, an egg provides a developing embryo with some shelter from the outside world, and with a valuable food source in the form a nutrient-rich yolk, which is often provided in abundance beyond the minimum required by the offspring to successfully hatch. For example, when herring gull mothers invest more of their own resources into producing heavier eggs, chicks that hatch from those eggs are more likely to survive to fledging than those hatched from smaller eggs (Davis 1975), but chicks from smaller eggs can and do survive to fledging, demonstrating that greater investment in a heavier egg is not necessary for offspring survival. Even in the case where a mother abandons her eggs after laying them, as is seen in many reptiles, she has still provided her offspring with care in the form of: a nutrient-rich yolk, a protective eggshell, selecting a laying site, and, potentially, constructing a nest. It may even be argued that selecting a high-quality mate constitutes parental care as this improves offspring fitness by providing them with superior genes. The extent of parental care varies from species to species, occurring before birth/hatching (e.g. gestation in mammals), and afterwards, with some parents providing extensive care for long time periods, which may include: provisioning the offspring, protection of offspring against predators and parasites, passing on information about the environment and even teaching the young behaviours which improve their survival.

Actively caring for offspring has implications for the risk of predation faced by parents. It is intuitive that any care behaviour which detracts from a parent's ability to carry out its usual anti-predator behaviours, such as vigilance, increases that parent's risk of being predated upon. Certain behaviours, such as guarding the young at a nest, may directly reduce predator avoidance and make the parent more vulnerable to attack (Pressley 1981, Winkelman 1996). For example, only male lumpsuckers (*Cyclopterus lumpus*) provide parental care by guarding the nest, resulting in roughly twenty times as many male lumpsuckers being caught by sea otters (*Enhydra lutris*) than females (Lissåker & Kvarnemo 2006). In eider ducks (*Somateria mollissima*), parental predation risk causes a major shift in parental care strategies from solitary care towards group care when predation risk is high (Jaatinken *et al*. 2011). Unfortunately, since predators of offspring often also pose a threat to parents, separating the effects of offspring versus parental predation risk can be difficult. So far, attempts to make such a distinction are rare in the literature. However, it is important for understanding the evolution of parental care to determine whether responses of parents to predators are driven by a reduction in the value of offspring or an increase in the cost of investment. If there is a high risk of offspring being depredated, then they are less valuable and the optimal level of parental investment in those offspring should be lower than in relatively safe offspring. Inversely, if the risk is instead to the parents themselves, the benefits of investment are unaffected, but the costs of investment may increase markedly.

It is possible to separate effects of offspring and parental predation risk when predators prey exclusively upon either the parents or the offspring. Such a scenario exists for blue tits (*Cyanistes caeruleus*), adults of which are the prey of sparrowhawks (*Accipiter nisus*). Sparrowhawks hunt by catching adult and fledgling blue tits on the wing, however, the small size of the cavities in which blue tits make their nests prevents the birds of prey, as well as most other potential predators, from accessing nestlings. The blue tit is a small, biparental passerine, resident throughout much of Europe. They are socially monogamous and typically raise one clutch of 7 – 12 eggs per breeding season, with laying taking place in April or May (Cramp & Perrins 1993). Both parents provision nestlings, although construction of the nest, incubation, brooding, and nestling parasite control are all exclusively female behaviours (Perrins 1979; Bańbura *et al*. 2001), which suggests differing optimal care strategies between the sexes.

In this thesis, I explore two aspects of parental care in the context of parental predation risk: faecal sac removal (Chapter 2) and coordination between parents (Chapter 3). To do so, I use observational and experimental data collected over two breeding seasons from a population of blue tits (*Cyanistes caeruleus*) breeding in nestboxes in Lancashire, Northwest England. The primary threat to blue tits is the sparrowhawk, followed by the domestic cat (*Felis catus*), and the great spotted woodpecker (*Dendrocopos major*), which uses its bill to break into the nest-cavity and predate upon nestlings. However, all nestboxes at the study site were fitted with a covering of wire mesh, which successfully prevents nest predation by woodpeckers (Mainwaring & Hartley 2008), and no signs of any nest predation were found throughout the two breeding seasons studied. To experimentally manipulate parents' perception of predation risk, I used a life-sized model of a large bird of prey, the peregrine falcon (*Falco peregrinus*), as well as a non-threatening wood pigeon (*Columba palumbus*) as a control species. The peregrine could certainly not pose a danger to nestlings inside the nestbox, but should be threatening to the parents attending the nest.

Nest sanitation, in the form of nestling faecal sac removal, is an often overlooked aspect of avian parental care despite occurring in more than 95% of species (Ibáñez-Álamo *et al*. 2017). While over 80% of birds provide biparental care (Cockburn 2006), it is usually only the females that engage in faecal sac removal (Guigueno & Sealy 2012), and blue tits are a rarity in that both parents contribute to the disposal of faecal sacs (Bańbura *et al*. 2001). Although investment in faecal sac removal has traditionally been thought of as a fixed trait (Herrick 1900), there is potential for parents to show plasticity of this behaviour (Gow *et al*. 2015), and in Chapter 2 I attempt to demonstrate the idea that parental predation risk may motivate them to do so.

Biparental systems of care are unparalleled in the extent to which they allow for complex interactions through parental conflict and cooperation (Houston *et al*. 2005; Harrison *et al*. 2009). In Chapter 3, I aim to test a theory for resolution of parental conflict over care which has so far received little research attention: the theory of conditional cooperation, which predicts that parents cooperate to coordinate care by taking turns to provision offspring (Johnstone *et al*. 2014). The presence of a parental predator near the nest provides a unique opportunity for parental cooperation to escalate, as parents may increase cooperation by coordinating visits to the nest to provide mutual lookout cover against the predator. To test this idea, I again experimentally manipulated perceived parental predation risk. Finally, in Chapter 4, I present a general discussion of Chapters 2 and 3 and draw conclusions.

2.0 Fear and fastidiousness: reduced faecal sac removal by blue tits (*Cyanistes caeruleus*) coincides with reduced provisioning when predation risk is elevated

2.1 ABSTRACT

The removal of faecal sacs from nests is an important yet understudied component of parental care. The rules governing the removal of faecal sacs remain unclear and whilst the fixed sanitation hypothesis predicts that their removal occurs at a fixed proportion of provisioning rate, the adjustable sanitation hypothesis predicts that their removal is flexible. Here, I present a novel hypothesis for explaining variation in sanitation rates, the ‘predation risk hypothesis’, which predicts investment in faecal sac removal decreases when parental predation risk is high. I performed a study in which I analysed faecal sac removal behaviours of adult blue tits (*Cyanistes caeruleus*) in relation to experimentally increased perception of predation risk which allowed me to test my hypothesis. First, an analysis of the removal of faecal sacs from 119 nests showed that faecal sac removal rates were determined by brood size and provisioning rate, while both sexes engaged in faecal sac removal equally. Second, the experimental presentation of a peregrine falcon reduced the rate at which parents removed faecal sacs in comparison to parents presented with a benign wood pigeon, who removed faecal sacs at unchanged rates. These results contrast with the findings of previous studies, probably because the parents were at risk in this study whereas the offspring were at risk in other studies. Lower faecal sac removal rates at experimental nests coincided with reductions in provisioning rates and so the rate of faecal sac removal per visit to the nest did not change. Thus, while my results support the predation risk hypothesis, they also support the idea of fixed sanitation, as parental faecal sac removal rate remained a fixed proportion of provisioning rate, including when their perception of predation risk was elevated.

Key words: Faecal sac, nest sanitation, predation risk, blue tit, parental care, offspring provisioning.

2.2 INTRODUCTION

Parental care consists of a trade-off for parents between maximising their own fitness gains from current offspring and minimising fitness costs in relation to future reproductive attempts (Nur 1984; Smiseth *et al*. 2012). In birds, parental care consists of a range of behaviours from selecting a nest site, nest building, incubating eggs and caring for offspring. Although parental care is often quantified using nestling provisioning rates, the removal of nestling faeces from the nest is also a common behaviour (reviewed in Guigueno & Sealy 2012; Ibáñez-Álamo *et al*. 2017). Nest sanitation has been identified as an important component of avian parental care for a long time (Herrick 1900; Thompson 1934; Blair & Tucker 1941) but has received much less empirical attention than other aspects of care, such as offspring provisioning (Wright *et al*. 1998; Smiseth *et al*. 2008). It is intuitive that the removal of faeces improves conditions experienced by nestlings within nests (Blair & Tucker 1941; Welty 1982; Bucher 1988; Kepler *et al*. 1996; Potti *et al*. 2007; Ibáñez-Álamo *et al*. 2014a) and such behaviours are likely to be energetically costly as parents carry them long distances away from nests (Blair & Tucker 1941; Weatherhead 1984; Petit *et al*. 1989; Lang *et al*. 2002). Therefore, removing nestling faeces is likely to be an important facet of parental care which influences parent and offspring fitness.

The removal of faecal sacs is ubiquitous amongst birds, with the behaviour being shown by more than 95 per cent of 400 species included in a phylogenetic study (Ibáñez-Álamo *et al*. 2017). This suggests that removing faeces has important fitness consequences and the evolution of faecal sac membranes, which securely hold the waste together (Blair & Tucker 1941; Welty 1982; Ibáñez-Álamo *et al*. 2014a, 2017), suggests that the removal of faecal sacs is an integral component of care. Moreover, faecal sacs are a bright white colour, which when left near the nest would make it more conspicuous to predators (Tinbergen *et al*. 1962). As well as guarding against sight-oriented predators, disposing of faecal sacs may minimise olfactory cues for some predators (Petit *et al*. 1989), although Ibáñez-Álamo *et al*. (2014b) found no support for this. However, nest predation is one of the most fundamental forces shaping the evolution of avian life-histories (Ricklefs 1969; Martin 1995; Lima 2009) and may well have contributed to the evolution of nest sanitation in birds.

Despite the prevalence of nest sanitation behaviours in birds, the factors determining rates of faecal sac removal are unclear because few studies have experimentally investigated their removal (Markman *et al*. 2002; Ibáñez-Álamo *et al*. 2013; Gow *et al*. 2015; Quan *et al*. 2015; Amo *et al*. 2017). Herrick (1900) first hypothesised that nest sanitation may be an intrinsic compulsion of parents to remove faecal sacs the moment they appear within the nest. Most defecation events occur immediately after a provisioning parent visits the nest (Gabrielson 1912; Shaver 1918; Smith 1942, 1943; Quan *et al*. 2015), even when feeding intervals were experimentally increased from around 12 minutes to 60 and 120 minutes (Quan *et al*. 2015), and parents either wait in the nest for defecation to occur or actively stimulate it (Selous 1933; Erickson 1938; Gill 1983; Glück 1988; Islam 1994). Parents may then dispose of faecal sacs by eating them, although this usually only occurs in the first half of the nestling period (Blair & Tucker 1941; Kluijver 1950; Glück 1988; Guigueno & Sealy 2012), or by carrying them away from the nest. The consumption of faeces may occur as an economic alternative to carrying them away (Hurd *et al*. 1991; McGowan 1995) or as a means of recovering nutrients and water (Morton 1979; Glück 1988; McGowan 1995). Either way, the instantaneous nature of faecal sac disposal implies that sanitation rate should principally be determined by factors such as brood size, provisioning rate, the age of nestlings and their digestive functionality. Gow *et al*. (2015) described this as the ‘fixed sanitation hypothesis’ which predicts that, controlling for brood size and age, sanitation rate should be a fixed proportion of provisioning rate.

Alternatively, though, because nest sanitation behaviours are associated with time and energy costs, the ‘adjustable sanitation hypothesis’ has also been proposed and predicts that parents adaptively trade-off faecal sac removal (Gow *et al*. 2015). The adjustable sanitation hypothesis differs from the fixed sanitation hypothesis in that parents are not restricted to always removing faecal sacs the moment they are produced. This would be evidenced by a reduction in the rate of faecal sac removal per unit of food provisioned, as provisioning rate determines defecation rate and in this scenario parents allow faecal sacs to remain in the nest rather than disposing of them immediately after defecation. Gow *et al*. (2015) tested these two hypotheses in northern flickers (*Colaptes auratus*) by manipulating brood sizes and found support for the fixed sanitation hypothesis, as provisioning parents did not adjust the proportion of visits where they removed faecal sacs. Interestingly though, widowed males provisioned nestlings at 1.61 times the rate of paired males (Figure 3 in Weibe 2005) but removed faecal sacs at roughly half the rate (Gow *et al*. 2015). Hence, they may have been trading off sanitation with increased provisioning due to the time and energy constraints of single parenthood. However, an explanation not considered is that hungry males may have consumed faecal sacs rather than carrying them away, as predicted by the parental nutrition hypothesis of faecal sac ingestion (Guigueno and Sealy 2012). Indeed, Kluijver (1950) describes a stressed single female great tit (*Parus major*) eating most faecal sacs produced by her nestlings more than 15 days into the nestling period and attributed such behaviours as follows: "The eating of the faeces of such older young was perhaps a sign that the above female was hungry" (Kluijver 1950).

Although they occur less frequently, each sanitation event is energetically more costly than a provisioning event because faecal sacs are heavier than prey items and parents may fly farther from the nest to dispose of them than when foraging (Brooke 1981; Weatherhead 1984; Hendricks 1987; Lang *et al*. 2002). Assuming that faecal sacs produced near to fledging are approximately 2.4% of nestling body mass (Morton 1979), an 11 g adult carrying the faecal sac of a 10 g nestling typically adds around 2.2% to their own mass, weighted at the bill. For comparison, an average sized caterpillar of the green oak leaf-roller (*Tortrix viridana*), a common prey of tits (Kluijver 1950; Perrins 1991), when close to pupation weighs 37.5 mg (Szujecki 2012), around 0.3% of adult blue tit mass. Therefore, in addition to time and energy costs, it is also possible that sanitation carries costs to aerodynamics and evasiveness. Furthermore, the bright white colour of faecal sacs may stand out against the darker background of a woodland environment, making parents more visible to predators while carrying a faecal sac. For all of the above reasons, I propose a novel hypothesis regarding parental removal of nestling faecal sacs: 'the predation risk hypothesis', which predicts that parents will reduce investment in faecal sac removal as parental predation risk increases.

In this study, I examined blue tit (*Cyanistes caeruleus*) sanitation rates over two breeding seasons, and present the first experiment testing the adjustable sanitation hypothesis in parents experiencing elevated levels of perceived predation risk from a predator of adults, as opposed to a nest predator. Birds are able to identify different predators and respond to them accordingly. For example, pied flycatchers (*Ficedula* *hypoleuca*) have different hormonal and behavioural responses to woodpeckers and weasels (Silverin 1998), and nuthatch species (*Sitta carolinensis* and *S. canadensis*) respond more strongly to either a nest predator or an adult predator in accordance with their own life histories (Ghalambor & Martin 2000). I predict that parents will adjust their faecal sac removal behaviours in response to the perceived risk of parental predation (predation risk hypothesis), whilst maintaining provisioning rates, because sanitation is of less immediate importance to nestling survival than provisioning and so the effect on sanitation will be disproportionately large compared to any reduction in nest visit rate. Additionally, males and females may place different value in the current brood because they have invested different amounts in it (Trivers 1972), or may be specialised in the type of care they provide (Bańbura *et al*. 2001; Markman *et al*. 2002), and so I also look for sex effects. For example, since female blue tits invest more than males in the early stages of parental care, they may be more willing to risk predation and continue removing faecal sacs. Alternatively, because males are typically larger than females, their flight may be less negatively influenced by carrying a faecal sac and as such, males may be less affected by the presence of the predator.

2.3 METHODS

2.3.1 Study area and routine data collection

I studied a population of nestbox breeding blue tits within three separate, small (< 30 ha) predominantly deciduous but intermittently mixed woodlands in northwest Lancashire, UK (54°0’N, 02°47’W; see Mainwaring *et al*. 2010 for details). Nestboxes (treated wood, 150 x 150 x 200 mm, with 25mm entrance hole) were spaced around 50 metres apart, secured to trees approximately 1.8 metres off the ground, and covered with wire mesh to prevent nest predation by great spotted woodpeckers (*Dendrocopos major*; Mainwaring & Hartley 1998). From the 1st of April, nestboxes were checked in the 2016 and 2017 breeding seasons at least once every three days to determine the date on which the first egg was laid, under the assumption that blue tits lay one egg per day (Cramp and Perrins 1993). After the sixth egg had been laid, nestboxes were subsequently checked daily to determine the date of the onset of incubation, and were then left undisturbed until daily checks for hatching commenced after 13-days of incubation had passed (Cramp and Perrins 1993).

2.3.2 Quantifying provisioning rates and nest sanitation

When the eldest nestlings were between 9 and 11 days old, in good weather conditions, which consisted of periods of warmth and sun as opposed to cold and rain, a video camera was placed on a tripod 5 - 10 metres from the nestbox to record natural provisioning and faecal sac removal behaviours of the parents for a period of one hour (following Mainwaring and Hartley 2016). At this stage of the nestling period, faecal sacs are unlikely to be eaten and parents should not need to stimulate nestlings to defecate (Guigueno & Sealy 2012). One to three days before filming, one of the parents was caught using a nestbox trap, and was sexed in the hand based on the presence (female) or absence (male) of a brood patch, before being marked with a prominent spot of white correcting fluid on the tail. This enabled the identification of sex of the provisioning bird from the videos. Brood size was also established at this time. Videos were recorded at 59 nestboxes in 2016 and 60 nestboxes in 2017. All of the videos were watched by one observer (AMB), who was blind to the sex of the birds, thereby preventing inconsistencies caused by inter-observer bias.

2.3.3 Predation risk experiment

In the 2017 breeding season, 27 of the nests were randomly assigned to a control or experimental treatment, and at these nests an additional experimental hour was recorded immediately following the first observational hour, to investigate responses in faecal sac removal and provisioning visits to perceived predation risk. Nests were randomly assigned to be presented with a model of one of two test species: a predator (peregrine, n = 13 nests) or a non-threatening control species (wood pigeon, n = 14 nests). When setting the camera before the first hour of filming, a plastic life-size model of the test species concealed by camouflage fabric was placed approximately 5 metres from the nest, oriented side-on to the front of the nestbox, and a wireless speaker was concealed nearby in a camouflaged bag. The model was revealed at the end of the observational hour by pulling on a long length of string attached to the covering fabric, such that the nest was not approached by a human observer to reveal the model. Simultaneously, playbacks of either peregrine or wood pigeon calls, corresponding to the model being presented, commenced through the wireless speaker. The call lasted for 30 seconds, followed by 15 seconds of silence, then another 30 seconds of call, and this was repeated at 20 and 40 minutes during the recording so that the blue tits were able to see and hear the threatening or non-threatening species. A dummy under camouflage fabric was left in place of the model the day before filming to allow the parents time to grow accustomed to the novel object. Three peregrine and two pigeon models were used, which were selected at random at the start of each day of filming. Neither mean brood size nor mean first egg date were significantly different between threat groups (brood size: pigeon 8.29 ± 0.398 standard error [SE] vs peregrine 8.31 ± 0.429 SE, $t_{25} = -0.038$, $p = 0.970$; first egg date: pigeon 21.07 ± 1.24 SE vs peregrine 20.31 ± 1.43 SE; $t_{25} = 0.404$, $p = 0.690$). From day 14 after hatching, nests were left undisturbed due to the risk of inducing premature fledging, until day 20 (±1) when fledging success was established.

2.3.4 Statistical analyses

Data were analysed using the SPSS v23.0 statistical package (SPSS, Chicago, IL, USA). A Linear Mixed Model (LMM) was used to examine variation in faecal sac removal rates, with 'nestbox number' as a random effect to control for the non-independence associated with two parents provisioning nestlings at one nest. Faecal removal rates were simply the number of faecal sacs removed by individual birds in the one-hour observational period, using data from both 2016 and 2017. Explanatory factorial variables were year, sex, and age (either first-year or older) and explanatory covariate variables were first egg date, brood size, and provisioning rate. All of the terms, and all of their interaction terms, were initially included in the model before the highest order non-significant effects were progressively removed until only fixed terms with significant effects, or which were involved in significant interactions, remained (Crawley 1993).

The results of the predation risk experiment were also analysed using LMM. Nestbox number was again used as a random effect and explanatory factorial variables were threat (predator or control) and hour ('prior to' versus 'during' model presentation), with brood size and provisioning rate included as explanatory covariates. The model was simplified as described above. All statistical tests were two-tailed, means are presented ±1 standard error and a critical *p*-value of 0.05 was applied throughout.

2.4 RESULTS

2.4.1 Observations of provisioning and faecal sac removal

Faecal removal rates ranged from 0 to 11 per hour with a mean of 3.91 ± 0.134 sacs removed. Sanitation rates were not significantly correlated between males and females within breeding pairs (Pearson's rho, $p = 0.145$), although there was a significant positive relationship between partners' provisioning rates to nestlings (Pearson's rho: $r = 0.296$, $p = 0.001$). There was no effect of parent sex on sanitation; males (3.99 ± 0.185) and females (3.92 ± 0.193) had similar sanitation rates (paired samples: $t_{117} = 0.236$, $p = 0.814$). There was, however, a highly significant difference in provisioning rates, with males (24.35 ± 0.991 feeds/h) provisioning more than females (20.98 ± 0.901 feeds/h; paired samples: $t_{117} = 2.99$, $p = 0.003$). Faecal removal rates increased with brood size (Figure 2.1) and provisioning rate, and decreased with the interaction between brood size and provisioning rate, whilst there was no effect of year, sex, age, or first egg date (Table 1.1).

Table 1.1 Summary of linear mixed effects model showing the effects of year, parental age (first year or older), parental sex, brood size, hourly nest visit rate, and first egg date on the hourly faecal sac removal rate of individual blue tit parents. Significant terms are highlighted in bold.

Predictor	d.f.	*F*	*p*	Estimate ± SE
Year	1,223	0.01	0.915	0.031 ± 0.285
Age	1,223	0.50	0.479	−0.177 ± 0.250
Sex	1,223	1.11	0.294	−0.242 ± 0.230
Brood size	**1,223**	**11.29**	**0.001**	**0.378 ± 0.113**
Visit rate	**1,223**	**17.03**	**< 0.001**	**0.180 ± 0.044**
First egg date	1,223	2.57	0.110	−0.042 ± 0.026
Brood size*visit rate	**1,223**	**5.41**	**0.021**	**−0.011 ± 0.005**

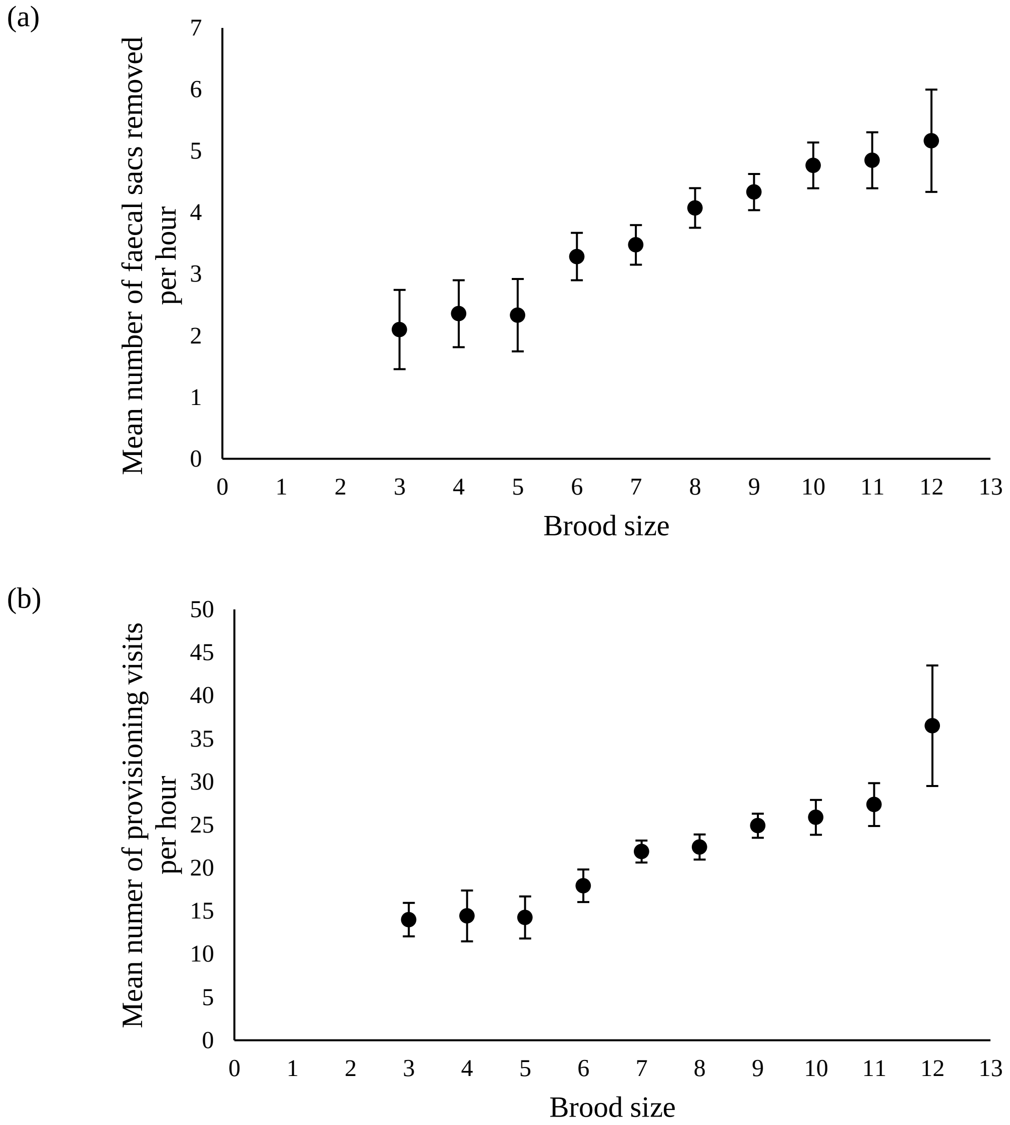


Figure 2.1. The mean number of faecal sacs removed (a) and provisioning visits to the nest (b) per hour by adult blue tits plotted against brood size. Data are combined for males and females as there was no significant effect of sex. Error bars show ± 1SE.

2.4.2 Predation risk, parental provisioning and faecal sac removal rates

Provisioning parents exposed to the peregrine falcon model reduced their faecal sac removal rates (3.08 ± 0.403) compared to the hour before exposure (4.42 ± 0.300; paired samples: $t_{25} = 3.33$, $p = 0.003$; figure 2.2a). These parents also had significantly lower faecal removal rates than pairs exposed to a wood pigeon (4.46 ± 0.376; independent samples: $t_{52} = 2.52$, $p = 0.015$). Parents exposed to a wood pigeon performed faecal sac removal at a similar rate to that seen in the hour beforehand (4.54 ± 0.306; paired samples: $t_{27} = 0.172$, $p = 0.864$) and there was no statistically significant difference in faecal removal rates recorded prior to the presentation of either test species model (independent samples: $t_{52} = 0.262$, $p = 0.794$).

Table 1.2. Summary of linear mixed effects model showing the effects of hour (before or during model presentation), model (pigeon or peregrine), brood size and hourly nest visit rate on the removal of faecal sacs by individual blue tit parents. Significant terms are highlighted in bold.

Predictor	d.f.	*F*	*p*	Estimate ± SE
Hour	1,97.7	1.42	0.236	−0.714 ± 0.445
Model	1,97.6	2.95	0.089	−0.872 ± 0.479
Brood size	**1,97.9**	**10.55**	**0.002**	**0.326 ± 0.100**
Visit rate	**1,101.9**	**25.26**	**< 0.001**	**0.075 ± 0.015**
Hour*model	1,97.6	1.33	0.252	−0.699 ± 0.607

However, faecal removal rate was not independently affected by hour or threat type, and the interaction between them was not significant (Table 1.2). The significant predictors of faecal sac removal were again brood size and provisioning rate, being higher for larger broods and for parents that visited the nest more often (Table 1.2). Brood sizes in each treatment were not significantly different (see Methods), nor were provisioning rates prior to presentation of the models (27.39 ± 1.81 visits per hour for pigeon vs. 27.08 ± 1.60 for peregrine; independent samples: $t_{52} = 0.130$, $p = 0.897$), however, provisioning rates during presentations depended on whether birds were exposed to a model peregrine (19.26 ± 2.46) or a model wood pigeon (26.64 ± 1.79; independent samples: $t_{52} = 2.45$, $p = 0.018$; figure 2.2b). Blue tits exposed to a peregrine provisioned nestlings at significantly lower rates than in the preceding hour (paired samples: $t_{25} = 4.11$, $p < 0.001$), whereas provisioning behaviours of birds exposed to a pigeon remained unchanged (paired samples: $t_{27} = 0.516$, $p = 0.610$). The rate of faecal sac removal per feed did not significantly differ for either treatment at any stage of the experiment (Repeated measures ANOVA on arcsine transformed data: all $p > 0.05$).

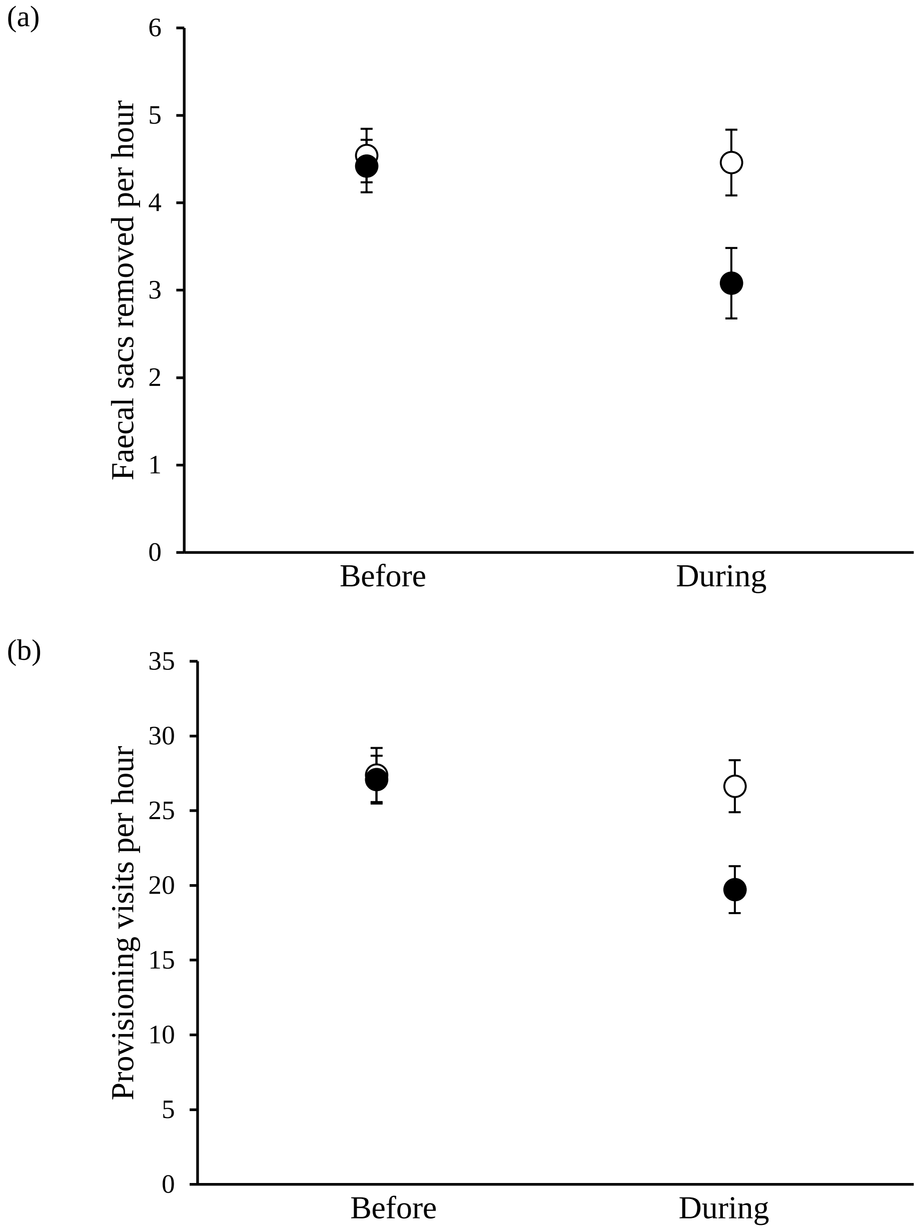


Figure 2.2. The mean number of faecal sacs removed (a) and provisioning visits to the nest (b) per hour by adult blue tits in the hour before and during model presentation. Either a model wood pigeon (open circles, n=28) or a model peregrine falcon (filled circles, n=26) was presented close to the nest throughout the presentation hour. Data are combined for males and females as there was no significant effect of sex. Error bars show ± 1SE.

2.5 DISCUSSION

The main finding of this study was that blue tit parents reduced nest sanitation rates when their perceived risk of predation was experimentally increased, supporting the hypothesis that faecal sac removal is, at least in part, mediated by parental risk of predation. This contrasts with two previous studies in which playback of Eurasian magpie (*Pica pica*) calls had no effect on common blackbird (*Turdus merula*) faecal sac removal (Ibáñez-Álamo *et al*. 2013), and visual or chemical cues of a ferret (*Mustela putorius*) near the nest did not result in blue tits altering faecal sac removal rate (Amo *et al*. 2017). These equivocal results may have occurred because in those studies, the predator posed a threat to the brood, whereas in this study, the threat was to the parents themselves. Clearly, examining how the risk of predation influences parental and offspring fitness warrants further research. It was not possible to entirely separate reductions in sanitation effort from reductions in provisioning effort, as lower faecal sac removal rates were associated with lower provisioning rates in the presence of the model peregrine falcon. Sanitation relative to visit rate did not decrease, indicating that parents did not necessarily forsake sanitation by allowing faecal sacs to accumulate in the nest, but instead continued to remove faecal sacs as soon as they were produced. This is surprising given that the parents appeared to be threatened by the model predator (AMB, personal observation), evidenced by less frequent visits to the nest and time spent alarm calling.

Three, non-mutually exclusive, hypotheses could potentially explain the observed lack of change in relative sanitation rate: first, the costs of removing faecal sacs are sufficiently low that relative sanitation rate need not be reduced under the risk of predation. Whilst removing faecal sacs is known to be energetically costly (Guigueno & Sealy 2012) these costs may not be excessive when birds are not exposed to predators on a regular basis. Other studies demonstrate that the direction in which birds fly and the distance they fly to forage differ when leaving nests with or without faecal sacs (Weatherhead 1988; Lang *et al*. 2002; but see Weitzel 2003, 2005) and further studies could usefully establish the extent of such differences in blue tits. Whatever the costs of sanitation are, they were to some extent regulated indirectly through provisioning rate. Second, the benefits of faecal sac removal, in the form of the avoidance of the costs of allowing faeces to remain in the nest, may be sufficiently high as to outweigh the costs, even in the face of increased predation risk. Third, whereas our study only quantified the removal of faecal sacs, the costs may have been mitigated by parents dropping faecal sacs closer to the nest when predation risk was elevated, thereby reducing distances flown whilst burdened by a faecal sac. This scenario is incongruous with the effort birds usually make to carry faecal sacs away from the nest, but is certainly worthy of research attention.

I also found that faecal removal rates were influenced by brood sizes and provisioning rates, which is consistent with the fixed sanitation hypothesis. Larger broods are likely to produce larger quantities of faecal sacs, and independently of this, nestlings which ingest more food are likely to defecate more frequently. Lombardo (1991) found that provisioning rates and faecal sac removal rates both increased with increasing brood sizes, and although potential correlations between faecal removal rates and provisioning rates were not investigated, the two exhibit similar patterns of increase when plotted against brood size (Figure 1 in Lombardo 1991). Other studies also report that increasing faecal sac removal rates coincide with elevated provisioning rates (Markman *et al*. 2002; Spencer 2005). The significant negative interaction I found between visit rate and brood size may be due to the diminishing returns of provisioning as brood sizes increase. In smaller broods, each food item equates to a greater intake of food per given nestling, stimulating them to defecate more frequently whereas in larger broods, greater numbers of feeds are required to achieve the same levels of per nestling provisioning. Parents also balance fitness gains from the current brood against future broods by investing less effort in each nestling when brood sizes are larger (Nur 1984). This is achieved by providing fewer feeds per nestling, resulting in lower mean weight of fledglings but reduced weight lost by parents over the nestling period (Nur 1984). Since the need to remove faecal sacs is essentially an additional cost of provisioning, the above strategy would be expected to produce the interaction between brood size and visit rate seen in this study.

We did not find any effect of parental sex on the rate at which faecal sacs were removed, which contrasts with Bańbura *et al*. (2001) who found that males removed more faecal sacs than females. Bańbura *et al*. (2001) attributed the sex differences in faecal sac removal rate to differences in prey selectivity, with larger prey better stimulating defecation (Royama 1966), resulting from a situation in which females' time budgets were constrained by undertaking intensive nestling parasite control behaviours in a population found to be highly infested by blowfly larvae. Average prey size is known to decline with increasing nest visit rate in tits (Nour 1998) and lower selectivity may be the cause of this. While males in our study visited the nest slightly more frequently than females, the difference appears to have been too small to influence sanitation rates. On the other hand, females may have visited the nest less frequently because they were more selective of prey, resulting in similar sanitation rates to males. However, I did not measure prey size and so further studies examining sex-specific patterns of faecal sac removal and other aspects of care are warranted.

The widespread occurrence of faecal sac removal amongst birds (Guigueno and Sealy 2012; Ibáñez-Álamo *et al*. 2017) clearly indicates that allowing faeces to remain in the nest is detrimental to fitness. But what causes this loss of fitness? Hypotheses were initially proposed when the removal of faecal sacs by parents was first described, but have scarcely been tested since. The nest predation hypothesis (Herrick 1900; Blair & Tucker 1941; Weatherhead 1984) predicts that the accumulation of faeces within the nest attracts predators, resulting in greater chance of nest predation. Only two studies have experimentally tested the nest predation hypothesis, and they produced conflicting results (Petit *et al*. 1989; Ibáñez-Álamo *et al*. 2014b). Petit *et al*. (1989) found that chicken faeces left next to artificial quail (*Coturnix* spp.) nests increased the chance of them being depredated, but when nestling faecal sacs were left suspended just below blackbird (*Turdus merula*) nests, Ibáñez-Álamo *et al*. (2014b) did not find any effect on nest predation rates. My own study on nestbox-breeding blue tits does not provide support for this hypothesis, as nest predation rates are minimal, although it is possible that the removal of faecal sacs reflects a behaviour that evolved prior to cavity nesting behaviour. Further experiments are necessary to determine the validity of the nest predation hypothesis, particularly as it remains the only hypothesis explaining why faecal sacs are carried long distances away from the nest.

Another hypothesis proposed to explain faecal sac removal is the parasitism hypothesis (Skutch 1976) which suggests that their removal reduces parasite loads within nests. The only empirical test of the parasitism hypothesis found that faecal sacs attracted flies (Order Diptera), but not ectoparasites, to nests and as the proximity to faecal sacs did not affect nestling ecto- or endoparasite loads, then it provided no support for the parasitism hypothesis (Ibáñez-Álamo *et al*. 2016). In that study, proximity to faecal sacs produced an immune system response in nestlings, which together with the attraction of flies points to a third explanation: that faecal sac removal is an anti-microbial behaviour. Faecal sac membranes act as a physical barrier to enteric bacteria contained within, but only for a period of around 20 minutes, meaning that they must be removed quickly to prevent contamination of the nest material (Ibáñez-Álamo *et al*. 2014a). Furthermore, nestling diet may influence the microbial content of faeces, and thus the fastidiousness of nest sanitation behaviours. For example, the Poo-uli (*Melamprosops phaeosoma*) feeds its nestlings on invertebrates and maintains strict faecal sac removal throughout the nestling period, whereas other Drepranidines which feed their nestlings on plant material allow faeces to accumulate towards the end of the nestling period (Kepler 1996). Blue tit nestlings which share a nest are known to have significantly more similar faecal bacterial species richness (Benskin *et al.* 2015). The microbial hypothesis thus provides a plausible explanation as to why the blue tit parents in my study did not adjust their relative sanitation rates. The potential spread of pathogens associated with forsaking faecal sac removal may outweigh any increase in parental predation risk associated with continuing it.

To conclude, I have provided strong evidence in support of the novel predation risk hypothesis of faecal sac removal. Parents responded to the simulated predator by reducing their investment in their current offspring, which included a reduction in sanitation rate, but not relative to nest visit rate. Therefore, sanitation rate may well be a fixed outcome of provisioning rate, as predicted by the fixed sanitation hypothesis. Sanitation rates in blue tits were determined by brood sizes and provisioning rates, and these rules still held when the parents experienced an experimental time period when their perceived risk of predation was elevated. We recommend that future studies include a measure of the volume and nutritional quality of food delivered to nestlings, as this is likely to permit a more complete explanation for variation in the occurrence of nest sanitation behaviour in response to parental predation risk.

3.0 Cooperative coordination of care: blue tit (*Cyanistes caeruleus*) parents alternate and synchronise provisioning visits

3.1 ABSTRACT

Parents undertaking biparental care must overcome parental conflict of interests if the system is to be evolutionarily stable. While past theoretical models for conflict resolution have resulted in suboptimal efficiency of fitness gains, the conditional cooperation theory predicts the most efficient outcome, as each parent withholds provisioning until its partner has taken its turn, resulting in alternation of parents visiting the nest. Here, I test this theory in the blue tit (*Cyanistes caeruleus*), as well as investigating another component of parental coordination, provisioning synchrony, and examining both in response to heightened perception of parental predation risk. A randomisation process was used to establish levels of alternation expected to occur by chance alone, and I devised a new method for determining a threshold for synchronous inter-visit intervals, which enabled the calculation of synchrony for each individual breeding pair based on their specific provisioning rates. Both sexes alternated on significantly greater proportions of provisioning visits than could be accounted for by chance alone, and females alternated on a significantly greater proportion of visits than males. Synchronised arrival occurred approximately five times the amount predicted if parents were provisioning randomly, while females again appeared to be the more coordinated sex, synchronising a greater proportion of their visits than did males. Females increased provisioning synchrony in the presence of a model peregrine, while males and birds exposed to a nonthreatening control species model did not. I provide support for the theory of conditional cooperation, and for coordination of parental care, with levels of coordination and responses to predation risk being sex-specific. Although previously coordinated care has primarily been attributed to species with long-term pair bonds, here I demonstrate the prevalence of coordination in a species with very low mate fidelity, and future studies should seek to investigate this in other such species.

Key words: parental coordination, parental conflict, conditional cooperation, provisioning synchrony, predation risk

3.2 INTRODUCTION

Biparental care is both a cooperative undertaking and a basis for conflict. Conflict arises between parents as each benefits from the other's investment in care, while only experiencing the costs of their own investment (Smiseth *et al*. 2012). Thus, each parent stands to gain by reducing their own investment at the expense of their partner (Trivers 1972), and potentially even to the detriment of the offspring (Parker 1985; McNamara *et al*. 2003; Lessells & McNamara 2012). Parental conflict should make systems of biparental care evolutionarily unstable, yet such systems are widespread, particularly in birds, where biparental care occurs in over 80% of species (Cockburn 2006). Clearly then, parents providing biparental care are able to resolve conflict, and one way this may be achieved is through cooperative coordination of care.

The question of how stable systems of biparental care can evolve despite parental conflict of interests is one that remains largely unanswered. Initial theoretical models, such as that proposed by Houston and Davies (1985), constituted a 'sealed-bid' scenario where investment in care was genetically determined and as such only changed over evolutionary time. Newer models have progressively developed the more biologically realistic idea that 'negotiation' between parents can resolve conflict and lead to evolutionarily stable biparental care (McNamara *et al*. 1999, 2003; Johnstone & Hinde 2006; Johnstone 2011; Lessells & McNamara 2012). These negotiation models allow for parents changing their investment in care on a behavioural timescale, and changes are in response to investment levels of the focal parent's partner. However, most negotiation models still predict a net fitness gain below the most efficient outcome (McNamara *et al*. 1999, 2003; Johnstone & Hinde 2006; Johnstone 2011; Lessells & McNamara 2012). Johnstone *et al*. (2014) addressed this issue with a 'conditional cooperation' model where each parent feeds the young only after the other has done so, or in other words, parents alternate provisioning visits to the nest. This tit-for-tat reciprocity leads to the most efficient fitness outcome because any change in one parent's rate of investment results in an equal change in that of its partner. That is the case in theory, however, Johnstone *et al*. (2014) also tested this idea empirically by observing provisioning behaviours of adult great tits (*Parus major*) and found alternation occurred more frequently than expected by chance, but not on every visit.

Strict alternation is only possible when partners' provisioning rates are equal, and the maximum possible alternation will decrease as the difference between provisioning rates increases (Bebbington & Hatchwell 2016). For this reason alone, strict alternation seems unlikely in reality. Further, strict alternation may also be impractical whenever there is the potential for a parent to fail to observe a provisioning visit made by its partner. In this scenario, if both parents followed strict alternation rules, provisioning would come to a standstill as each would be indefinitely waiting for the other to take their turn (Johnstone *et al*. 2014). As such, parents can be expected to enforce alternation only to a certain extent, and they may try to directly monitor provisioning by their partner as a signal for when to take their own turn. One way that this may be achieved is through synchronisation of provisioning visits to the nest, whereby parents intentionally time their own offspring provisioning to coincide with that of their partner (Mariette & Griffith 2012, 2015).

So far, three non-mutually exclusive explanations have been proposed for why carers may synchronise visits to the nest, with the majority of supporting evidence coming from studies of cooperative breeders. First, provisioning synchrony may serve some kind of signalling function, either of an individual's quality or contribution towards a common good as 'payment' for group membership (Koko *et al.* 2002; Doutrelant & Covas 2007; but see McDonald *et al.* 2008). Second, by synchronising provisioning visits parents or carers may be able to improve the efficiency of food delivery by partitioning food more equally amongst nestlings (Shen *et al*. 2010), or by using information on provisioning by other individuals to better estimate nestling hunger (Johnstone & Hinde 2006). Third, synchronised arrival at the nest may lower predation risk, either by reducing conspicuousness of the nest or parents/carers to predators (Martin *et al*. 2000; Fontaine & Martin 2006; Raihani *et al*. 2010; Bebbington & Hatchwell 2016) or by enabling provisioning adults to provide increased joint vigilance against attack. Alternatively, provisioning synchrony may arise from coordination of other behaviours, such as foraging (Masello *et al*. 2006), or may facilitate them, as could be the case with alternation.

In this study, I investigated two forms of coordination between pairs of breeding blue tit (*Cyanistes caeruleus*) parents: provisioning alternation and provisioning synchrony. I test the hypothesis that parents take turns provisioning nestlings by alternating visits to the nest, as is predicted by the conditional cooperation theory for resolving parental conflict over care (Johnstone *et al*. 2014). I test whether parents actively synchronise their provisioning visits and, by experimentally manipulating perceived predation risk, how provisioning synchrony is affected by risk of predation. As synchronised provisioning may be an attempt by parents to reduce conspicuousness to predators (Martin *et al*. 2000; Fontaine & Martin 2006; Raihani *et al*. 2010; Bebbington & Hatchwell 2016), or may be associated with providing mutual vigilance cover when risk of predation is high, I make the prediction that parents will increase provisioning synchrony during a period of elevated predation risk.

3.3 METHODS

3.3.1 Routine data collection and observations

Data were collected from April to June in 2016 and 2017 from a population of blue tits nesting in treated wooden boxes distributed throughout three distinct, small (< 30 ha), mostly deciduous but occasionally mixed woodlands in northwest Lancashire, UK (54°0’N, 02°47’W; see Mainwaring *et al*. 2010 for details). Nestboxes (150 x 150 x 200 mm, with 25mm entrance hole) were spaced around 50 metres apart, secured to trees approximately 1.8 metres off the ground, and covered with wire mesh to prevent nest predation by great spotted woodpeckers (*Dendrocopos major*; Mainwaring & Hartley 1998). Nestboxes were checked at least once every three days commencing on the 1st of April to determine when the first egg was laid, under the assumption that blue tits lay one egg per day (Cramp and Perrins 1993). Once the sixth egg was laid, nests were checked daily to determine the onset of incubation, and were then left undisturbed until daily checks for hatching commenced based on an expected 13-day incubation period (Cramp and Perrins 1993). When the eldest nestlings were 9 – 11 days old, in good weather, which comprised periods of warmth and sun as opposed to cold and rain, a video camera was placed on a tripod 5 – 10 metres from the nestbox to record natural provisioning behaviours of the parents for a period of one hour (following Mainwaring and Hartley 2016). One to three days before filming, one of the parents was caught, using a nestbox trap, and was sexed in the hand based on the presence (female) or absence (male) of a brood patch, before being marked with a prominent spot of white correcting fluid on the tail. This enabled the identification of sex of the provisioning bird from the videos. Brood size was also established at this time. After discarding videos where one parent performed > 90% of the total provisioning visits (< 1% of total nests), as such instances do not provide useful information for the study of alternation or synchronisation, sample sizes of nests observed in 2016 and 2017 were 57 and 58, respectively. All videos were watched by one observer (AMB), who was blind to the sex of the birds, thereby preventing inconsistencies caused by inter-observer bias.

3.3.2 Calculating alternation of provisioning visits

For each pair, alternation, A, was calculated as $A = F/(t - 1)$, where t is the total number of provisioning visits and F is the number of visits where one parent followed the other in order of delivery. For each parent, alternation was calculated one of two ways depending on whether the bird was the first of its pair to provision the young in the observation period. Alternation by the first provisioning parent was calculated as above, $A = F/(t - 1)$, were F and t correspond to alternated and total feeds by that bird. Alternation by the other parent was calculated as $A = F/t$. One is subtracted from the total number of provisioning visits made by the first parent because it cannot be known whether the first visit of the observation period was alternated, as the previous visit occurred outside of the observation period.

If each parent provisions randomly and independently of the other, a certain degree of alternation will still occur by chance alone. To evaluate the strength of this effect, I generated expected values of alternation for each nest by randomising the sequence of visits to the nest thirty times, and calculating alternation for each randomisation. The expected alternation was then the mean of all thirty randomly generated alternation values. This procedure allowed me to compare observed alternation levels to those expected by chance.

3.3.3 Analysis of provisioning synchrony

To determine whether two birds arrive at the nest synchronously, it is necessary to set a threshold for the length of the interval between the visits above which the visits are not classed as synchronous, and below which they are. I called this threshold C, so that if one bird arrived at the nest within C seconds of the other, that visit was deemed to be synchronous. In other studies, values of C seem to have been selected arbitrarily and have been applied to every nest equally, despite differences in parental provisioning rates at each nest. Here, I calculated C separately for each nest, based on the provisioning rates of both parents, as the length of interval between alternate feeds with a 5% likelihood of occurring by chance, under the assumption that each parent visits independently of the other.

I began with the assumption that the probability of a bird arriving at the nest at any given second is equal to the total number of visits made by that bird in the observation period, t_i, divided by the total number of seconds in the observation period, which was always 3600 as I filmed for one hour. This can be expressed as $p = t_i/3600$, and for a partner provisioning at rate t_j, $p = t_j/3600$, where in each case p represents the probability of that bird arriving at the nest at any given second. It follows that the probability of a bird arriving within a period of C seconds is equal to $Ct_i/3600$, and the probability of both parents provisioning within C seconds, p_c, is given by

$$p_c = \frac{Ct_i}{3600} \times \frac{Ct_j}{3600}$$

Assigning p_c a value of 0.05, meaning that there is a 5% chance of both parents randomly provisioning the young within a period of C seconds, the above equation can be solved for C to

$$C = \sqrt{\frac{0.05 \times 3600^2}{t_i \times t_j}}$$

By inputting male and female provisioning data into this formula, I was able to calculate a synchrony threshold, C, for individual nests. I then calculated provisioning synchrony, S:

$$S = V/(t - 1)$$

where V is the number of visits made by alternate birds within C seconds of one another, and t is the total number of provisioning visits in the observation period. As with alternation, values of S were calculated differently for each parent depending on which made the first visit of the observational period. The mean (± standard deviation) value of *C* from all 115 nests was 42.7 ± 20.48 seconds.

3.3.4 Predation risk experiment

In the 2017 breeding season, 27 of the nests were randomly assigned to a control or experimental treatment, and at these nests an additional experimental hour was recorded immediately following the first observational hour, to investigate responses in alternation and synchrony of provisioning visits to perceived predation risk. Nests were randomly assigned to be presented with a model of one of two test species: a predator (peregrine, n = 13 nests) or a non-threatening control species (wood pigeon, n = 14 nests). Due to very low return rates at some nests presented with a model peregrine, the sample size for this treatment was reduced to 7 nests. When setting the camera before the first hour of filming, a plastic life-size model of the test species concealed by camouflage fabric was placed approximately 5 metres from the nest, oriented side-on to the front of the nestbox, and a wireless speaker was concealed nearby in a camouflaged bag. The model was revealed at the end of the observational hour by pulling on a long length of string attached to the covering fabric, such that the nest was not approached by a human observer to reveal the model. Simultaneously, playbacks of either peregrine or wood pigeon calls, corresponding to the model being presented, commenced through the wireless speaker. The call lasted for 30 seconds, followed by 15 seconds of silence, then another 30 seconds of call, and this was repeated at 20 and 40 minutes during the recording so that the blue tits were able to see and hear the threatening or non-threatening species. A dummy under camouflage fabric was left in place of the model the day before filming to allow the parents time to grow accustomed to the novel object. One of three different peregrine and two different wood pigeon models were selected at random at the start of each day of filming. Neither mean brood size nor mean first egg date were significantly different between threat groups (brood size: pigeon 8.29 ± 0.398 standard error [SE] vs peregrine 8.31 ± 0.429 SE, $t_{25} = -0.038$, $p = 0.970$; first egg date: pigeon 21.07 ± 1.24 SE vs peregrine 20.31 ± 1.43 SE; $t_{25} = 0.404$, $p = 0.690$). From day 14 after hatching, nests were left undisturbed due to the risk of inducing premature fledging, until day 20 (±1) when fledging success was established.

3.4 RESULTS

3.4.1 Observations of parental coordination

Parents on average alternated 54.6 ± 1.0% of provisioning visits to the nest, which was significantly higher than levels of alternation expected by chance (47.6 ± 0.4% of visits per hour; paired samples *t*-test on arcsine transformed data: $t_{114} = -8.84$, $p < 0.001$). Males provisioned the brood more frequently than females (24.35 ± 0.991 visits per hour vs. 20.98 ± 0.901; paired $t_{117} = 2.99$, $p = 0.003$) and accordingly showed a significantly lower propensity to alternate (53.6 ± 1.5% vs. 63.7 ± 1.7%; paired samples *t*-test on arcsine transformed data: $t_{114} = 3.81$, $p < 0.001$; figure 3.1). Logically, when provisioning rates are not equal the parent which visits the nest less frequently is expected to have a greater proportion of alternated visits (47.4 ± 1.3% expected for males vs. 55.3 ± 1.3% expected for females; figure 3.1), however, both sexes showed a significantly stronger tendency to alternate than expected by chance (paired samples *t*-test on arcsine transformed data: males, $t_{114} = -6.65$, $p < 0.001$; females, $t_{114} = -8.47$, $p < 0.001$).

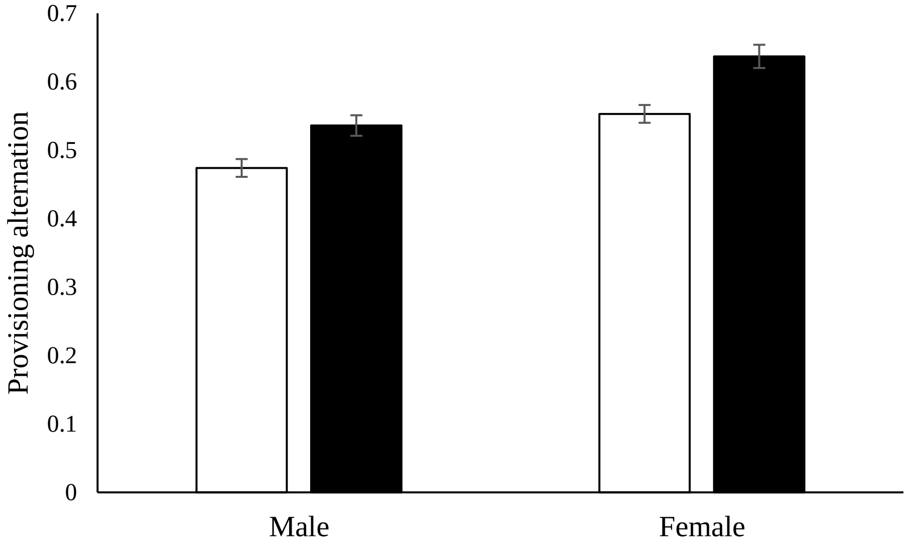


Figure 3.1. Expected (white columns) versus observed (black columns) provisioning alternation by male (n = 115) and female (n = 115) adult blue tits. Alternation is scored as the proportion of nestling provisioning visits made by a bird where the previous visit was by its partner. Error bars show ± SE.

Females also synchronised a greater proportion of their visits than males (paired samples *t*-test on arcsine transformed data: $t_{114} = 4.73$, $p < 0.001$; figure 3.2), arriving within *C* seconds of their male partner on 32.2 ± 1.5% of nestling feeding visits, compared to males which synchronised their arrival on 22.2 ± 1.0% of feeds (figure 3.2). In both sexes, alternation and synchronisation were significantly correlated (Pearson's rho: females, $r = 0.669$, $p < 0.001$; males, $r = 0.635$, $p < 0.001$; figure 3.3). However, within pairs, male and female alternation were significantly negatively correlated (Pearson's rho: $r = -0.473$, $p < 0.001$), as were male and female synchronisation (Pearson's rho: $r = -0.456$, $p < 0.001$).

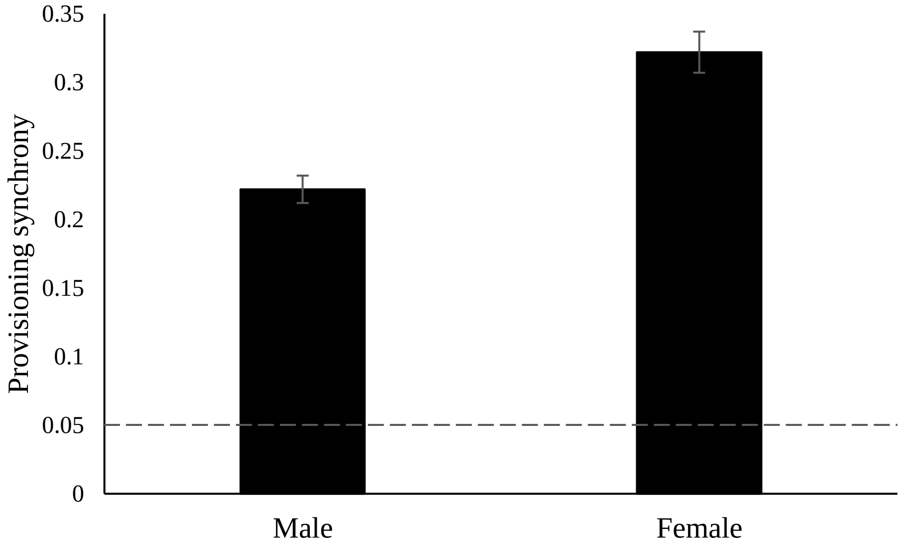
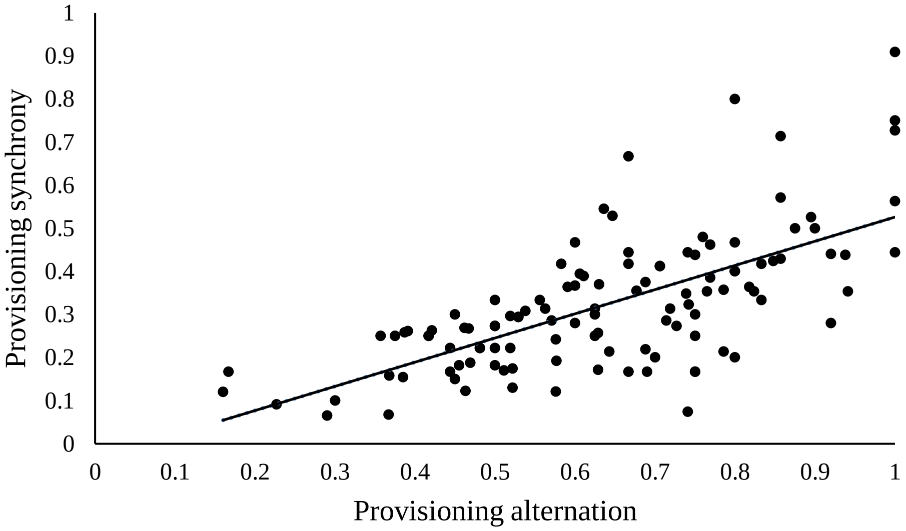


Figure 3.2. Provisioning synchrony by male (n = 115) and female (n = 115) adult blue tits. Synchrony is scored as the proportion of nestling provisioning visits made by a bird within *C* seconds of a visit by its partner (see Methods for calculation of *C*). The dashed line shows a synchrony score of 0.05, the expected level of synchrony if provisioning were random. Error bars show ± SE.

(a)



(b)

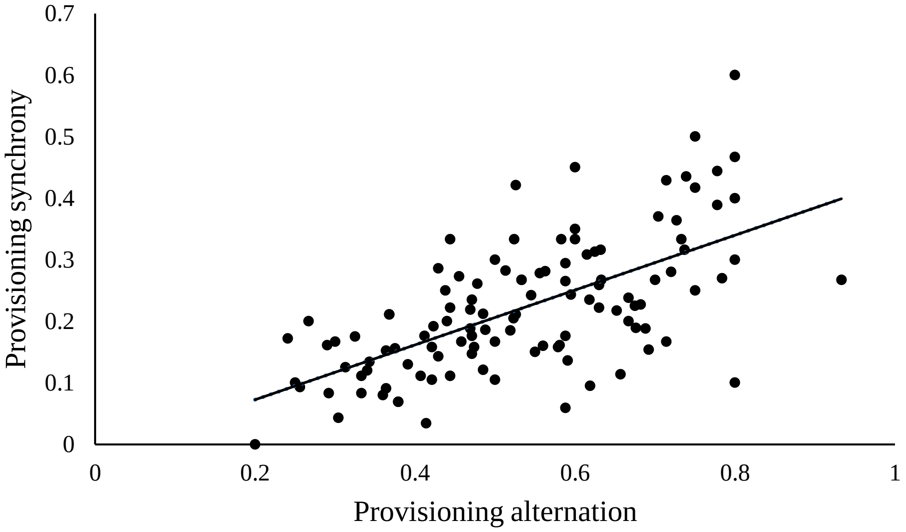


Figure 3.3. Nestling provisioning alternation correlated against provisioning synchrony in (a) female and (b) male adult blue tits. Alternation is scored as the proportion of nestling provisioning visits made by a bird where the previous visit was by its partner, and synchrony is the proportion of nestling provisioning visits made by a bird within *C* seconds of a visit by its partner (see Methods for calculation of *C*). Lines are plotted as a best fit with (a) $R^2 = 0.447$ and (b) $R^2 = 0.403$, $p < 0.001$ for both.

3.4.2 Predation risk experiment

The mean visit synchrony of females exposed to a model pigeon decreased from 24.0 ± 3.5% before model presentation to 20.8 ± 2.1% of visits during model presentation, which was similar to males presented with the same test species, whose synchrony decreased from 23.0 ± 2.4% to 19.6 ± 1.9% (figure 4). The opposite occurred in females exposed to a model peregrine, whose synchrony increased from 25.5 ± 3.8% to 32.9 ± 4.7%, while the corresponding males showed synchrony of 20.4 ± 3.4% before and 20.8 ± 2.2% during model presentation (figure 4).

Provisioning synchrony was analysed using a repeated measures ANOVA on arcsine transformed data with experiment stage (before or during model presentation) as a within-subjects factor and model species and bird sex as between-subjects factors. The analysis revealed a significant interaction between the test model species and whether data were recorded before or during presentation ($F_{1,44} = 4.17$, $p = 0.047$), with no independent effects of test species, experiment stage, or sex, and no other significant interactions (all $p > 0.05$). Combining data from both sexes, synchrony was confirmed to be similar in the hour before models were presented (independent samples *t*-test on arcsine transformed data: $t_{46} = -0.272$, $p = 0.787$), but significantly higher when birds were presented with a model peregrine compared to a model pigeon (Welch's *t*-test on arcsine transformed data: $t_{29.5} = 2.05$, $p = 0.049$).

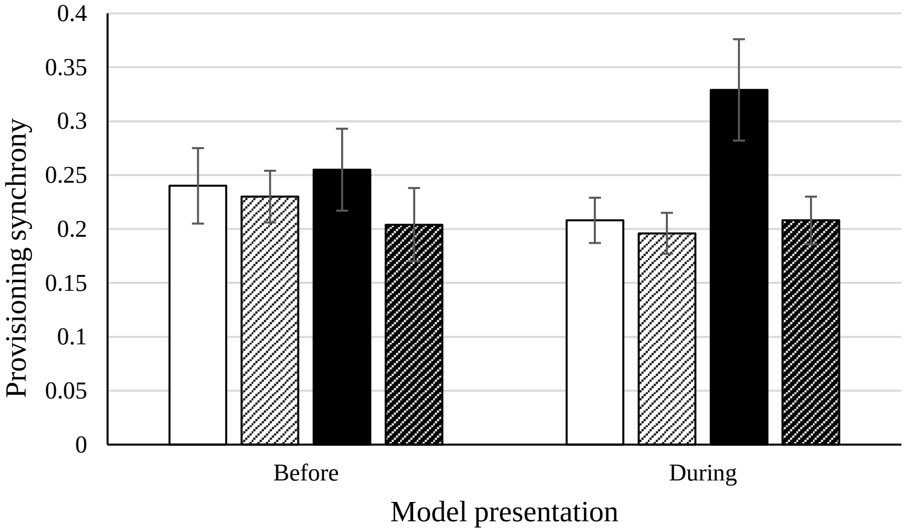


Figure 3.4. Nestling provisioning synchrony of adult blue tits before and during presentation with either a model wood pigeon or a model peregrine falcon. From left to right, bars show: females presented with a pigeon (n=14), males presented with a pigeon (n=14), females presented with a peregrine (n=10), males presented with a peregrine (n=10). Error bars show SE.

A similar analysis of provisioning alternation indicated that this behaviour was unaffected by the experiment (repeated measures ANOVA on arcsine transformed data: all $p > 0.05$; figure 3.5). However, observed alternation was still significantly higher than expected during model presentation (paired samples t-test on arcsine transformed data: $p < 0.001$ for both sexes; figure 3.5).

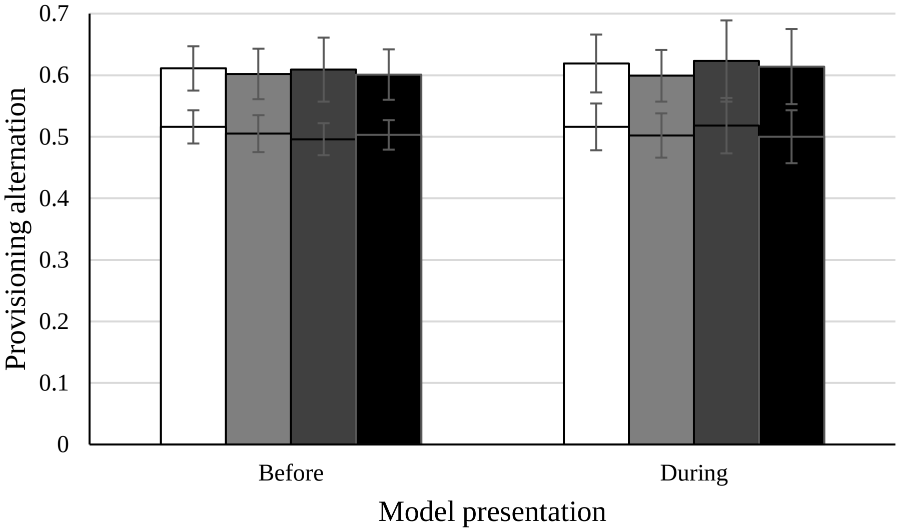


Figure 3.5. Nestling provisioning alternation of adult blue tits before and during presentation with either a model wood pigeon or a model peregrine falcon. On each bar, the lower line indicates expected alternation based on random provisioning, whereas the higher line shows observed alternation. From left to right, bars show: females presented with a pigeon (n=14), males presented with a pigeon (n=14), females presented with a peregrine (n=10), males presented with a peregrine (n=10). Error bars show SE.

3.5 DISCUSSION

This study supports the hypothesis that parents take turns when provisioning their young, and provides evidence in support of the conditional cooperation theory of biparental care proposed by Johnstone *et al*. (2014), which predicts that parents alternate visits to the nest as a means of resolving conflict over care (Johnstone *et al*. 2014; Bebbington & Hatchwell 2016). Although the theory predicts strict alternation, occurring on 100% of visits, the empirical investigation carried out by Johnstone *et al*. (2014) using great tits (*Parus major*) revealed levels of alternation at around 58% (see corrigendum Johnstone *et al*. 2016), which is comparable to the alternation score of 54.6% observed in blue tits in this study. The theoretical 100% alternation is presumably not met when there is potential for parents to be imperfectly informed on which bird last fed the young, as if both parents were to observe strict alternation, but one fails to notice the other visiting the nest, then provisioning would come to a complete standstill. As such, parents are not expected to entirely resolve conflict through a strategy of conditional cooperation, but evidence from this study and those by Johnstone *et al*. (2014) and Bebbington & Hatchwell (2016) indicates that alternation of provisioning visits may play an important role in resolving some of the conflict over care experienced by parents providing biparental care.

Females showed a higher tendency to coordinate care, both alternating and synchronising provisioning visits more than males. This result differs from the findings of Johnstone *et al*. (2014), who reported that male and female great tits were equal in their propensity to alternate when provisioning nestlings. This may be because great tit parents provision the young at equal rates (Johnstone *et al*. 2014), whereas, in this study, male blue tits provisioned more often than females. This difference could explain why females were found to alternate proportionally more visits, as they visited the nest less frequently overall, and why they deviated from expected levels of alternation to a greater extent than males, which alternated 6.2% more provisioning visits than expected by chance, while females alternated 8.4% more. Examining the numbers of alternated visits confirms that the average male and female are each expected to alternate around 11 visits per hour, and are both observed to alternate approximately 13. Therefore, while females are both expected and observed to alternate a greater proportion of their provisioning visits, this translates to an equal total number of alternated visits as expected and observed in males. Future investigations may provide useful insight into sex-specific patterns of provisioning alternation in other species.

The calculation of provisioning synchrony used in this study meant that parents provisioning nestlings independently of each other would arrive at the nest ‘synchronously’ with their partner on 5% of provisioning visits. Instead, the proportions of visits synchronised by males and females were 4.5 and 6.5 times this value, respectively. Females were more synchronous than males suggesting that the sexes operate under different rules for synchronising nest visits with their partner, and highlighting the need for future studies of provisioning synchrony in biparental animals, in particular, studies investigating sex differences. If the purpose of visit synchrony is to reduce the conspicuousness of the nest to predators (Martin *et al*. 2000; Fontaine &

Martin 2006; Raihani *et al*. 2010; Bebbington & Hatchwell 2016), then this may be evidence that concealing the location of the nest is more important to females, perhaps because they invest more in the current brood (Trivers 1972; Smiseth *et al*. 2012). However, my own experimental results concerning provisioning synchrony were in response to heightened parental predation risk, rather than nest predation risk.

Indeed, females presented with a model peregrine did increase synchrony with their partner, while males did not, again suggesting that synchrony may be more important to females, and that this may be driven by predation risk. The significant interaction between test species model and stage of the experiment, suggested that increased predation risk caused an increase in visit synchrony for females. Female blue tits may be more vulnerable to predation than males at this stage of the nestling period, and this may cause them to react more strongly to potential predators. Female blue tits are often in worse body condition than males while provisioning nestlings (Nur 1984), due to investing more in the early stages of care by constructing the nest, producing and incubating eggs, brooding chicks and keeping them free of parasites, all of which are activities performed only by the female (Perrins 1979; Cramp & Perrins 1993; Bańbura *et al*. 2001). Another potential explanation is that females may be providing vigilance cover for males. The females showed an increased tendency to enter the nest shortly after males, which may have come about through females arriving with males and perching nearby to provide vigilance cover. Males typically spend less than 15 seconds in the nest per visit (AMB, unpublished data), and once they leave, under the watch of the female, she then enters, perhaps now receiving vigilance cover from the male. Further experiments measuring provisioning synchrony under elevated parental predation risk in systems of biparental care are clearly warranted.

Birds which alternated a greater proportion of visits to the nest also tended to synchronise a greater proportion of visits. This could arise from the fact that, by definition, a visit which is synchronised must also be alternated. Or, these may potentially have been birds which had paired together in previous years, over which time their coordination improved through the mate familiarity effect (Black 1996). Alternatively, parents may possess a tendency to coordinate care in general, making them more likely to both alternate and synchronise visits to the nest with their partner, particularly if coordination has direct fitness benefits for offspring (Raihani *et al*. 2010). Synchronised arrival at the nest may serve a similar purpose to alternation in mutually ensuring that parental effort is reciprocated, or may function as a display of effort performed for one's partner, signalling an individual's willingness to care for joint offspring, similar to the 'pay to stay' hypothesis in cooperative breeders (Kokko *et al*. 2002). The negative correlation seen here between male and female alternation is surprising, because in conditional cooperation theory, the more one parent satisfies the condition of having cooperated then the more the other should do so (Johnstone *et al*. 2014). A negative correlation was also found between male and female synchrony, which may be indicative of coordination driven by conflict, rather than cooperation. If, for example, females benefit from synchrony as it allows them to monitor investment by their partner, while males lose from synchrony as they are less able to exploit their partner, it may be expected that the more females attempt to achieve synchrony, the more males strive to avoid it. However, if this really were the case, it seems unlikely that the observed levels of male synchrony would be so far above those expected from random provisioning. More studies looking at coordination by each sex, rather than the pair as a whole, are necessary to develop this idea further.

Coordination of care activities has been shown to occur in cooperatively breeding species (Raihani *et al*. 2010; Shen *et al*. 2010), and in biparental species with long-term pair bonds (Black 1996; Mariette & Griffith 2012, 2015), and here we show that it is important even in a biparental species where pairs typically only mate together once, the blue tit. Blue tit parents coordinate their provisioning efforts by alternating and synchronising visits to the nest. That these events occurred significantly more often than can be accounted for by chance demonstrates that parents do not perform nestling provisioning independently of one another, but instead monitor their partner's provisioning and adjust their own behaviour accordingly.

4.0 Overall discussion

The aims of this thesis were to investigate two aspects of blue tit parental care, which were faecal sac removal and parental coordination, within the context of parental predation risk. To achieve these goals, I: produced an analysis of some of the factors influencing sanitation rates; experimentally tested the novel hypothesis that nest sanitation rate is sensitive to parental risk of predation; tested the conditional cooperation theory (Johnstone *et* al. 2014) by comparing observed alternation to that expected by chance, under ambient and elevated parental predation risk; devised a new method for measuring provisioning synchrony, and implemented it under conditions of ambient and elevated parental predation risk.

While the risk of nest predation has been widely discussed as a major force in shaping the evolution of parental care (Ricklefs 1969; Martin 1995; Lima 2009), the effects of predators targeting parents themselves have rarely been considered, even though this can clearly alter the balance between costs and benefits of parental investment. The 'predation risk hypothesis' predicts that, based on costs of reduced crypticity and aerodynamic capabilities, as well as increased energy expenditure, which may logically be associated with faecal sac removal, parents will remove fewer faecal sacs when they are at a greater risk of being predated upon. My results supported this hypothesis, although nest sanitation may have been indirectly managed through provisioning rate, which also decreased under parental predation risk, such that the number of faecal sacs removed per feed did not change. These findings, as well as brood size and provisioning rate being significant predictors of sanitation rate, support the notion that faecal sac removal is an instantaneous compulsion in parent birds, and as such is determined primarily by brood size and provisioning rate (Herrick 1900; Thomson 1934; Smith 1942, 1943; Guiegueno & Sealy 2012; but see Gow *et al.* 2015). It is important to highlight that measuring provisioning rate alone omits effects of load size, which can be variable, even in single-prey-loading species (Van Balen 1973; Nour 1998), and may greatly affect rate of faecal sac removal (Bańbura *et al.* 2001). In future studies, a measure of the mass of food provisioned to nestlings, rather than simple provisioning rate, may prove useful when differentiating between effects of provisioning and predation risk.

The fact that provisioning alternation was more frequent than could be expected by chance in both males and females provides strong support for the theory of conditional cooperation (Johnstone *et al*. 2014), and my results were similar to those seen in great tits (Johnstone *et al*. 2014), with the exception of a sex difference in that female blue tits alternated on a greater proportion of visits than males. Conditional cooperation may prove an incredibly important theory to the study of parental care, as it is the first theory of its kind to successfully model resolution of parental conflict from a perspective of cooperation. While this study provides a valuable addition to the scant research on this topic, more studies of conditional cooperation are necessary to fully understand how this theory applies to real-world animals.

Where in previous studies seemingly arbitrary thresholds for synchronous inter-visit intervals have been applied (Mariette & Griffith 2012, 2015; Bebbington & Hatchwell 2016), here I have justified a measure of synchrony, which is derived from the variable provisioning rates of individual blue tit parents. Future studies of provisioning synchrony in animals with rapid provisioning rates may benefit from employing similar techniques. Levels of synchrony in this study were relatively low compared to the highly coordinated zebra finch (*Taeniopygia guttate*; Mariette & Griffith 2012, 2015), and some cooperative breeders (Martin *et al*. 2000; Fontaine & Martin 2006; Raihani *et al*. 2010), but were higher than seen in long-tailed tits (*Aegithalos caudatus*; Bebbington & Hatchwell 2016), and were several times those expected if both parents were provisioning randomly. This demonstrates the importance of determining a proper baseline for what can be considered synchronous. For example, although Bebbington & Hatchwell (2016) concluded that their long-tailed tits were not particularly synchronous, by inputting values interpreted from their data into my formula, I suspect that their synchrony threshold of one minute was in fact overly stringent, and a re-analysis may reveal higher levels of synchrony.

By showing that blue tit parents reduce faecal sac removal, and females increase synchrony, in the presence of a predator which can only pose a threat to adults, I have provided evidence for parental predation risk as a selective pressure influencing the evolution of parental care.

5.0 References

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