Cracking egg investment: Maternal investment in cuckoos and their hosts

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Declaration

All work in this thesis is my own original work except where credit is given to other contributors in the text. I declare that no part of this work has been submitted previously for assessment for a doctoral degree.

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Photo credit: Swimming Wombat

Abstract

Maternal investment is important for the growth and survival of offspring. Maternal investment is influenced by the environment of the mother, including both the physical environment (the climate), and the social environment (the number of individuals that will care for the offspring). Females may vary their investment in offspring relative to environmental conditions to optimise the trade-off between investment in current and future offspring. This thesis examines how environmental factors affect maternal investment and the impact of maternal investment strategies on offspring, using the superb fairy-wren *Malurus cyaneus* and a brood parasitic cuckoo that exploits them, the Horsfield's bronze-cuckoo *Chalcites basalis,* as a model system.

In the second and third chapters of my thesis, I used superb fairy-wrens as a study species. Superb fairy-wrens are an ideal species to investigate maternal investment as their egg size varies according to the climatic and social conditions. The second chapter of this thesis presents a field experiment that explored the mechanism underlying adaptation of egg size to the environment. Using a food supplementation experiment, I ascertained that food availability is a limiting factor in egg investment in superb fairy-wrens. The third chapter investigated the impact of maternal investment in egg size on chicks, through a whole-clutch cross-fostering experiment. I found that egg investment had a positive effect on chick growth, larger eggs size buffered chicks against extreme conditions, and females breeding in groups tailored their post-natal investment to their pre-natal investment.

In the fourth and fifth chapters of my thesis I investigated an atypical method of maternal investment using the primary brood parasite of superb fairy-wrens, the Horsfield's bronze-cuckoo. Brood parasitic cuckoos are an interesting case study for maternal investment as their entire investment is in their eggs, which they dump in the nest of a host to be raised exclusively by the host. In addition, cuckoos face selection pressure from their hosts, because raising cuckoos is costly for hosts and selects for host

defences against parasitism, including rejection of foreign eggs. Thus, cuckoos face the competing selection pressures of maximising investment in their offspring, whilst minimizing detection of the foreign egg by the host. The fourth chapter of this thesis explores these competing selection pressures on cuckoo egg investment and concludes that the need to evade detection by hosts exerts the strongest selection pressure on Horsfield's bronze-cuckoo egg morphology. Moreover, the size of cuckoo eggs doesn't show the same fine-tuned adaptations to climate and social circumstances as their hosts.

Another complicating factor for migratory Horsfield's bronze-cuckoo maternal investment is coordinating arrival at the breeding site with the breeding season of their host. Climate change has made this relationship more precarious by increasing the likelihood of phenological mismatches between cuckoos and their hosts. Chapter 5, using a long-term dataset of cuckoo and host breeding dates, found little evidence of a phenological mismatch between Horsfield's bronze-cuckoos and their superb fairy-wren hosts. However, trends suggest there is potential for a mismatch in the future and negative consequences for cuckoo breeding success associated with this mismatch.

Thesis Outline

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Chapter 2: Food availability and egg investment: a food supplementation experiment in superb fairy-wrens.

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Chapter 3: Maternal investment in eggs, helpers and temperature influence nestling mass gain in cooperatively breeding superb fairy-wrens.

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Chapter 4: How do brood-parasitic cuckoos reconcile conflicting environmental and host selection pressures on egg size investment?

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Chapter 1

General Introduction

Individuals have finite resources to invest, which results in trade-offs between investment in their survival and current and future reproduction (Bernardo 1996). The resources individuals have to invest depend on both their physical (e.g., temperature, rainfall, habitat quality) and social environment (e.g., number of individuals caring for the offspring). The impact of the physical and social environment on maternal investment in offspring has been investigated in a range of taxa from mammals to reptiles and invertebrates. Studies have suggested that variability in the physical environments may select for plasticity in egg size due to adaptive effects for young, in many species such as the oriental fire-bellied toad *Bombina orientalis* (Kaplan 1992) and seed beetles *Stator limbatus* (Fox et al. 1997). In addition, the social environment has been found to be important for maternal investment in cooperatively breeding mammals, insects and fish (Russell and Lummaa 2009), with helpers often allowing females to invest less in their offspring without a detrimental impact to offspring (known as load lightening; Crick 1992; Hatchwell 1999; Langmore et al. 2016), such as in meerkats *Suricata suricatta* (Sharp et al. 2013) and cichlids *Neolamprologus pulcher* (Taborsky et al. 2007). Past research has investigated how in some bird species females vary their maternal investment to the physical and social conditions by modifying their clutch size, egg size or timing of breeding (see Chapter 2; Meijer et al. 1990; Robertson 1995; Russell et al. 2007; Tarwater and Arcese 2017). Maternal investment in egg size is important in many species, but further research is required to understand maternal investment strategies in birds and their consequences within the changing environment.

Maternal investment in egg size is one-way mothers can influence their offspring survival. Egg size investment has important consequences for both mothers and offspring. Egg size has been found to be important for offspring in many species of birds, particularly for their hatching success, chick survival and growth rate (Williams 1994; Mousseau and Fox 1998; Krist 2011). There is great variation within species in egg size, with the biggest egg generally being a minimum of 50% bigger than the smallest (Christians 2002). However, there is generally more variation between individuals then

within individuals (Potti 1993; Smith et al. 1993; Christians 2002), despite this females in some species show adaptive manipulation of egg investment to the environment (Aslan and Yavuz 2010; Langmore et al. 2016). In birds, there is strong evidence for an effect of egg size in the nestling period, however there is less research about whether these effects continue with chick growth and into later life (see Chapter 3; Williams 1994; Krist 2011). Whilst larger egg size seems to be more beneficial to offspring, this comes at a cost to mothers, as large eggs are more costly to produce and may constrain future reproduction or the mother's survival due to the availability of finite resources (Trivers 1974). Evidence that egg production is costly has been found in experimental studies that manipulate the mother's environment (i.e., heated or cooled nest boxes, or ambient temperature; Meijer et al. 1999; Salvante et al. 2007, Yom-Tov and Wright, 1993) and the observed advance of lay date with warmer spring temperatures (Crick et al. 1997; Dunn and Møller 2014). The environmental conditions change the cost of self-preservation of the mother and therefore the resource she has to trade-off between survival and reproduction, with more challenging environmental conditions often resulting in lower or relatively later egg investment. These findings highlight the trade-offs mothers face in allocating resources to maternal investment and self-preservation and survival. Investment anywhere within the viable range of egg size for a species requires trade-offs and has consequences for both the mother and her offspring.

As females have finite resources to invest in eggs, a trade-off might be expected between the size and number of eggs within each clutch, representing a trade-off between egg quality and quantity (Potti 1993; Nager et al. 2000). However, a survey of the literature by Christians (2002) found egg size was not related to clutch size in 64% of studies investigated. Lack (1947) suggested that clutch size is below the maximum number individuals are physically able to produce, which has found some support (Nager et al. 2000). This indicates that clutch size is often related to other factors, such as predation risk (as provisioning could attract attention to the nest, limiting the number of visits by parents; Skutch 1949), the level of care required by the young after hatching

and the parent's ability to provide adequate care for the number of offspring produced (often investigated using cross-fostering experiments, see Chapter 3). As females have finite resources to invest it may be beneficial for them to optimize their egg investment to their environment and the resources available.

The environment, including both the physical and social environment, impact maternal investment in egg size and clutch size (Russell and Lummaa 2009; Savage et al. 2015). The physical environment, rainfall and temperature, can have both direct and indirect effects on egg investment. Direct effects of the environment on egg investment include thermoregulatory costs, which can affect the timing of breeding and the amount of resources that mothers have to allocate to self-maintenance for survival and therefore cannot be used for egg investment (Salvante et al. 2007; Visser et al. 2009). The physical environment can also have indirect effects on egg investment through food availability. Food availability in most species is susceptible to environment, but as the focal species of this thesis are insectivorous birds, the effect of rainfall and temperature on the biomass and activity of insects (Denlinger 1980; Porcelli et al. 2017; Sánchez-Bayo and Wyckhuys 2019) is particularly relevant here, as it influences the food availability and thereby resources of insectivorous bird species. Egg production requires a large amount of protein, which in passerine birds is largely sourced from diet (usually from eating insects), indicating that food availability may constrain egg investment (Meijer et al. 1999). The effect of temperature on egg size has been analysed in many bird species (Järvinen 1994; Nager and Zandt 1994; Robertson 1995; Williams and Cooch 1996; Tryjanowski et al. 2004; Aslan and Yavuz 2010; Langmore et al. 2016) but it is difficult to determine if any effects found are due to direct (such as thermoregulatory costs) or indirect (such as food availability) environmental variables (see Chapter 2). In most species the direct and indirect effects of the environment are tightly correlated. To separate the effect of direct and indirect impacts of the environment on maternal investment in egg size and clutch size, food supplementation experiments have been used (Christians 2002; Ruffino et al. 2014). Food

supplementation experiments dissociate the direct and indirect effects of climate, because females experience the same direct effects (temperature and rainfall), but different indirect effects (food supply, see Chapter 2). Species which have male-only care (Dzialowski and Sotherland 2004) or those that have plentiful food availability (such as frugivores) also provide valuable insights into the effect of the environment on maternal investment, as some effects are naturally uncoupled (i.e., pre-natal and post-natal investment, and resource availability and maternal investment respectively). It is important to distinguish between these mechanisms to fully understand the causal effect of the environment on maternal investment in eggs, particularly to predict future effects of climate change (Visser et al. 2009).

There have been a number of maternal investment strategies proposed as to how females optimize their egg size to their environmental conditions. The 'silver spoon' hypothesis predicts mothers should capitalise on favourable conditions by increased investment in egg size to optimise their offspring's condition above what is usually possible (Grafen 1988). This also requires further additional investment at the nestling stage because larger offspring require more food (Clutton-Brock et al. 1985). Similarly, the head start hypothesis predicts that in good conditions females may produce larger eggs but decrease provisioning levels with less negative impacts on offspring (Savage et al. 2015). In contrast, the 'bigger is better' hypothesis predicts that females should invest more in eggs when conditions are poor, to provide their offspring with a buffer against the adverse conditions and increase their likelihood of survival (Krist 2011).

In addition to the physical environment, the social environment may also affect egg size and clutch size investment (Russell and Lummaa 2009; Savage et al. 2015). Females may vary their investment depending on the source of care and the number of carers her offspring will have. In cooperatively breeding species, helpers contribute to the care of offspring. This gives the females two options; 1) provide the same level of care as they would in a pair and increase the overall level of care for offspring (additive care) or; 2) decrease their level of care, allowing helpers to compensate, and reserve their

resources for self-maintenance or future broods (known as load lightening; Crick 1992; Hatchwell 1999; Langmore et al. 2016).

Both the physical and social environment have been found to influence investment in egg size in cooperatively breeding superb fairy-wrens *Malurus cyaneus* (Russell et al. 2007; Russell et al. 2008; Langmore et al. 2016). Females that breed in groups lay smaller eggs (load lighten) compared to females breeding in pairs in cool conditions. This increases female survival and does not have a detrimental effect on offspring, because helpers compensate for the shortfall in offspring size by increasing the overall provisioning rate (Russell et al. 2007; Langmore et al. 2016). In contrast, in hot and dry conditions females breeding in groups lay larger eggs than those in pairs. This may reflect the ability of females with helpers to invest more in offspring to buffer them against harsh conditions, as a result of conserving resources through load lightening earlier in the breeding season. While there is evidence that superb fairy-wrens adapt their egg size to their physical and social environment, it is unknown what cues trigger these adaptations to the environment. Chapter 2 in this thesis explores whether direct environmental cues (temperature and rainfall) or indirect environmental cues (food availability) are important for superb fairy-wren egg investment using a food supplementation experiment. Furthermore, the impact of the adaptation of egg size to the environment for chick growth and survival, is unknown. Chapter 3 in this thesis investigates the impact of maternal investment in eggs compared to investment at the chick stage using a cross-fostering experiment. In addition, Chapter 3 tests if superb fairy-wren egg size has a lasting effect on chick growth and survival, as few studies have looked at the long-term effects of egg size on offspring (Krist 2011).

Superb fairy-wren nests are preferentially parasitized by Horsfield's bronze-cuckoos *Chalcites basalis* (Langmore and Kilner 2007). Obligate brood parasitic cuckoos lay their eggs in the nest of a host, abandoning their young to be raised entirely by the host (Davies 2000). This makes cuckoos an interesting study species for maternal investment, as their maternal investment is restricted entirely to their eggs, so it may be

particularly important for cuckoos to optimize their egg size to the environmental conditions. While the young of parasitic cuckoos are reared in the same physical and social conditions as their hosts, we don't know if the same maternal investment strategies exist in the brood parasite. In addition, egg investment in parasitic cuckoos is influenced by selection pressure from hosts. Raising a cuckoo is costly for hosts, selecting for the ability to detect and reject cuckoo eggs in hosts (Marchetti 2000; Langmore 2013; Attard et al. 2017). In turn, this selects for cuckoo eggs that mimic those of their host and thereby evade detection (Brooke and Davies 1988; Davies 2011; Spottiswoode and Stevens 2012). Cuckoos are larger than their hosts, so selection for mimicry of host eggs results in relatively small eggs for their body size (Davies 2000). Thus, cuckoos experience competing selection pressure to adapt their egg size to the environmental conditions but also to avoid egg detection by the host through egg mimicry. Chapter 4 explores the effect of the competing selection pressures from the environment and hosts on Horsfield's bronze-cuckoo egg shape and size.

The environment can influence maternal investment, not only through effects on the quality and quantity of eggs, but also by driving the timing of breeding. This effect is becoming increasingly evident as the environment is affected by climate change, which has caused phenological mismatches of many migratory species with their resources (Both and Visser 2001; Møller et al. 2011; Studds and Marra 2011). For example, resident hosts of the migratory European cuckoo *Cuculus canorus* have begun to breed earlier as spring temperatures increase. This has not been matched by earlier migration in the cuckoo, so cuckoos are arriving at the breeding grounds too late to parasitise the nests of their hosts (Møller et al. 2011). In Australia, Horsfield's bronze-cuckoos are also migratory, and are therefore particularly at risk of experiencing mismatches with the timing of breeding of their hosts. This has the potential to detrimentally effect the breeding success of obligatory brood parasitic cuckoos if mismatches with host breeding occur (Saino et al. 2009; Douglas et al. 2010). Chapter 5 will use a long-term dataset to explore if the timing of arrival on breeding grounds in migratory cuckoos shows direction

change over the last two decades, and whether this matches changes in the timing of breeding in hosts. Potential impacts of phenological mismatches with hosts on cuckoo reproductive success are also assessed. This study will extend our knowledge of the impact of climate change on host-parasite interactions, which have been documented in northern hemisphere but are yet to be explored in species in the southern hemisphere (Saino et al. 2009; Douglas et al. 2010; Avilés et al. 2014; Yun et al. 2020).

Broadly, this thesis aims to investigate direct and indirect environmental effects on maternal investment in superb fairy-wrens (Chapter 2) and the consequences of variation in maternal investment in eggs and feeding of chicks for chick growth and survival (Chapter 3). In addition, it explores the competing selection pressures on the maternal investment of Horsfield's bronze-cuckoos (Chapter 4) and assesses if a mismatch is occurring between the timing of breeding of these migratory cuckoos and their resident hosts (Chapter 5). Furthermore, it investigates whether the timing of breeding of the cuckoo relative to the host affects cuckoo breeding success (Chapter 5). This thesis will 1) contribute to an understanding of the extent to which maternal investment in egg size has carry-over effects throughout the offspring's lifetime, 2) determine the relative importance of direct and indirect environmental effects on maternal investment in superb fairy-wrens, 3) decipher whether environmental or host selection pressure exerts a greater influence on cuckoo egg morphology, and 4) determine if a phenological mismatch is occurring in the timing of breeding of migratory cuckoos and their resident host, and whether this has an impact on cuckoo reproductive success. Each chapter of this thesis was prepared as a stand-alone piece for publication, as such there may be some overlap in the content of the chapters.

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Chapter 2

Food availability and egg investment: a food supplementation experiment

in superb fairy-wrens

This chapter details the food supplementation experiment I conducted on superb fairy-wrens during one field season. NEL and CJT conceived the idea together, CJT conducted the experiment, analysis, wrote the manuscript and both authors contributed to revising the manuscript and approved the final manuscript.

Food availability and egg investment: a food supplementation experiment in superb fairy-wrens

Claire J. Taylor and Naomi E. Langmore

Abstract

Individuals can benefit by varying their investment in offspring. Theory predicts that females may benefit by investing more in offspring either during favourable environmental conditions, because of the relatively greater fitness on offer (the 'silver spoon' or 'head start' hypothesis), or during harsher or more competitive conditions, when extra resources can buffer offspring against survival costs (the 'bigger is better' hypothesis). Female birds often vary their investment in eggs by adapting their egg size, clutch size or lay date to prevailing conditions. However, the precise cues used to manipulate maternal investment and the mechanisms that drive these manipulations are often unknown. Cooperatively-breeding superb fairy-wrens (*Malurus cyaneus*) have previously been found to adapt their egg size to climatic and social conditions. Females with helpers produce smaller eggs than females without helpers in cool conditions, but larger eggs in hot conditions. Superb fairy-wrens also produce larger eggs in dry conditions, which is predicted to buffer chicks against insect shortages. However, the mechanism underlying these adaptations is unknown. During a drought year, we artificially replicated the abundant food supply typical of wet conditions, using a food supplementation experiment, to determine if food availability is the limiting factor in superb fairy-wren egg investment. We found that superb fairy-wrens didn't advance their lay date or increase their clutch size with supplemental food. However, females with supplementary food produced significantly heavier eggs. This supports the silver

spoon or head start hypothesis, and suggests that food availability is a limiting factor in egg investment in superb fairy-wrens.

Key words: Maternal investment, food supplementation, egg size, clutch size, breeding phenology

Introduction

In many bird species, females vary their investment in eggs within and among breeding seasons (Hiom et al. 1991; Christians 2002; Hargitai et al. 2005; Horvathova et al. 2012). Variation in investment may take the form of modifications to clutch size or egg size, or breeding for a longer period of time (Meijer et al. 1990; Robertson 1995; Russell et al. 2007; Tarwater and Arcese 2017). Several hypotheses have been proposed that suggest ways in which females may vary their investment at both the pre-natal and post-natal stages of their offspring development to suit the environmental conditions (Grafen 1988; Verboven et al. 2003; Bolund et al. 2009; Krist 2011; Table 1). First, two hypotheses predict that females should increase their investment in favourable conditions. The 'silver spoon' hypothesis predicts that females will invest more at the pre-natal stage and thereby produce offspring of a higher quality than is usually accessible (Grafen 1988; Savage et al. 2015), but this may also require greater investment at the post-natal stage. The 'head start' hypothesis predicts that females will invest more at the pre-natal stage to give their offspring a 'head start', thereby decreasing the consequences of reduced provisioning at the post-natal stage (Savage et al. 2015). The 'silver spoon' and 'head start' hypotheses both predict high investment at the pre-natal stage in favourable conditions but differ in investment levels at the post-natal stage (Table 1). Alternatively, the 'bigger is better' hypothesis predicts that females should increase investment in their eggs when conditions are unfavourable, to buffer their offspring against the harsh conditions and increase the likelihood of their

survival (Krist 2011). In addition to the impacts of the physical environment on female investment, the social environment, such as the presence of helpers in cooperatively breeding species, may also influence the maternal investment strategy (Russell et al. 2007).

Table 1. Hypothesised strategies for pre- and post- natal maternal investment to adapt to the environmental conditions, a brief explanation of the hypothesis (rational) and a quick practical guide to of differences between the hypotheses in birds.

Hypothesis	Environment conditions	Investment Pre-natal	Post-natal	Rational	Differentiate
Silver-spoon	Good	High	High	Capitalise on good conditions	Large eggs in good condition, high feed rate of chicks
Head start	Good	High	Low	Invest more in eggs to invest less in chicks	Large eggs in good conditions, low feed rate of chicks
Bigger is better	Poor	High		Invest a lot to increase chance of survival	Large egg in poor conditions

While studies have found support for all of these hypotheses, it is less clear what cues females use to determine the optimal investment strategy. Females may respond to climatic cues such as rainfall and temperature, which are good predictors of future food availability (Denlinger 1980; Zann et al. 1995; Hidalgo Aranzamendi et al. 2019). By timing egg laying to coincide with periods of rainfall and favourable temperatures, females may ensure that the eggs hatch when food is most available for chicks (the 'anticipation' hypothesis; Lack 1954). Alternatively, variation in investment may be constrained by female body condition, such that females invest in smaller eggs and/or smaller clutches at times when food availability has been low, causing females to have fewer reserves to dedicate to egg production (constraint hypothesis; Perrins 1970). Egg production requires large amounts of protein and, as protein is largely sourced from diet in passerine birds, food availability may impact maternal investment in eggs (Meijer and Drent 1999). The constraint hypothesis, where females wait for an increase in insect biomass to begin breeding or invest less when resources are scarce, may be more adaptive in species where the time lag between egg laying and egg hatching is short, as food availability during egg laying is likely to be a good predictor of food availability at

the time the nestlings hatch. Maternal investment may also be affected by the trade-off between investment in young and the survival of the mother, as well as the opportunity for future maternal investment (Bernardo 1996).

Here we aim to determine the factors underlying egg investment in insectivorous superb fairy-wrens, by disassociating the effects of climate and food availability experimentally. Superb fairy-wrens are cooperative breeders, in which a breeding pair are sometimes assisted in rearing young by helpers (Cockburn et al. 2016). Egg investment in superb fairy-wrens varies in relation to the environmental and social conditions of the breeding female (Russell et al. 2007; Langmore et al. 2016). Females with helpers produce smaller eggs in cool conditions, and larger eggs in hot conditions, than females without helpers. Cooperative breeding has been suggested to be strategy to buffer the costs of breeding in highly variable environments (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011), we were unable to explore this in our study due to the low number of groups with helpers. Furthermore, females lay larger eggs with decreasing mean rainfall (consistent with the bigger is better hypothesis; Langmore et al. 2016). It is predicted that bigger egg size in hot, dry conditions buffers chicks against a shortage of insect prey, as insect biomass declines in dry conditions and extreme heat (Denlinger 1980; Woinarski and Cullen 1984; Porcelli et al. 2017; Sánchez-Bayo and Wyckhuys 2019). Conversely, rainfall during the sensitive period before egg laying may allow females to invest relatively less in egg size as they will have a reliable food source to provision chicks after hatching. The time window during which rainfall was associated with superb fairy-wren egg size was found to be the 20-59 days prior to egg laying (Langmore et al. 2016). This period is likely to be influential because it allows sufficient time after rainfall for insect abundance to increase, and for this to influence female body condition, prior to egg laying (Denlinger 1980; Langmore et al. 2016). It is also likely to result in a greater insect biomass for provisioning of chicks, because the positive effect of rainfall on superb fairy-wren chick body mass was found to have at least a two-week lag (Kruuk et al.

2015). Similarly, in zebra finches, *Taeniopygia guttata*, a delay was found between rainfall and the beginning of breeding, thought to align breeding with grass seed availability (Zann et al. 1995). However, these studies are correlative, and to establish a causative relationship between food availability and egg size, an experimental study is needed.

Superb fairy-wrens have a long breeding season, usually running from September to February, but can begin as early as late August and extend to late March (Cockburn et al. 2016). An earlier study on superb fairy-wren breeding season start times and duration, over five breeding seasons, found that higher than average rainfall advanced the start date and increased the duration of the breeding season (Nias and Ford 1992). However, a more recent study which analysed almost 30 years of data, found start date of breeding of superb fairy-wrens advanced with higher minimum temperatures in early spring, which also resulted in a longer breeding season duration, but rainfall was not found to have an influence at this irrigated study site (Lv et al. 2020). Breeding seasons were found to have a shorter duration and end earlier when there was less rainfall and when there were more heatwaves in late summer (Lv et al. 2020). Lv et al. (2020) proposed that lower minimum temperatures in early spring may constrain the start of breeding due to low food availability in these conditions, but this is yet to be tested. In addition, as the site in Lv et al. (2020) is irrigated superb fairy-wrens breeding here may be influenced differently by rainfall than a study site, such as the one in this paper, that is not irrigated, warranting further investigation.

Food supplementation experiments have been used to explore if female birds produce larger eggs or larger clutches, or start breeding earlier when more food is available. Many studies have found an advance in lay date and an increase in clutch size with supplementary feeding, with varying evidence of improved breeding success (Meijer and

Drent 1999; Robb et al. 2008; Schoech et al. 2008; Harrison et al. 2010). There is also conflicting evidence about the effect of supplementary feeding on egg size, with some studies finding an increase (Hiom et al. 1991; De Neve et al. 2004) and others finding no effect (Ruffino et al. 2014) or inconclusive evidence (Christians 2002). To test whether superb fairy-wrens vary breeding phenology, clutch size, or egg size in relation to food abundance, we used a food supplementation experiment during a drought year (the fifth driest and the second hottest spring on record; Bureau of Meterology 2020), when background food availability was likely to be low (Denlinger 1980). Some fairy-wren groups received food supplementation on alternate days, while others were not supplemented. We predicted that, if females use climatic cues to modify their investment in eggs, there will be no difference in egg investment or timing of breeding between supplemented and unsupplemented females. Conversely, if egg investment is determined by food availability or female body condition, supplemented females will invest more than unsupplemented females and this effect will be more pronounced in females that have received supplementary food for longer. Finally, if females vary their investment in relation to resource availability at the time of egg laying, supplemented females will invest more in eggs than unsupplemented females, irrespective of how long they have received supplementary food. This experiment also allows us to test predictions of the 'silver spoon', 'headstart' and 'bigger is better' hypotheses. The silver spoon and head start hypotheses predict that supplemented females will invest more in offspring than unsupplemented females to capitalise on the favourable conditions. By contrast, the 'bigger is better' strategy predicts that unsupplemented females should invest more in offspring than supplemented females, to buffer their chicks during harsh conditions. If food availability influences the commencement of breeding, supplemented females should also commence breeding earlier than their unsupplemented counterparts.

Methods

Study species

Superb fairy-wrens are small insectivorous passerines that breed both cooperatively, in groups with helpers (usually male offspring from previous broods and most often only one helper at the study site), or as pairs without helpers. The breeding season typically runs from September-February, however during our study (during the 2019 breeding season) superb fairy-wrens stopped breeding at the end of December (Lv et al. 2020). They can have up to nine nesting attempts with up to as many as three clutches fledging, however it is more common to have only one successful brood annually, if any, due to a high depredation rate (Langmore et al. 2003; Cockburn et al. 2016). Superb fairy-wrens lay clutches ranging from one to five eggs, with most clutches consisting of three eggs (Cockburn et al. 2016). Breeding seasons with higher rainfall have proportionately more clutches with four eggs than three eggs (Nias and Ford 1992). This is thought to be due to the positive relationship between food availability and rainfall (Nias and Ford 1992), but this requires further investigation. While previous studies have revealed that females vary their egg investment in relation to the presence of helpers in their group (Russell et al. 2007; Langmore et al. 2016), we were unable to explore this aspect of their investment as there were insufficient groups that had helpers during the study period. Only 5 of the 29 territories (17% of all territories) had at least one helper and 2 of these territories with helpers (7% of all territories) received supplementary feeding. All nesting attempts of females in the study population were monitored, subsequent nests were found when nests were depredated or fledged providing high certainty that attempts were monitored. During the 2019 breeding season 45 nest were built and laid in, compared to an average of 94 nest per season over 13 seasons (1999, 2000, 2001, 2002, 2006, 2011, 2012, 2013, 2014, 2015, 2017, 2018, 2019; range 45-155 nests).

Study site

The experiment was conducted from July to December 2019 in an open eucalypt woodland in Campbell Park, Canberra in south-eastern Australia (149°10'E, 35°16'S;

Langmore et al. 2016). Most individuals were colour-banded for identification and territories and group sizes were mapped during July – September. Breeding activities of all females were monitored daily or every second day until nest building commenced and once nest building commenced, nests were checked every three days.

Food supplementation experiment

Feeders were established in 14 out of 29 territories from July – September (Figure 1). To reduce the possibility that unsupplemented females might visit feeders on the territories of supplemented females, the study site was divided into treatment and control zones (Figure 1). These zones were separated by areas of habitat that were unsuitable for superb fairy-wrens as they were not found in these areas.

Feeders consisted of a plastic saucer resting on rocks above a dish filled with water. The water prevented ants from accessing the food and also provided experimental individuals with access to water. The feeder was surrounded by a wire cage with 5cm mesh, so that the superb fairy-wrens could easily access the feeder but larger birds were unable to gain access. This provided protection from predators for the study species and limited the amount of food lost to other species. Rocks and sticks were placed in the feeder for the birds to perch on. The feeders were placed in areas that the individuals frequented and always next to low vegetation so that individuals could flee to safety if they felt threatened. Feeders were replenished every second day with mealworms *Tenebrio molitor* (three per bird in the group) and insectivore mix (a mixture of Vetafarm Insecta Pro and boiled egg with a ratio of 20g insectivore mix to 50g boiled egg, as per packet instructions, mashed together into small pieces). Each feeder was monitored throughout the breeding season to determine if it was being used by the superb fairy-wrens. Females were classified as using the feeder when they had been observed taking food from the feeder at least once. However, in practice, feeder usage was very obvious, because once individuals (both females and males used the feeder) discovered a feeder

they were almost always waiting nearby when the researcher replenished the food and came in to feed as soon as the researcher moved away. While mealworms were usually consumed very quickly, the insectivore mix was observed to be eaten at a slower rate and some remained when the feeders were restocked, suggesting supplementary food was constantly available. Female condition was not measured during this study, however the probable low background food availability suggests supplementary food was likely to be beneficial and has been found to improve female body condition in other studies (Schoech 1996; Verboven et al. 2003). Females that were never observed to use their feeder were included in the control group (N=5 females), these females, and all other superb fairy-wrens on these territories, never seemed to recognise the feeder as a food source and didn't enter it. We used linear mixed models to confirm that there was no significant difference in traits between females that did not use feeders and those that did not have feeders (supplementary material). The control group did not receive any supplementary food or water (N=15 females, therefore the total number of control females including those that didn't use their feeders=20).

Previous studies (including one on superb fairy-wrens; Russell et al. 2007) have shown that egg weight and volume are highly correlated, and that both are positively related to the nutritional content of the egg (OnbaŞilar et al. 2011; Hegab and Hanafy 2019). We measured the eggs from every nesting attempt of every female for the entire breeding season. Egg length and breadth were measured using Vernier callipers (±0.1 mm) and eggs were weighed using electronic scales on day two of incubation. The egg volume was calculated using Hoyt's formula (Hoyt 1979). An average of the egg volume and egg weight measurements was calculated for each clutch. The observer was not blinded to the treatments when measuring eggs, however great care was taken with every measurement and the scales were electronic meaning weight measurements were not subjective. Daily maximum temperature and rainfall measurements were collected by the Australian Bureau of Meteorology (http://www.bom.gov.au/climate/data/) at a weather station approximately 4km from the study site. From these measurements we

calculated the sum of the rainfall in the 20-59 day period and the mean maximum temperature in the 12-2 day period before the first egg of the clutch was laid, as these periods have previously been found to influence egg volume (Langmore et al. 2016). The lay date was recorded as the date the first egg was laid by each female. This is not always exactly representative of when females are first ready to breed, because some
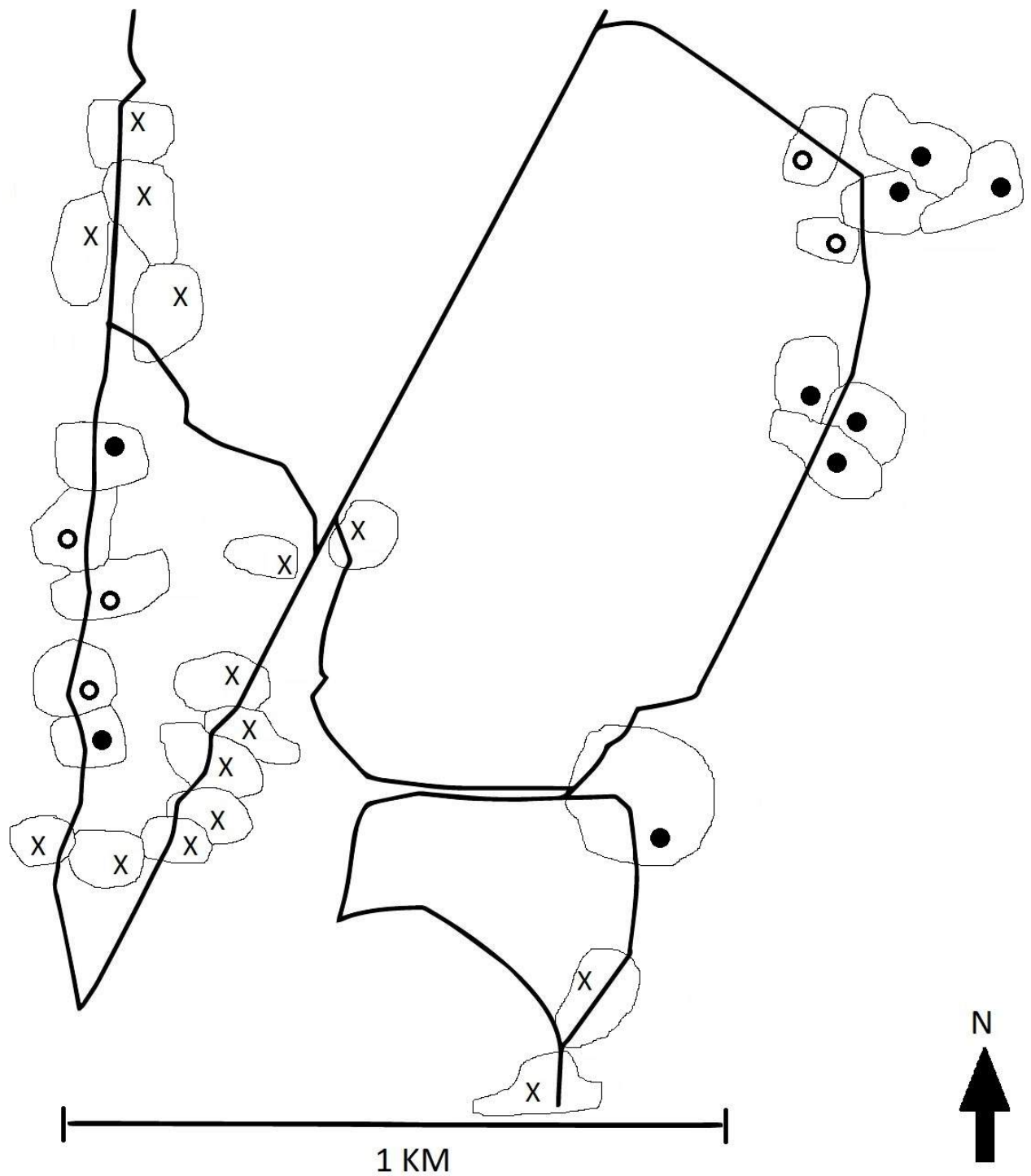
Figure 1. Map of Campbell Park showing superb fairy-wren territories (irregular circles) and the principal tracks through the study site (solid lines). Territories that were provided with supplemental food are indicated by a filled circle, territories that weren't provided with supplemented food are indicated by a cross and those that were provided with supplemental food that did not use the feeders are indicated by an unfilled circle.

first nests are destroyed before they are laid in. However, it is the least arbitrary measure, as the time to build the first nest and between nest building and laying is variable for the first nest. This research was conducted under approval of the Australian National University Animal Ethics Committee Protocol Number A2017/35 and ACT Government Licence number LT20187.

Statistical analysis

Breeding Phenology

We investigated whether superb fairy-wrens advance their lay date (the number of days after the first of September that the first egg of the first clutch was laid by each female [N=25, range =20-76 days]) when they are provided with supplemental food (fed [N=8] or unfed [N=17]). We were unable to determine the lay date of the first egg of the first nest for three females, and there was a possible 5-day period for the lay date of one female, so they were excluded from this analysis. When there were two possible days for the exact lay date the earliest date was chosen (N=3 females). There was no significant difference in variance ($F_{7,16}$=1.46, P=0.50) so an equal variance two sample t-test was used.

Egg size

To investigate whether food supplementation and climate had an effect on egg size we used a linear mixed model (LMM) with Restricted Maximum Likelihood (REML). Egg volume (N=44) and egg weight (N=22) were the response variables tested in separate models. Sample sizes for egg volume and weight differ because only eggs weighed on day two of incubation were included, this is to account for egg mass decreasing through incubation due to evaporative water loss (Hoyt 1979). The fixed effects for both models were 'fed' (yes [egg volume N=13; egg weight N=8] or no [egg volume N=31; egg weight N=14]), 'rainfall' (sum of rainfall in 20-59 days prior to egg laying), 'temperature' (average maximum temperature in the 12-2 days prior to egg laying) and 'julian date for

day 2 of incubation of eggs' to account for seasonality. All fixed effects were scaled. The random effect was mother ID (as some mothers had multiple clutches [egg volume $N=29$; egg weight $N=17$]).

An LMM REML was used to investigate whether egg volume ($N=42$) varied with clutch size. Egg volume was the response and clutch size (2 eggs $N=8$, 3 eggs $N=30$, 4 eggs $N=4$) was the fixed effect. Mother ID was included as a random effect to account for multiple clutches from some females ($N=28$). We used a linear model (LM) to investigate whether the number of days between when the female first ate supplementary food and when she laid the first egg of the clutch affected egg volume. Clutch average egg volume ($N=13$) was the response and the number of days between adopting the feeder and egg laying was the fixed effect. Three females had multiple clutches ($N=7$ clutches), however mother ID wasn't included as a random effect in the final model due to the small sample size making accurate prediction of both fixed and random effects difficult. Prior analysis with the inclusion of this random effect, indicated exclusion in the final model did not alter the overall outcome of the analysis.

Clutch size

To test whether clutch size varied in relation to food supplementation, a generalised linear mixed model (GLMM) with quasi-Poisson distribution (with a log link function) to account for under-dispersion in the count data was used. Clutch size ($N=42$; 2 eggs $N=8$, 3 eggs $N=30$, 4 eggs $N=4$) was the response and treatment (fed $N=11$ or unfed $N=31$) was the fixed effect. The random effect was mother ID to account for multiple clutches from some females ($N=28$; 16 females with 1 clutch, 10 mothers with 2 clutches and 2 mothers with 3 clutches). Two clutches from two different females (one clutch from each female, two clutches total) were excluded as the females were depredated during the egg laying period, so the clutches may have been incomplete.

To investigate whether the number of days between when the female first ate supplementary food and when she laid the first egg of the clutch affected clutch size, we used a GLMM with quasi-Poisson distribution (with a log link function) to account for under-dispersion in the count data. Clutch size (N=11) was the response, the number of days between adopting the feeder and egg laying was the fixed effect and mother ID was included as a random effect (N=8).

All analyses were conducted using the statistical package R (R Core Team, 2019). LM and LMM's were conducted using the lme4 package (Bates et al. 2014) and the GLMM's were conducted using glmmTMB package.

Results

Breeding Phenology

Lay date of the first egg did not differ between females that were provided with supplementary food before the beginning of the breeding season and females that were not (two sample t-test: t_{23}=-0.65, P=0.52; Figure S1). However, females that were provided with supplementary food laid their first egg an average of two days earlier than unsupplemented females (average lay date ± SE=48 ± 5.70 days after first September for fed females, 50 ± 3.52 days for unfed females).

Egg size

Supplemented females laid significantly heavier eggs than unsupplemented females (Table 2, Figure 2). Eggs of supplemented females were on average 0.09g (7%) heavier than those of unsupplemented females (average supplemented female egg weight ± SE=1.46 ± 0.03, average unsupplemented female egg weight ± SE=1.37 ± 0.02g). Although eggs of supplemented females generally also had a larger volume (mean ±

SE=1434.31 ± 25.23 mm^3), this was not significantly different from the volume of eggs of unsupplemented females (mean ± SE=1386.52 ± 13.98 mm^3; Table 2; Figure S2). Neither egg weight nor egg volume differed significantly with rainfall, temperature or julian date (Table 2).

Consistent with previous studies (Russell et al. 2007; Langmore et al. 2016), egg volume did not differ significantly with clutch size (LMM: $F_{1,19.84}$=0.19, P=0.67), but varied significantly with mother ID ($P < 0.001$). The time between the female first using the feeder and laying the first egg of the clutch did not affect the average clutch egg volume (LM: $F_{1,11}$=0.38, P=0.55). Mean date of first egg laid was the 19th of October (SE=2.95, range=27th of September to 15th of November).

Table 2. Summary of the linear mixed model (REML) of egg weight and of egg volume in relation to supplementary feeding (fed), rainfall, temperature and Julian date.

Response term	Fixed effects	Estimate ± s.e	F	n.d.f	d.d.f	P
Egg volume	Fed	26.89±15.73	2.92	1	26.98	0.10
	Rainfall	-2.63±7.61	0.11	1	14.53	0.74
	Temperature	1.95±11.09	0.03	1	16.59	0.87
	Julian date	-0.51±11.58	0.002	1	15.85	0.97
Egg weight	Fed	0.05±0.02	5.25	1	11.70	0.04*
	Rainfall	-0.03±0.02	1.49	1	15.68	0.24
	Temperature	-0.04±0.03	1.51	1	16.89	0.24
	Julian date	0.06±0.03	2.45	1	16.11	0.14
Response term	Random effect	Variance				
Egg volume	Mother ID	6545				
Egg weight	Mother ID	0.002				

This table shows estimate and standard error (s.e), numerator and denominator degrees of freedom (n.d.f and d.d.f respectively) and significance levels with a threshold of 0.05 (*P*) determined using F-statistics (*F*). Significant values are indicated using (*).
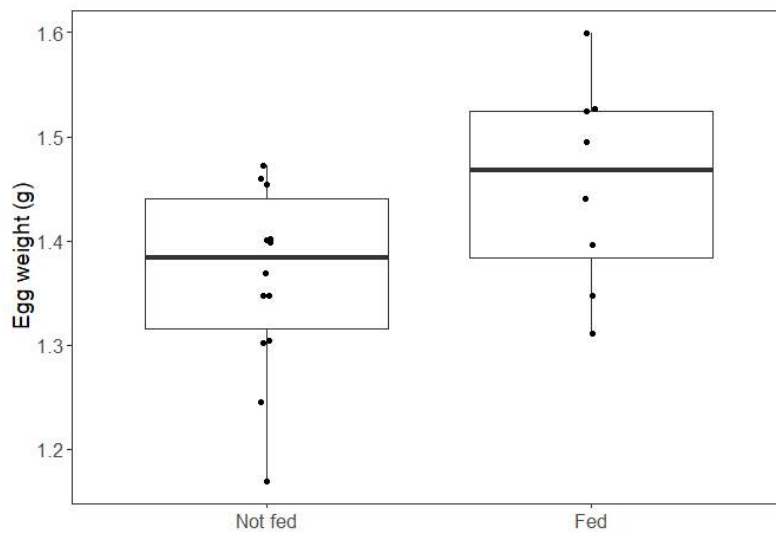
Figure 2. Females fed supplementary food (Fed [N=8]) produced heavier eggs than females that were not fed supplementary food (Not fed [N=14]). The median is indicated by the thick line in each box, minimum and maximum quantiles are shown by the upper and lower lines of each box, the minimum and maximum values are represented by the whiskers and points indicate the data spread.

Clutch size

Clutch size (mean clutch size ± SE=2.90 ± 0.08) did not differ significantly between females that were fed (mean fed clutch size ± SE=2.91 ± 0.16) and those that weren't (mean unfed clutch size ± SE=2.90 ± 0.10; GLMM: X^2_1=0.15, P=0.70; Figure S3). The time between the female first using the feeder and laying the first egg of the clutch did not affect the clutch size (GLMM: X^2_1=0.14, P=0.70; Figure S4).

Discussion

We found that female superb fairy-wrens provided with supplementary food showed some differences in their egg investment compared to females that were not given supplementary food. Female superb fairy-wrens with supplementary food laid heavier eggs. This suggests that variation in egg investment is related to food availability, and

possibly female condition, rather than directly related to climatic cues. Laying heavier eggs with increased food availability also supports the silver spoon or head start hypotheses, as females increased their egg investment in comparably favourable conditions. Superb fairy-wrens did not vary their clutch size or advance their lay date with increased food availability.

The increase in egg weight with supplementary food found in our study suggests that food availability is a limiting factor for superb fairy-wren egg production. Many studies have found no effect of food supplementation on egg size (review by Christians, 2002: no effect in 71% of studies; review by Robb *et al.*, 2008: no effect in 62% of studies; meta-analysis by Ruffino *et al.*, 2014: showed no effect of food supplemntation on egg size). However, the lack of effect of supplementary food in other studies may be due to differing ecological conditions, such as relatively high background food availability (Hiom et al. 1991; Schultz 1991; Ruffino et al. 2014). Our food supplementation experiment was conducted in the context of an extreme drought (the fifth driest and the second hottest spring on record; Bureau of Meterology 2020), suggesting there was low background food availability (Denlinger 1980). Thus, the contrast in food supply between the supplemented and unsupplemented treatments is likely to be greater than in most other studies.

The increase in egg weight with higher food availability indicates that resources from supplementary food were allocated to egg investment (Drent and Daan 1980; Hogstedt 1981; Williams 1996). However, the time between the female first using the feeder and laying the first egg of the clutch did not affect the average clutch egg volume or clutch size. This suggests that either food availability has a limited capacity to influence maternal investment in eggs, or supplemented females reached the nutritional threshold or body condition needed to increase their egg size within a very short time period, or that females varied in the amount of supplementary feeding they needed to increase egg investment due to difference in female quality (Perrins 1970; Schultz 1991). While there

is indirect evidence in our study that food supplementation had a positive influence on female body condition, measuring female condition in future studies would help assess the direct effect of food supplementation on female condition (Podofillini et al. 2019; Schoech 1996; Verboven et al. 2003).

Our finding that females provided with supplementary food produced significantly heavier eggs than those without supplementary food suggests that superb fairy-wrens with supplementary food either capitalised on the opportunity to produce offspring of a quality above what is normally achievable (the 'silver spoon' hypothesis; Grafen 1988; Savage et al. 2015), or buffered their offspring against a potential decrease in food availability in the post-natal stage (head start hypothesis; Savage et al. 2015). Larger egg mass and volume are associated with increased nutritional content in the egg (Hegab and Hefany 2011; Russell et al. 2007), which leads to improvements in hatchability, nestling weight, growth rate, immunity and survival (Krist 2011). This suggests that females use supplementary food to improve the chances of survival of their young, but further study on investment in the post-natal stage is required to differentiate between the two hypotheses (Table 1).

In accordance with our findings for egg weight, females with supplementary food generally produced eggs with a larger volume than those that did not receive supplementary food, although not significantly so. Measurement precision may be greater when measuring egg weight using electronic scales than when measuring egg volume by measuring egg length and breadth using vernier callipers. As superb fairy-wren eggs are very small in size (~16 x 12mm; Langmore and Kilner 2009), achieving precise measurements with callipers is challenging and any small amount of human error could account for the weaker effect for egg volume.

Whilst we did find an increase in egg weight, we did not detect an increase in clutch size, suggesting that superb fairy-wrens increase egg quality but not egg quantity with supplementary food. Evidence for an increase in egg quality rather than quantity with food supplementation has also been found in Ural owls *Strix uralensis* (Karell et al. 2008). As in our previous studies (Russell et al. 2007; Langmore et al. 2016), and consistent with studies in other taxa (Arnold 1992; Christians 2002), there was no evidence of a trade-off between egg volume and egg number. We had insufficient data to test for a relationship between clutch size and egg weight due to not all eggs being weighed on day 2. A trade-off between egg weight and number is yet to be tested for, so cannot be ruled out (Potti 1993; Williams 1996; Nager et al. 2000).

There is conflicting evidence from previous studies as to whether food supplementation increases clutch size. Christians (2002), when considering only statistically significant effects, found clutch size increased with supplemental food in 63% of studies investigated, however Robb et al. (2008) found it was unrelated in 54% of studies and Ruffino et al. (2014) found an effect of food supplementation on clutch size only in birds that cache food, which superb fairy-wrens do not. Consistent with this we found that superb fairy-wrens did not increase their clutch size with supplemental food. This is unsurprising, as while superb fairy-wren clutch size can range between 1-5 eggs it has a strong mode of three eggs (Cockburn et al. 2016), which was also found in this study. Yellow-headed blackbirds *Xamthocephalus xanthocephalus*, also show little variation in clutch size and did not lay larger clutches with supplemental food (Arnold 1992). Our results suggest that food availability is not the mechanism through which increased rainfall has an effect on clutch size, as suggested by Nias and Ford (1992), or that rainfall itself is required to trigger this response.

Superb fairy-wrens did not significantly advance their lay date, even though supplemental food was provided as early as 31st July, with the mean start date of the superb fairy-wren breeding season found in a 28 year study conducted in the same region to be October 18th (Lv et al. 2020), which is a day earlier than the mean lay date in our study, October 19th. While supplemented females laid on average two days earlier than unsupplemented females, this difference is low in comparison to the variation in the lay date for the first egg (SE of 6 days for supplemented females and 4 days for unsupplemented females). Greater variation in lay date than advancement in lay date has been found in other studies (Nager et al. 1997; Ramsay and Houston 1997), and suggests that food availability does not constrain lay date in these species, or only constrains lay date to a point, after which it is subject to the environment (Schultz 1991).

Temperature may have a direct effect on onset of breeding due to thermoregulation costs, rather than through an indirect effect of food abundance (Meijer et al. 1999; Salvante et al. 2007; Visser et al. 2009). Thermoregulatory costs may prevent females from being able to build up sufficient resources to begin breeding, as females require more resources to both breed and maintain body condition in colder conditions (Pendlebury et al. 2004; Salvante et al. 2007). Studies using temperature-controlled aviaries or nest boxes with a warm and cool condition have generally found females in warmer conditions lay earlier (Meijer et al. 1999; Salvante et al. 2007; Visser et al. 2009; but see Nager and Noordwijk 1992). Thermoregulatory costs are likely to be a contributing factor to the timing of breeding in superb fairy-wrens as minimum late winter and early spring temperatures were found to predict the start date of the breeding season (Lv et al. 2020), with earlier lay dates occurring with higher minimum temperatures. Experimental manipulation of temperatures before laying would be ideal for testing if thermoregulation is the important factor for determining lay date.

In conclusion, our study suggests that, during a drought, egg investment in superb fairy-wrens is constrained by food availability, because females supplied with supplementary food laid heavier eggs compared to females who were not. However, food supplementation did not affect clutch size or lay date, suggesting these breeding parameters respond to variables other than the food availability. Instead, direct environmental conditions, such as rainfall, may be more important for clutch size and lay date. Several questions arise from this study which require investigation. Further research is needed to differentiate between the silver spoon and head start hypotheses by looking at investment of females at the post-natal stage to ascertain if they continue to provide a high level of care consistent with the silver spoon hypothesis or decrease their care as predicted by the head start hypothesis. Also, an experimental study investigating the thermoregulatory costs potentially constraining the lay date of superb fairy-wrens would help determine if this is the case. In addition, future studies would benefit from the inclusion of the effect of helpers on maternal investment, as female superb fairy-wren have been found to tailor their investment to helper presence (Russell et al. 2007), this level nuance has likely been missed in our study.

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Supplementary Material

Control females (who did not have a feeder; $N=15$ females, 21 data points) and females with a feeder who didn't used it ($N=5$ females, 10 data points) did not differ significantly in clutch size (LMM: $F_{1,15.82}=0.07$, $P=0.80$; average clutch size of females that used the feeder $=2.91$, that didn't use the feeder $= 2.88$, and that didn't have a feeder $= 2.91$), egg volume (LMM: $F_{1,17.41}=0.07$, $P=0.79$), or egg weight (LMM: $F_{1,8.95}=0.75$, $P=0.41$). The sample size was too low to test for a difference in lay date between control females and those that didn't use their feeder ($N=17$; control $N=13$, feeder but didn't use $N=4$).
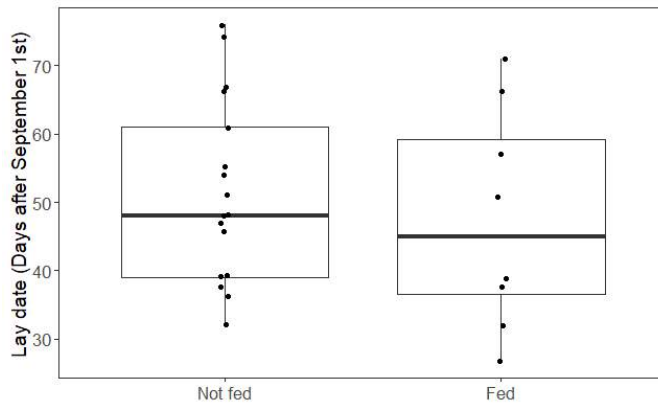
Figure S1. Lay date of the first egg of the season (calculated in Julian days from September 1st) did not differ significantly between females fed supplementary food (Fed [$N=8$]) and females that were not fed supplementary food (Not fed [$N=17$]). The median is indicated by the thick line in each box, minimum and maximum quantiles are shown by the upper and lower lines of each box, the minimum and maximum values are represented by the whiskers and points indicate the data spread.

Figure S2. Egg volume did not differ significantly between females fed supplementary food (Fed [N=13]) and females that were not fed supplementary food (Not fed [N=31]). The median is indicated by the thick line in each box, minimum and maximum quantiles are shown by the upper and lower lines of each box, the minimum and maximum values are represented by the whiskers and points indicate the data spread.
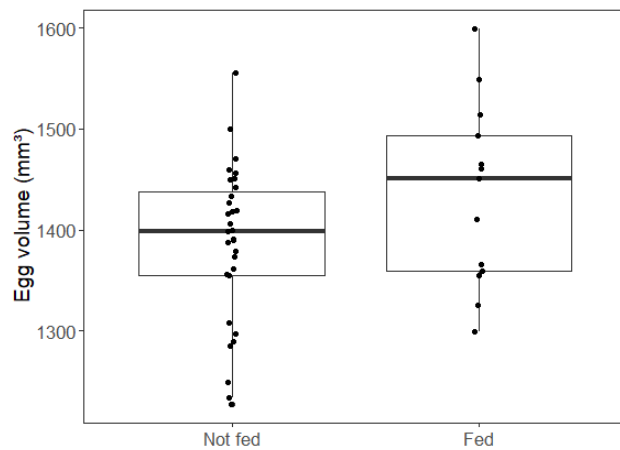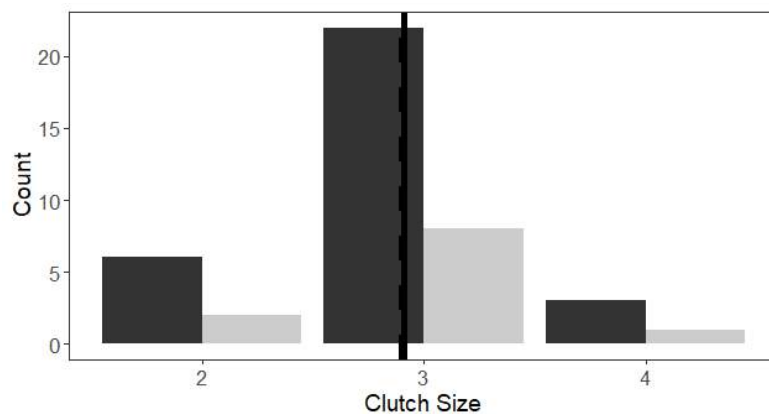

Figure S3. Clutch size did not differ significantly between females fed supplementary food (light grey bars [N=11]) and females that were not fed supplementary food (dark grey bars [N=31]). The mean clutch size for each treatment is indicated by the vertical lines (fed females is indicated by the filled line and unfed females by the dashed line).
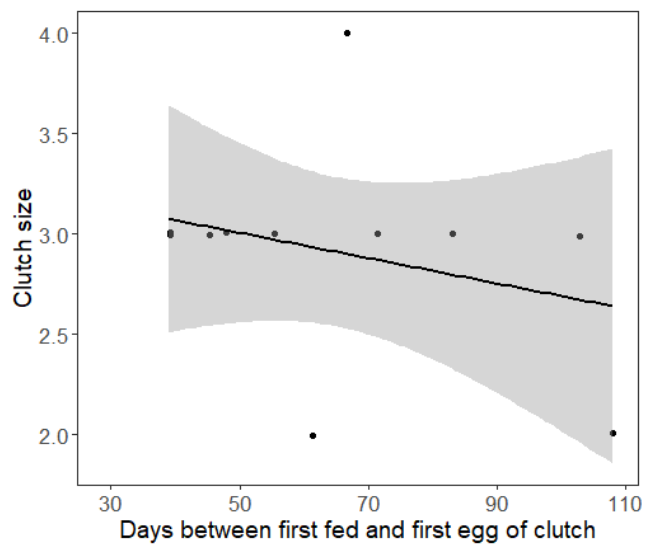
Figure S4. Clutch size was not significantly affected the number of days between the female first using the feeder and laying the first egg of the clutch. The trend line is shown in black, and the standard error is indicated by the grey shading.

Chapter 3

Maternal investment in eggs, helpers and temperature influence nestling mass gain in cooperatively breeding superb fairy-wrens.

This chapter details the cross-fostering experiment I conducted on superb fairy-wrens over four breeding seasons. NEL and CJT conceived the idea together, CJT conducted the experiment, analysis, wrote the manuscript and both authors contributed to revising the manuscript and approved the final manuscript.

Maternal investment in eggs, helpers and temperature influence nestling mass gain in cooperatively breeding superb fairy-wrens.

Claire J. Taylor and Naomi E. Langmore

Abstract

Optimal strategies for maternal investment in young are often influenced by the physical and social environment. The physical environment, such as temperature and rainfall, affect the resources individuals have to invest, whereas social conditions may influence how many carers offspring have. Here we investigate the influence of the social and physical environment on maternal investment in cooperatively breeding superb fairy-wrens *Malurus cyaneus*, using a cross-fostering experiment to decouple the effects of pre- and post-natal investment on offspring growth and survival. We cross-fostered clutches of eggs between synchronous nests of pairs and groups, throughout the varying climate of the five month breeding season. We found that egg volume was positively correlated with nestling mass and growth rate. Heat waves and the average maximum temperature during the nestling period interacted with egg size to affect chick mass gain, such that chicks that hatched from larger eggs were more buffered against extreme temperatures. Investment strategies differed between females breeding in pairs versus groups; females in groups tailored their post-natal investment in chicks to their prior investment in eggs, whereas females that lacked helpers showed relatively constant post-natal investment regardless of their prior egg investment. There was a non-significant tendency for offspring that hatched from larger eggs to have a greater probability of surviving to the next breeding season. These results extend our understanding of the benefits of cooperative breeding, indicating that the assistance

provided by helpers allows females to more finely modulate their investment in eggs and nestlings to the prevailing environmental conditions.

Keywords: Maternal investment, egg size, cross-fostering, climate, temperature, nestling mass, nestling survival

Introduction

Individuals allocate finite resources in a series of trade-offs to optimise their survival, growth and reproduction within their environment (Bernardo 1996). Environment can refer to an individual's physical environment such as climatic conditions, or an individual's social environment such as breeding group dynamics, or both. Allocation of maternal resources in offspring represents a trade-off between investment in current offspring, future offspring and self-maintenance (Williams 1996). The optimal solution to this trade-off is influenced by the individual's environmental context, both physical and social (Smith and Fretwell 1974). Physical climatic conditions such as temperature and rainfall have been shown to impact the resources available to individuals and the resulting energy they have available to invest. Social conditions can impact the amount and source of care the offspring will receive (Russell and Lummaa 2009).

The physical environment impacts parental and offspring condition both directly, by affecting thermoregulation costs to individuals, and indirectly, through its influence on resource availability such as food (King and Farner 1961). For example, in insectivorous birds, rainfall and temperature have been shown to influence the biomass and activity of insects (Denlinger 1980; Woinarski and Cullen 1984; Winkler et al. 2013), which in turn influence female body condition (Selman and Houston 1996). Female body condition is, in turn, positively related to propagule size (Fox and Czesak 2000; Christians 2002; Kvalnes et al. 2013; Podofillini et al. 2019). The way in which resource availability

translates to individual resource acquisition dictates the amount of energy individuals have available for self-maintenance and parental investment (such as egg size and provisioning of young, Hiom et al. 1991; Richardson and Smiseth 2019), in turn, influencing the individuals' optimal investment strategy. Ultimately, the physical environment affects offspring survival, as a consequence of both the environment's effect on carers and its direct impact on offspring (Arlettaz et al. 2010; Mainwaring et al. 2010).

Several studies have found that offspring that hatch from larger sized eggs have greater mass, skeletal size and survival, but only for a limited time after hatching; this effect disappears with age (Bolton 1991; Smith and Bruun 1998; Einum and Fleming 2000; Christians 2002). It has been proposed that this disappearance may result from parental investment (i.e. provisioning) and the environment in the post-natal stage overriding maternal investment in the pre-natal stage as time goes on (Smith and Bruun 1998). During benign environmental conditions, provisioning by carers in the post-natal stage may compensate for the effects of smaller egg size (Einum and Fleming 2000; Russell et al. 2007). However, in harsh conditions provisioning rate decreases in some species (Wiley and Ridley 2016; Nomano et al. 2019). If post-natal investment is insufficient to compensate for small egg size during harsh conditions, investment in larger eggs may be more important at these times (Einum and Fleming 2000; Fox 2000; Fox and Czesak 2000; Langmore et al. 2016).

Large egg size has a beneficial effect on many offspring traits, but is not always the optimal investment option for the mother, potentially resulting in parent-offspring conflict (Janzen and Warner 2009; Krist 2011). Females may vary their relative investment in the pre-natal stage (egg investment) or post-natal stage (investment in provisioning), to adjust to present and predicted future environmental conditions. Environmental factors such as temperature have been found to correlate with egg size in past studies (Nager and Zandt 1994; Kvalnes et al. 2013), indicating that maternal investment is influenced by the environment. There have been several theories proposed

as to how females may maximise maternal investment within their environment (Grafen 1988; Yampolsky and Scheiner 1996; Savage et al. 2015). The 'silver spoon' hypothesis predicts mothers should capitalise on favourable conditions by increasing their investment in both pre-natal and post-natal stages to optimise their offspring's condition above what is usually possible (Grafen 1988). In contrast, females may give their offspring a 'head start' by investing more in the pre-natal stage, thereby decreasing the consequences of less investment at the post-natal stage (Savage et al. 2015). Alternatively, the 'bigger is better' hypothesis predicts that increased maternal investment in offspring will have a greater impact on offspring quality in harsh conditions than in benign conditions (Parker and Begon 1986).

In cooperatively breeding species, optimal investment strategies may be influenced by the presence of helpers in the group (Russell and Lummaa 2009; Savage et al. 2015). Helpers provide care to offspring in addition to that provided by the breeding pair. The presence of helpers gives the breeding females two options; 1) provide the same level of care as they would in a pair, which results in greater overall investment in offspring in cooperative groups relative to pairs; 2) decrease their own level of care and allow helpers to compensate, which results in the same level of care in cooperative groups and pairs. The latter strategy allows females to conserve their resources for self-maintenance or future broods (known as load lightening; Crick 1992; Hatchwell 1999; Hammers et al. 2019). The investment in young must be considered within the care giver's environment, as there is evidence that parental investment can differ with temperature variation (Langmore et al. 2016; Wiley and Ridley 2016). To have a comprehensive view of the costs and benefits of maternal investment, all stages of offspring development should be considered, through to recruitment into the population (Ridley 2007; Zhao et al. 2019).

One cooperatively breeding species for which the effect of climate and helper presence on egg size has been investigated is the superb fairy-wren (Russell et al. 2007; Russell et al. 2008; Langmore et al. 2016). This species is a particularly suitable for studies of maternal investment in relation to environmental variables, because there is natural

variation in both helper presence and climate during the breeding season. Superb fairy-wrens breed either in pairs or in groups, in which offspring from previous breeding seasons assist with provisioning nestlings (Pruett-Jones and Lewis 1990). The superb fairy-wren breeding season spans a 5-month period, which progresses from spring to summer, during which the average maximum daily temperature increases.

Superb fairy-wren provisioning rate of nestlings has consistently been shown to increase in the presence of helpers (Dunn and Cockburn 1996; Russell et al. 2007). However, despite this extra provisioning, nestlings in groups are no bigger than those provisioned by pairs (Dunn et al. 1995). This is due to variation in the investment of breeding females; females produce smaller eggs if they have helpers, concealing the additive care provided by helpers (Russell et al. 2007). However, this decrease in egg size only occurs in cool conditions. In hot conditions ($>25°C$), females with helpers produce larger eggs (Langmore et al. 2016). This may provide nestlings with a buffer against reduced parental provisioning and dehydration during hot conditions. Larger eggs result in bigger nestlings (Russell et al. 2007), which may compensate for the decreased growth rate that superb fairy-wren nestlings experience during heat waves (eg. an average maximum temperature of $>35°C$ in the two days prior to measurement was associated with a 0.37 g decrease in mean body mass of superb fairy-wren nestlings; Kruuk et al. 2015). However, it is unknown whether hatching from a larger egg translates into a growth or survival benefit for these offspring.

To our knowledge, interaction of climate and helpers on investment in young has not been investigated simultaneously in all stages of development in a cooperatively breeding species. We aim to test whether the different maternal strategies used by superb fairy-wrens provide fitness benefits to their offspring, by investigating the effects of egg size, climate and helpers on chick growth and survival to the next breeding season. To separate the effects of maternal investment in eggs from the investment of carers in chicks, we cross-fostered clutches of eggs between synchronous nests, both

with and without helpers, throughout the different climate conditions during the breeding season (Krist 2011). Based on previous research, we predict that:

1) Hatching from a large egg should improve nestling size and survival.

2) Hatching from a large egg should provide a greater benefit to nestling growth and survival in hot and dry weather than in cool and wet weather (the 'Bigger is Better Hypothesis').

3) Heat waves (average maximum temperature >35℃ in 2 days prior to measurement; Kruuk et al. 2015) will have a negative impact on chick mass and survival.

4) If the size of the cross-fostered eggs reared by the pair or group differs from that laid, there will be a fitness cost to chick mass, growth and survival, which will vary in relation to climate and group size.

Methods

Study species

Superb fairy-wrens are insectivorous, cooperatively breeding passerines that raise young in either social breeding pairs or groups comprising one breeding pair and 1-5 helpers (Cockburn et al. 2016), usually only 1 helper in our study (of the 189 egg volume data points collected, 72% were from groups with 0 helpers, 19% from groups with 1 helper, 7% from groups with 2 helpers, 1% from groups with 3 helpers and 2% from groups with 4 helpers). Females have multiple breeding attempts across the breeding season due to high nest depredation rates, and may build as many as eight nests, although no more than three, and more commonly one at the most, survive to fledge (Cockburn et al. 2008). Incubation commences upon clutch completion and is performed only by the female, but all group members contribute to provisioning of young and nest defence (Cockburn et al. 2016). After reaching independence (able to independently forage at around four weeks post fledging), males are strongly philopatric, whereas females show

obligate, relatively long-distance (average 11.8 superb fairy-wren territories) dispersal in their first year (Mulder 1995).

Field methods

The field site for this study was Campbell Park in Canberra, south-eastern Australia (149°10′E, 35°16′S; Langmore et al. 2016), an open eucalyptus woodland. Data collection occurred during the 2017, 2018, 2019 and 2020 breeding seasons (September to February). Most individuals in the population were colour banded for identification and monitored to determine group composition and to track nesting attempts.

Clutches of eggs were cross-fostered between synchronous nests to test the effect of two environmental factors, helper presence and climate, on nestling growth and apparent survival (as females often disperse to outside the study site, so recorded survival is likely to be lower than actual survival) to the next breeding season. The experiment decouples pre-natal and post-natal investment as the egg size laid differs from that reared when clutches are cross-fostered. Clutches were swapped between unassisted pairs and groups with one or more helpers, and from pairs to pairs, and from groups to groups (number of clutches swapped, that were measured as chicks, between each condition is specified in Table S1). Some clutches were not fostered to act as controls (Table S1). The nests used in cross-fostering exchanges were selected based on synchrony of the start of incubation (within two days of one another). Cross-fostering occurred throughout the breeding season in both hot and cool conditions. We categorized 'hot' and 'cool' conditions based on the biological significance of temperature to egg size. Our previous work showed that fairy-wren egg size was influenced by maximum temperatures in the twelve to two days prior to egg laying; females laid larger eggs when there were more than four days above 25℃ (classified as 'hot' conditions), and smaller eggs when there were less than four days above 25℃ (classified as 'cool' conditions) during that time window (Langmore et al. 2016). During the swap the first clutch was temporarily

replaced with a clutch of three fake eggs (made from non-toxic polymer clay approximately matching the size, shape and colour of superb fairy-wren eggs), while the real eggs were measured and transported by hand to the nest they were swapped with. The eggs of the second nest were then measured and taken to the first nest to replace the fake eggs. Whole clutches were swapped even if there was a mismatch in clutch size. Superb fairy-wren clutch size has a strong mode of three eggs but can vary from 1-5 eggs (Cockburn et al. 2016). Clutches were usually reciprocally cross-fostered, but sometimes when three nests were synchronous ring swaps occurred so that all three nests could be included in the treatment rather than two. The fake eggs were always accepted by the female and no nest abandonment events occurred the same day that the eggs were cross-fostered.

Temperature and rainfall measurements were taken from a Bureau of Meteorology weather station (http://www.bom.gov.au/climate/data/) located at the Canberra Airport, 4 km from the field site (Langmore et al. 2016). Cool conditions typically occur at the beginning of the breeding season from September to early November, when the temperatures in Campbell Park usually have mean maximum below 25º C. Hot conditions occur later in the breeding season from mid-November to February. The average maximum temperature was calculated over the 12-2 days prior to egg laying and the 6-25 days prior to weighing seven day old chicks, as these were previously found to be the most influential temperature windows for egg volume and chick mass respectively (Kruuk et al. 2015; Langmore et al. 2016). The average maximum temperature was calculated over the 0-7 day nestling period as nestling measurements were taken on day 1 and day 7 (hatch day = day 0). The average maximum temperature in the two days prior to weighing day 7 chicks was also calculated, as a heatwave of average maximum temperature over 35℃ was previously found to negatively affect chick mass gain (Kruuk et al. 2015). The sum of rainfall in the 57-13 day period before chicks were weighed on day 7 was calculated, as this rainfall window was previously found to be the most influential on chick mass (Kruuk et al. 2015). The

sum of rainfall in the 59-20 days before the first egg of the clutch was laid was calculated as this rainfall window was previously found to be the most influential on egg volume (Langmore et al. 2016).

To reduce depredation of nests, we protected them with a wire mesh cage (5 x 5 cm mesh size), which allows access by superb fairy-wrens but prevents access by larger avian predators such as pied currawongs *Strepera graculina* (Langmore and Mulder 1992). This has been shown to reduce nest depredation from 66% to 28% at this field site previously (Langmore et al. 2003).

Egg length and breadth of 669 eggs were measured using Vernier callipers (± 0.1 mm) by CJT and NEL (there was no difference in egg size measurements by CJT and NEL [variance: $F_{9,7}=1.03$, $p =0.99$; equal variance two sample t-test: $t_{16}=0.09$, $P=0.93$]). From these measurements egg volume was calculated using Hoyt's formula (0.51 x length x breadth^2; Hoyt 1979). Egg volume was averaged for each clutch to account for variation of egg size within the clutch which in most species is less than between clutches (Christians 2002). In addition, we did not record which egg each chick hatched from as this would have involved multiple nest checks on hatch day which would have been very invasive and increase the risk of incidentally alerting predators to nest presence.

Nestling mass and tarsus length (a proximate measure of skeletal growth) were measured when chicks were 1 and 7 days old (Russell et al. 2007). Nestling mass and tarsus length was averaged for each clutch to produce a single value for each variable for day 1 and 7 for each nest. All nestlings were colour banded for individual identification on day 7 to allow monitoring of survival after fledging.

We conducted one hour nest observations from a hide when the nestlings were 5 days old to determine provisioning rates (Russell et al. 2007). The hide was erected 10 – 50 metres from the nest at least 30 minutes before observations commenced, to allow the birds to habituate to its presence. Provisioning rate was the number of adult visits per

hour. We also calculated the number of adult visits to the nest per hour per chick to correct for brood size. The provisioning watch start time did not differ significantly between pairs and groups so was not included in further analyses (variance test: $F_{56,22}=0.96$, $P=0.88$; equal variance two sample t-test: $t_{78}=1.29$, $P=0.20$).

Survival of nestlings to fledging was recorded on fledging day or the day after. The apparent survival of fledglings to the following breeding season was recorded for the 2017 and 2018 breeding seasons. Apparent survival data was categorised as at least one fledgling per nest surviving to the following breeding season versus no individuals from the nest surviving to the following breeding season.

This research was conducted under the approval of the Australian National University Animal Ethics Committee Protocol Number A2017/35, A2020/12 and ACT Government Licence numbers WT201746, LT2017928, and LT20187.

Statistical analysis

The statistical package R (R Core Team 2019) was used to run all analyses. We used a Bayesian Markov chain Monte Carlo (MCMC) approach by utilising the package MCMCglmm to fit all Generalised Liner Mixed Models (GLMM) and Generalised Linear models (GLM; Hadfield 2010). As we had no prior information about the parameter distributions of the models, we used weakly informative priors (for priors and model specifications see supplementary materials). Models were run for 325000 iterations with a burn-in period of 75000 and thinning interval of 250 (except in 4 models which required more iterations to deal with auto-correlation, these instances are specified in the model descriptions below). The 'autocorr' function of MCMCglmm was used to check all models for auto-correlation between samples and convergence of the chains was visually inspected. Variables were considered significant when the 95% confidence intervals (CI) did not include 0 and the MCMCglmm equivalent of p values (*p*MCMC) were less than 0.05. In all models the variable 'egg volume' was scaled and centred [the

mean of the effect was subtracted from each value and the resulting value was divided by the standard deviation of the effect; Becker et al. 1988]). As most groups only had 1 helper, 'helper presence' rather than the number of helpers was used. For all models, with the response variables 'egg volume', 'day 1 mass', 'day 7 mass', 'mass change', 'tarsus change' and 'provisioning rate', where cohort was included as a fixed effect there were four levels (2017/2018, 2018/2019, 2019/2020 and 2020/2021). However, when apparent survival was the response variable there were only 2 levels in cohort (2017/2018 [N=27] and 2018/2019 [N=21]) as apparent survival was only recorded for two breeding seasons. No random effects were included for the apparent survival model as most mothers were only represented once in the dataset (>78% for all models), the model fit was better without random effects and exclusion of mother identity did not affect the overall outcome in any model (tested with the prior inclusion of mother ID as a random effect).

Prediction 1. Hatching from a large egg should improve nestling size and survival.

To investigate if egg volume influences nestling size and apparent survival, we used a GLMM with a Gaussian distribution for the response variables; day 1 mass (N=117), day 1 tarsus length (N=117), day 7 mass (N=73), day 7 tarsus length (N=72), and a GLM with a binomial distribution to investigate apparent survival to the next breeding season (at least one individual from the nest survived to the following breeding season [yes N=14, no N=34]). For all models the fixed effects were egg volume and cohort. The identity of the mother that laid the clutch was included as a random effect for the mass and tarsus response models to account for multiple clutches from the same mother (mother ID: day 1 mass and tarsus length N=73 females, and day 7 mass and tarsus length N=54 females). No random effect was included for the apparent survival analysis as explained previously.

To investigate if provisioning rate influences chick mass or growth, we used a GLMM with response variables; day 7 mass ($N=67$), day 7 tarsus length ($N=65$), 'mass gain from day 1 to day 7' ($N=67$) and 'tarsus length difference from day 1 to day 7' ($N=62$) with a Gaussian distribution. The fixed effects were provisioning rate per hour per chick, helper presence at rearing nest (yes [day 7 mass $N=16$, day 7 tarsus $N=15$, mas change $N=15$, tarsus change $N=15$] or no [day 7 mass $N=51$, day 7 tarsus $N=50$, mas change $N=49$, tarsus change $N=47$]) and cohort. The random effect was rearing mother ID (day 7 mass $N=56$, day 7 tarsus $N=55$, mas change $N=53$, tarsus change $N=52$).

Prediction 2. Hatching from a large egg should provide a greater benefit to nestling growth and survival in hot and dry weather than in cool and wet weather.

To investigate whether large egg size is more beneficial to nestling growth and apparent survival in hot weather than in cool weather, we used a GLMM with a Gaussian distribution for the response variables; difference in mass between day 1 and day 7 of the nestling period ($N=69$), difference in tarsus length between day 1 and day 7 of the nestling period ($N=69$) and a GLM with a binomial distribution for the response variable apparent survival to the next breeding season ($N=48$). The fixed effects were egg volume, a temperature variable (the average maximum temperature in the 6-25 day period before day 7 chick weighed [Kruuk et al. 2015] and the average maximum temperature during the 0-7 day nestling period, were tested in separate models as they were highly correlated [Pearson's r (67)=0.79, P=<0.001]) fitted with a quadratic term due to nonlinearity, cohort and an interaction between egg volume and temperature. The identity of the female that reared the brood was included as a random effect for nestling growth models (mass gain [$N=56$] and tarsus growth [$N=54$]), because multiple broods reared by each female were included in the analysis. A random effect was not included in the apparent survival model as explained previously.

To investigate if large egg size is more beneficial to nestling growth and apparent survival in dry conditions, the same model was used as prediction 2 with the sum of rainfall in the 57-13 day period before day 7 of the nestling period (Kruuk et al. 2015) replacing the temperature variable.

Prediction 3. Heat waves (average maximum temperature >35℃ in 2 days prior to measurement; Kruuk et al. 2015) will have a negative impact on chick mass and survival.

To investigate if heat waves affect chick mass, we used a GLMM with a Gaussian distribution for the response variables; day 7 mass (N=73) and difference in mass between day 1 and day 7 of the nestling period (N=69). The fixed effects were 'experienced a heat wave' (yes [the average maximum temperature >35℃ in the 2 days before weighing at day 7] or no [the average maximum temperature below 35℃ in the 2 days before weighing at day]) and cohort. The random effects were rearing mother ID (day 7 mass N=59, mass change N=56) and Julian date (date chicks were weighed at day 7 [number days since the first August]).

Due to the small sample size, a Fisher's exact test was used to look at the effect of experiencing a heatwave on apparent survival to the next breeding season (heatwave [survived=0, died=6], no heatwave, [survived=14, died=28]).

To assess if experiencing a heatwave has an indirect effect on chick mass and apparent survival through a reduced provisioning rate, we used a GLMM with a poisson distribution with the number of feeds per hour as the response. The fixed effect was maximum temperature on the day of the provisioning watch (the provisioning watch occurred when chicks were 5 days of age, so within the 2-day period used to determine if chicks experienced a heatwave). The random effect was the identity of the mother that reared the clutch (N=59). Differing from the models specifications above, this model was run for 455000 iterations with a burn-in period of 105000 and thinning interval of 350.

Prediction 4. If the size of the cross-fostered eggs reared by the pair or group differs from that laid, there will be a fitness cost to chick mass, growth and survival, which will vary in relation to climate and group size.

Langmore et al. (2016) found that the number of days above 25°C and helper presence influenced superb fairy-wren egg size. As our fifth hypothesis is based on these findings, we first investigated if the same interaction existed within our data. We used a GLMM with a Gaussian distribution with egg volume (N=189) as the response variable. The fixed effects were helper presence (yes N=53, no N=136), the sum of rainfall in the 59-20 days before the first egg of the clutch was laid, the number of days above 25°C in the 12-2 days before the first egg of the clutch was laid, and an interaction between helper presence and temperature. The random effect was mother ID (N=91) to account for multiple clutches from some mothers in the dataset.

To investigate if helpers affect provisioning rate we conducted a GLMM with the response variable 'number of feeds per chick per hour' with a Gaussian distribution. The fixed effects were cohort and helper presence at the rearing nest (yes [N=22] or no [N=67]). The random effect was rearing mother ID (N=59). Differing from the model specifications above, as more iterations were required to deal with auto-correlation of successive samples in the Markov chain, this model was run for 455000 iterations with a burn-in period of 105000 and thinning interval of 350.

Clutches of eggs were cross-fostered between synchronous nests to test the effect of two environmental factors, helper presence and climate, on nestling growth and apparent survival to the next breeding season.

To investigate the effects of temperature, helper presence and the cross-fostering treatment (decoupling pre-natal and post-natal investment) on chick mass, growth and apparent survival we used GLMM, with a Gaussian distribution for the response variables; day 7 mass, day 7 tarsus length, mass change from day 1 to day 7 and tarsus

length change from day 1 to day 7. The models with day 7 mass and day 7 tarsus length as the response variables were run for 650000 iterations with a burn-in period of 150000 and thinning interval of 500. The fixed effects for all models (sample sizes for categorical variables in Table S2) were helper presence (group or pair), egg size of reared eggs compared to egg size of the laid clutch of eggs (reared eggs are bigger, the same size or smaller than the eggs laid by the foster mother), average maximum temperature in the 0-7 day nestling period fitted with a quadratic term due to nonlinearity, cohort and an interaction between helper presence and egg size. The random effect was rearing mother ID (N=91). To control for seasonal effects Julian date (date clutch hatch [number days since the first August]) was added as a random effect to the mass and tarsus models.

Due to the small sample size, a Fisher's exact test was used to look at the effect of helper presence and cross-fostering treatment (difference in egg size between eggs laid by the female and egg reared by the female) on apparent survival to the next breeding season (reared by pair [survived=11, died=26] and reared by group [survived=3, died=8]; and reared eggs bigger [survived=8, died=16], reared eggs same size [eggs not fostered, survived=4, died=9] and reared eggs smaller [survived=2, died=9]).

Results

During this study, 189 clutches, comprising 669 eggs, were measured, of which 124 clutches (66%) survived to hatch (117 of these clutches were measured the day after hatching at day 1 of age). Of the 124 clutches that hatched, and survived to 1 day of age, at least one individual in 74 clutches (60%) survived to 7 days of age (day 7 mass was recorded for 73 of these clutches and day 7 tarsus length was recorded for 72 of these clutches) and at least one individual in 61 clutches (49%) survived to fledge. Apparent survival to the following breeding season was only recorded for the 2017/2018 and 2018/2019 breeding season (48 clutches). 20 individuals from 14 clutches out of a total of 48 clutches survived to the following breeding season. For 34 out of 48 clutches

that survived to fledge, no individuals survived to the following breeding season. 35% of the individuals that survived were female and 65% were male. In the following breeding season 50% of the survivors were helpers on their natal territory, while 50% took the dominant position on a breeding territory.

Prediction 1. Hatching from a large egg should improve nestling size and survival.

Consistent with this prediction, nestling day 1 mass (pMCMC<0.001; Figure 1A; Table S3) and tarsus length (pMCMC<0.001; Figure 1B; Table S3) and day 7 mass (pMCMC=0.02; Figure 1C; Table S3) and tarsus length (pMCMC=0.04; Figure 1D; Table S3) increased with increasing egg size. However, they did not differ significantly in apparent survival to the next breeding season (pMCMC=0.20; Table S3), although there was a non-significant trend for clutches that survived to the following season to have larger egg volume on average (Figure 1E). Provisioning rate did not significantly affect day 7 chick mass (pMCMC=0.22; Table S4), tarsus length (pMCMC=0.83; Table S4), mass gain (pMCMC=0.12; Table S4), or tarsus growth (pMCMC=0.30; Table S4).
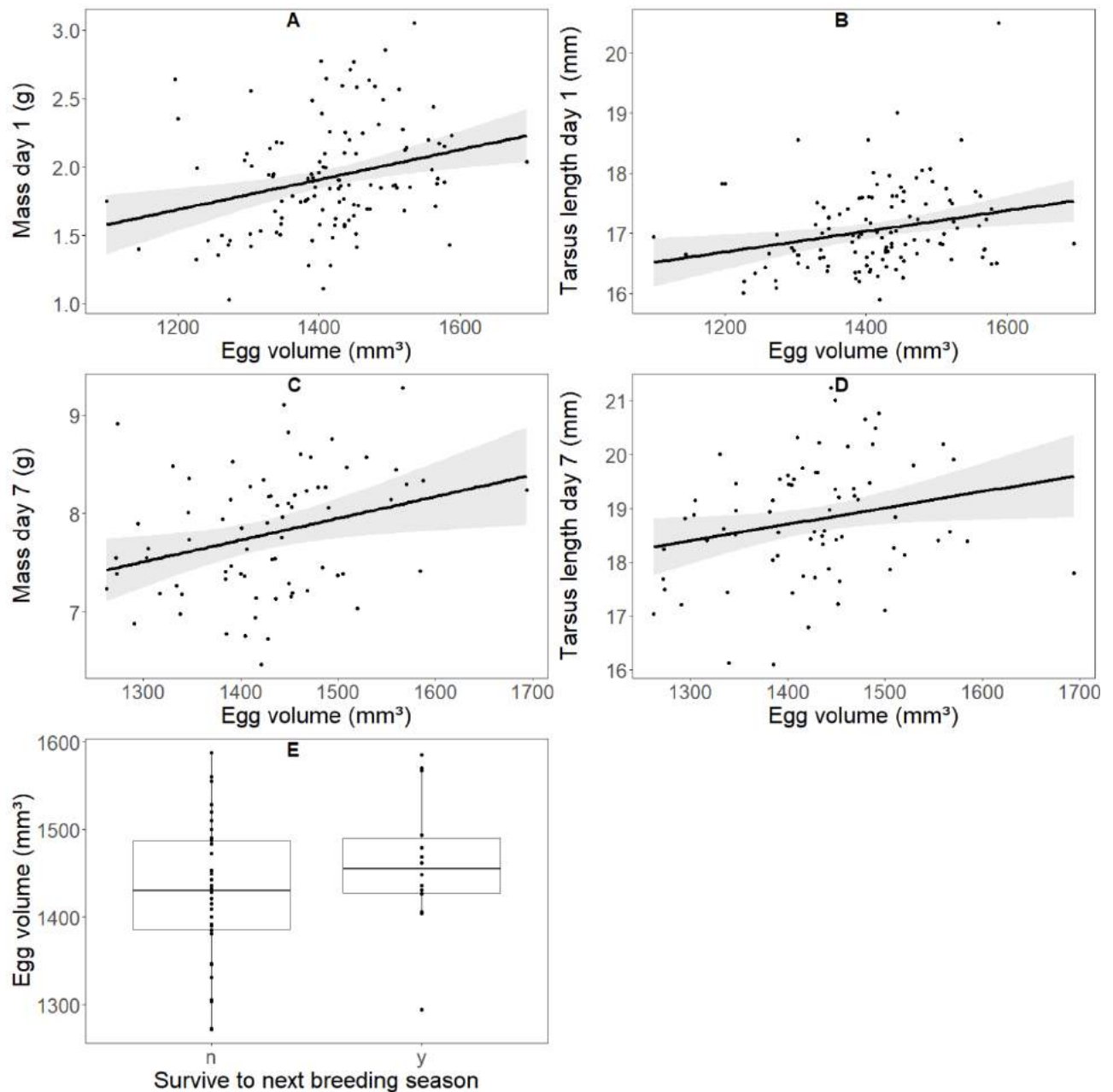
Figure 1. Raw data and model predictions (shown by the solid line with confidence intervals indicated by shading) showing that chick day 1 mass (A) and day 7 mass (C), and tarsus length at day 1 (B) and day 7 (D) increase with egg volume. The egg volume of clutches in which at least one individual in the clutch survived to the next breeding season (n=died, y=survived) was on average, but not significantly, larger than those in which all individuals died (E). The median is indicated by the line in each box, minimum and maximum quantiles are shown by the upper and lower lines of each box, whiskers extend to the furthest point or 1.5 times the interquartile range at the maximum, and points indicate the data spread.

Prediction 2. Hatching from a large egg should provide a greater benefit to nestling growth and survival in hot and dry weather than in cool and wet weather.

Chick mass gain was significantly affected by the interaction between egg volume and temperature in the 0-7 day nestling period (pMCMC=0.03; Figure 2). When temperatures were around the mean temperature experienced during the 0-7 day nestling period, chicks from all egg sizes had similar mass gain. However, in more extreme temperatures (both cooler and hotter) chicks that hatched from larger eggs gained greater mass (Figure 2). In contrast, the interaction between egg volume and temperature in the 0-7 nestling period had no effect on chick tarsus growth or apparent survival (Table S5; tarsus difference: pMCMC=0.28; survival: pMCMC=0.98). Similarly, hatching from a large egg did not result in significantly different chick mass gain, tarsus growth or apparent survival with varying temperature in the 6-25 days before weighing the brood at 7 days of age. The interaction between egg volume and average maximum temperature was not significant for any response variable for this temperature variable (Table S5; mass gain: pMCMC=0.56; tarsus difference: pMCMC=0.61; survival: pMCMC=0.98).

Hatching from a large egg did not provide a greater benefit to chick growth or apparent survival in dry conditions relative to wet conditions. The interaction between egg volume and rainfall was not significant for any response variable (Table S6; mass gain pMCMC=0.65; tarsus growth pMCMC=0.34; survival pMCMC=0.55).
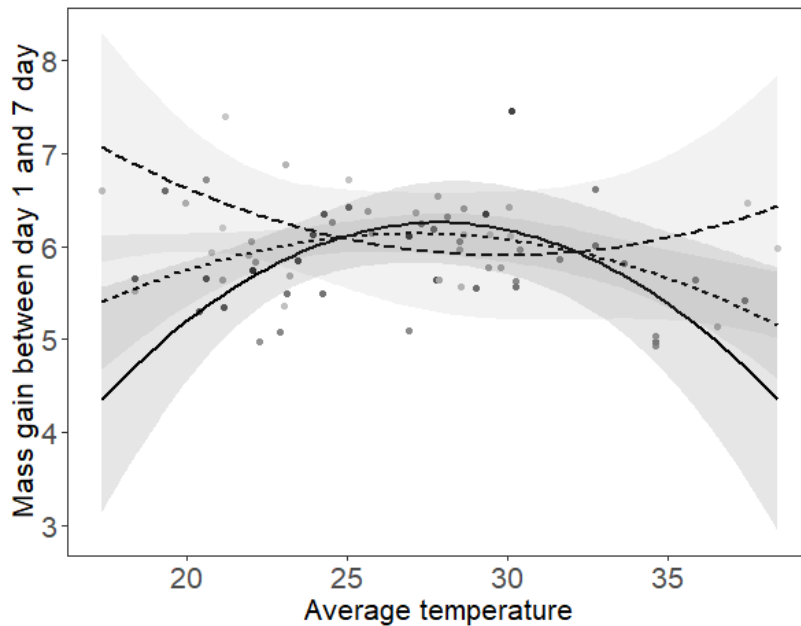
Figure 2. Raw data and model preditions showing that in hot and cool conditions during the 0-7 day nestling period, chicks from larger eggs (dashed line [based on the maximum egg volume in the dataset, 1694.39mm^3]) gained more mass than those from average sized (dotted line [based on the minimum egg volume in the dataset, 1262.09mm^3]) and small eggs (solid line [based on the mean egg volume in the dataset, 1428.17mm^3]). In contrast in mild temperatures chicks from all egg volumes experienced similar mass gain. Points are shaded in relation the egg volume with points representative of smallest eggs being the darkest and becoming progressively lighter as egg volume increases.

Prediction 3. Heat waves (average maximum temperature >35℃ in 2 days prior to measurement; Kruuk et al. 2015) will have a negative impact on chick mass and survival.

Heat waves had a negative impact on chick mass gain (Table S7; pMCMC=0.02); chicks that experienced a heatwave had significantly lower mass gain than those that did not experience a heat wave in the 2 days before weighing (Figure 3). However, experiencing a heat wave did not significantly affect day 7 mass (Table S7; pMCMC=0.32), although there was a non-significant trend for those exposed to a heatwave to have lower day 7 mass on average (no heat wave average day 7 mass ± SE=7.83±0.08g, heatwave average day 7 mass ± SE=7.58±0.13g). No chicks that experienced a heat wave survived to the next breeding season, but there was no significant effect of heatwave on

apparent survival (Fisher's exact test, p=0.16). The number of feeds provided to the brood per hour was significantly affected by the maximum temperature on the day of the provisioning watch (nestlings 5 days old, which is within the period looked at for an effect of heatwave; pMCMC=0.04; Table S8), with the provisioning rate dropping as the maximum temperature increased (Figure 4).
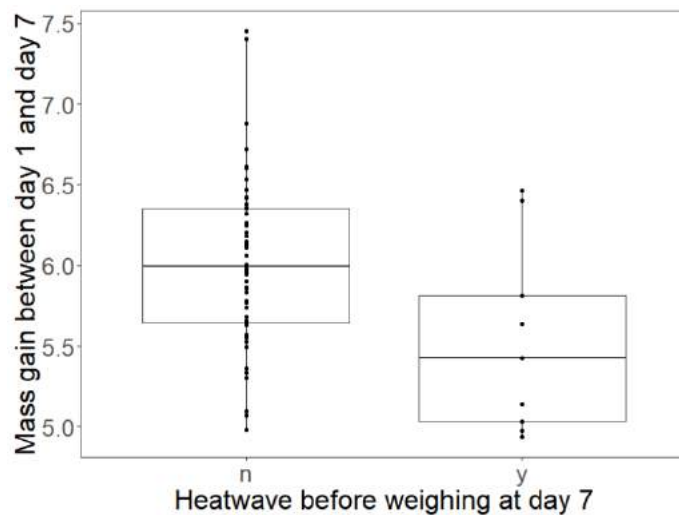

Figure 3. Chicks that experience a heatwave (y) before being weighed at 7 days of age gained less mass, on average, than chicks that did not experience a heatwave (n). The median is indicated by the line in each box, minimum and maximum quantiles are shown by the upper and lower lines of each box, whiskers extend to the furthest data point and points indicate the data spread.
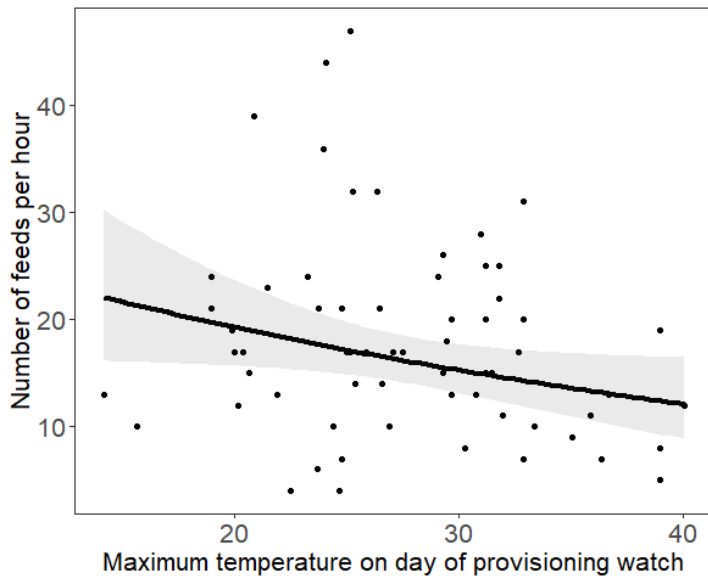
Figure 4. Raw data and model predictions showing that the number of feeds provided to the brood per hour decreased with increasing maximum temperature on the day of the provisioning watch.

Prediction 4. If the size of the cross-fostered eggs reared by the pair or group differs from that laid, there will be a fitness cost to chick mass, growth and survival, which will vary in relation to climate and group size.

The results showed a broadly similar pattern to those of our previous studies (Russell et al. 2007, Langmore et al. 2016), as females in both groups and pairs laid significantly larger eggs in hot conditions than in cool conditions (pMCMC=0.03; Table S9; Figure 5). However, females did not produce significantly different sized eggs when breeding in groups and pairs (pMCMC=0.69; Table S9; Figure 5). Also, in contrast to previous studies (Dunn and Cockburn 1996; Russell et al. 2007), we found no difference in provisioning rate between pairs and groups (pMCMC=0.73; Table S10). Mass gain was significantly affected by the interaction between helper presence and reared egg size relative to laid egg size (pMCMC=0.02; Table S11). This suggests a different pattern of post-natal investment in chicks by groups and pairs in relation to prior egg investment (Figure 6). Chicks reared by groups, from eggs smaller than those laid by the female, gained less mass than chicks from eggs that were larger than those laid by the female.

Chicks raised by pairs gained a similar amount of mass regardless of if reared eggs were, bigger, the same or smaller than laid eggs. However, the interaction between helper presence and difference between egg size laid and reared did not have a significant effect on mass or tarsus length at 7 days of age or tarsus growth (Table S11). Apparent survival to the next breeding season was not significantly related to helper presence (Fisher's exact test, $P=1$) or the difference between the egg size laid and reared (Fisher's exact test, $P=0.71$). The sample sizes for the survival analyses were small so this warrants further investigation.
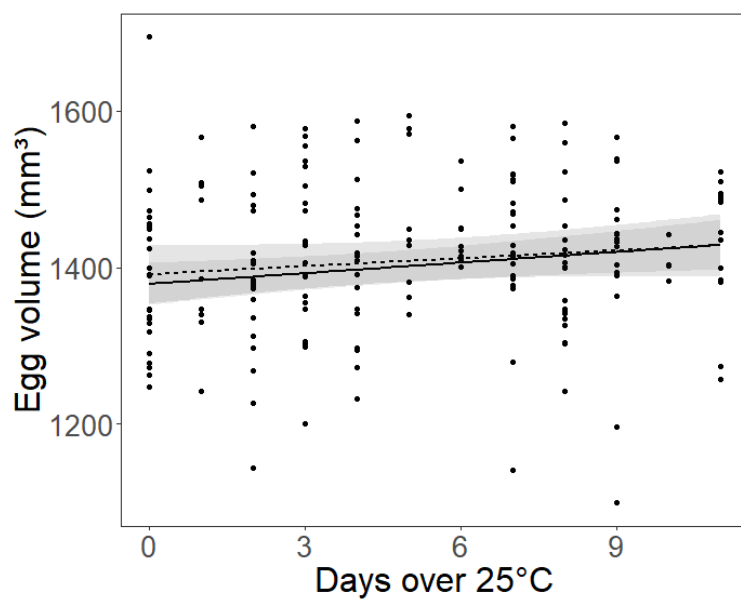
Figure 5. Raw data and model predictions showing that egg volume laid by both group-living (dotted line) and pair-living (solid line) females increased with the number of days over 25°C in the 12-2 day period before egg laying.
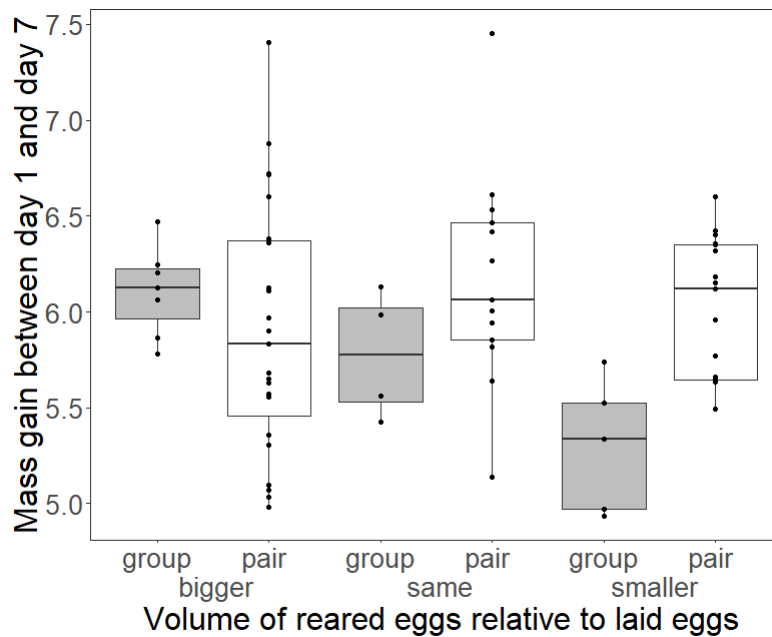
Figure 6. Chicks raised by pairs (white box plots) gained a similar amount of mass regardless of whether the eggs they raised were bigger, smaller or the same as those that they laid. In contrast, the mass gain of chicks raised by groups (grey box plots) differs in relation to the volume of eggs they raised relative to those the female laid. The median is indicated by the line in each box, minimum and maximum quantiles are shown by the upper and lower lines of each box, whiskers extend to the furthest point or 1.5 times the interquartile range at the maximum, and the points indicate the data spread.

Discussion

Our study found that egg volume, climate and helper presence had an effect on superb fairy-wren chick growth. Egg volume had a positive relationship with nestling mass and size from hatching through to 7 days of age. Temperature experienced by nestlings, both mean maximum temperature during the nestling period and heatwaves, had a significant quadratic and negative effect, respectively, on chick mass gain, but, surprisingly, rainfall did not affect chick growth or apparent survival. Egg volume interacted with temperature in the nestling period to influence chick mass gain, such that larger egg volume led to relatively greater mass gain in more extreme temperatures. Helper presence and the volume of the egg reared in relation to the egg laid also influenced chick mass gain; in groups, chicks gained relatively greater mass when they hatched from an egg that was

larger than that laid by the female, and relatively less mass when they hatched from an egg that was smaller than that laid by the female, whereas, in pairs, nestling mass gain was fairly constant regardless of relative egg size. This result suggests that post-natal care of chicks differs between pairs and groups, with groups fine-tuning their provisioning rate in relation to prior investment in eggs, whereas pairs provide a fixed level of care regardless of prior egg investment.

Prediction 1. Hatching from a large egg should improve nestling size and survival.

Consistent with many previous studies, egg volume was positively associated with chick mass and skeletal size on the first day after hatching and continued to have a positive effect at 7 days of age (Bolton 1991; Smith and Bruun 1998; Christians 2002; Krist 2009; Krist 2011). Our findings indicate that maternal investment in egg volume is important for chick mass and growth in superb fairy-wrens. The effect of egg volume on chick mass and growth is likely to be a direct effect rather than an estimate of parental quality, because most clutches were cross-fostered, removing the relationship between pre-natal and post-natal investment (Amundsen and Stokland 1990; van de Pol et al. 2006).

Contrary to our predictions, the size of the egg that an individual hatched from did not significantly influence its probability of survival to the following breeding season. This is consistent with other studies that have looked for an effect of egg investment on survival (Blomqvist et al. 1997; Styrsky et al. 1999; Krist 2009). However, individuals that survived to the following breeding season hatched, on average, from larger eggs than those that died, suggesting egg volume may continue to be important for survival (Pelayo and Clark 2003; Sedinger et al. 2018). A lack of significant effect of egg volume on apparent survival of chicks to the next breeding season indicates either that other factors, such as post-natal care (Amundsen and Stokland 1990; Blomqvist et al. 1997; van de Pol et al. 2006) or environment (Smith and Bruun 1998), may override the effect

of egg volume on chick survival as they age, or that a larger sample size is needed to detect a carry-over effect of egg size to adulthood. The sample size of survivors to the next breeding season was probably lower than the true number for females (35% of survivors were female), because female superb fairy-wrens generally disperse out of the study area to find breeding vacancies (Cockburn et al. 2016).

Parental investment at the post-natal stage, such as provisioning, is suggested in some species to progressively override the effects of pre-natal investment with increasing nestling age (Amundsen and Stokland 1990). However, in our study provisioning rate did not affect chick mass gain, growth or mass and skeletal size at 7 days of age, although we cannot rule out the possibility that provisioning rate may have impacted chick mass and growth after 7 days of age. These findings suggest that either egg volume or the physical environment, such as rainfall and temperature, has a greater influence than provisioning rate on chick mass, growth and survival (Sauve et al. 2021). An alternative explanation for the lack of a significant effect of provisioning rate is that provisioning watches were too short (one hour per nest) to give an accurate representation of provisioning behaviour over the whole nestling period (Krist 2009), even though this procedure has been used to detect difference in provisioning rate in this species in the past (Dunn and Cockburn 1996; Russell et al. 2007). Furthermore, we only measured provisioning rate and did not record prey quality or size, this could be problematic if there is a trade-off between prey size and frequency of feeding trips (Cauchard et al. 2021), but we were unable to test for this in our study. It seems more likely in our study that longer provisioning watches were required as we found evidence of a significant effect of helper presence on chick mass gain (explained below), indicating that there is a difference in post-natal care between groups and pairs.

Prediction 2. Hatching from a large egg should provide a greater benefit to nestling growth and survival in hot and dry weather than in cool and wet weather.

As predicted from our previous work (Langmore et al. 2016), we found evidence that larger egg size buffers chicks against harsh conditions. During extreme conditions, both hot and cold, chicks that hatched from larger eggs gained more mass than those from smaller eggs, whereas in more moderate temperatures all chicks had similar mass gain regardless of egg size. Larger egg size and larger chick mass may be beneficial because larger chicks have more reserves that can buffer them against harsh temperatures (Pelayo and Clark 2003) and because they have a lower surface area to volume ratio, so may be less susceptible to changes in the ambient environment (McKechnie and Wolf 2010). Evidence has been found in other species that nestling size is particularly important in harsh (Cunningham et al. 2013) or competitive environments (Krist et al. 2015).

Contrary to a previous study (Kruuk et al. 2015), we did not find that the maximum temperature in the 25-6 days before nestling weighing had a significant effect on nestling mass gain, growth or apparent survival in our study. It is not immediately evident why we did not also see this relationship in our study population, as these populations are close in geographical terms (10 km; Russell et al. 2008), so presumably experience similar temperatures. However, we also did not find support for the rainfall window (57-13 days before chicks were measured at 7 days of age) detected by Kruuk et al. (2015), as having an impact on nestling mass gain, tarsus growth and survival. The superb fairy-wren population studied by Kruuk et al. (2016) resides in the Australian National Botanic Gardens, which is irrigated, whereas our study site is not. As rainfall and the resulting moisture in the environment can affect how temperature is experienced (Gerson et al. 2014), it is possible that the same temperature has a different impact on thermoregulation in superb fairy-wrens and on insect abundance (therefore food availability, which was the proposed mechanism for the effect of rainfall in this time period) at our study site. If so, the same climate windows may not be meaningful in our population. Therefore, whilst we did not detect an effect of rainfall on

chick growth and apparent survival in our study, it is possible that the effect of rainfall in a different time period may be influential, given the strong relationship between rainfall and insect biomass (Denlinger 1980; Woinarski and Cullen 1984; Winkler et al. 2013), and between insect biomass and body condition in insectivores (Selman and Houston 1996; Hidalgo Aranzamendi et al. 2019). In addition, our study ran over a much shorter time frame that the previous study, and the sample size may have been too low to detect effects of rainfall on chick mass and growth.

Prediction 3. Heat waves (average maximum temperature >35℃ in 2 days prior to measurement; Kruuk et al. 2015) will have a negative impact on chick mass and survival.

Consistent with a previous study (Kruuk et al. 2015), maximum temperature with an average above 35°C in the two days before weighing (heat wave) had a negative effect on chick mass gain, and trends suggest a negative effect on day 7 mass (Kruuk et al. 2015). Supporting this trend, we found that superb fairy-wrens laid heavier eggs in hotter conditions (Figure 5) and larger egg size buffered chicks against harsh conditions (Figure 2). Provisioning rate decreased as maximum temperature increased on the day of the provisioning watch (which was during the two days before weighing), indicating an indirect effect of temperature on chick mass gain through lower provisioning rates of carers. This may be due to increased thermoregulatory costs for carers, decreased prey availability or decreased foraging efficiency in hot weather (du Plessis et al. 2012; Wiley and Ridley 2016). However, heat waves may also have had a direct effect on chick mass gain through dehydration of chicks or increased thermoregulatory demands (Cunningham et al. 2013; Salaberria et al. 2014). Negative impacts of temperatures over certain thresholds has also been found in common fiscals (*Lanius collaris;* Cunningham et al*.* 2013). Spotless starlings (*Sturnus unicolor*) experienced negative effects of ambient temperatures and heat exposure on nestling mass and size in second broods, which overall experienced higher temperatures (Salaberria et al. 2014). This is

comparable to our study as temperature also increases over the breeding season, with heatwaves only experienced during the latter part of the breeding season.

Furthermore, our study provides some indication that the negative effect of experiencing a heatwave in the nestling period impacts apparent survival, because no chicks that experienced a heatwave survived to the following breeding season. Although there was not a statistically significant effect of experiencing a heatwave on apparent survival, the sample size was low so further research with larger sample size is needed. Future research in this area is particularly important as there is growing evidence of the negative impacts of sub-lethal high temperatures (Murphy 1985; Cunningham et al. 2013; Kruuk et al. 2015), but a lot of this research focuses on the semi-arid and arid zone (Gardner et al. 2016; Andreasson et al. 2020). More research is required into the impacts of sub-lethal temperatures in temperate species where global warming is causing more frequent heatwaves (Dosio et al. 2018).

Prediction 4. If the size of the cross-fostered eggs reared by the pair or group differs from that laid, there will be a fitness cost to chick mass, growth and survival, which will vary in relation to climate and group size.

Previous studies found that superb fairy-wren egg investment varied with climate and helper presence, such that females breeding in groups produced smaller eggs in cool conditions and larger eggs in hot conditions than females breeding in pairs (Langmore et al. 2016), and in addition that groups have a higher nestling provisioning rate than pairs (Dunn and Cockburn 1996; Russell et al. 2007). Based on these findings we predicted that being raised by a group would be more beneficial than being raised by a pair for chick mass, growth and survival. We also predicted that larger egg size would be beneficial for chick mass, growth and survival. However, when we looked for these relationships in the data collected in our study we did not find a difference in provisioning rate between groups and pairs, and whilst the trend for females to lay larger eggs in hot conditions was consistent with previous work and the load lightening

hypothesis (Langmore et al. 2016), we didn't find evidence of a significant difference in egg volume between group-living and pair-living females.

Consistent with our previous work (Russell et al. 2007; Langmore et al. 2016), we found that females in groups showed more fine-tuned modulation of their investment strategies than females in pairs. Our previous work found that in benign conditions, females reduce their investment in eggs if they have helpers that can compensate for the shortfall by providing additional provisioning (Russell et al. 2007), and that they benefit from this load lightening through increased survival (Russell et al. 2007) and by having surplus resources to invest in producing larger eggs during harsh conditions (Langmore et al. 2016). By contrast, females with no helpers showed little modification of egg size in relation to climatic conditions (Langmore et al. 2016). Similarly, in this study we found that females with helpers modified their post-natal investment according to the size of the eggs they laid, whereas females without helpers did not. In groups, when cross-fostered eggs were larger than the female's own eggs, the chicks hatching from those eggs gained significantly more mass than when the cross-fostered eggs were smaller than a female's own eggs (Figure 6). By contrast, in pairs there was no significant difference in mass gain in relation to the relative size of the cross-fostered eggs. These results suggest that females with helpers tailor their post-natal investment to suit their pre-natal investment. Our findings provide support for load lightening by females breeding in groups (Hatchwell 1999; Russell et al. 2008) and a tightly coupled relationship between pre-natal and post-natal investment in females breeding in groups (Grafen 1988; Krist 2011; Savage et al. 2015), which was disrupted by our cross-fostering treatment often to the detriment of chicks reared by groups (Amundsen and Stokland 1990; Krist 2011). Previous studies have found that females reduce their provisioning rate in the presence of helpers (Russell et al. 2008). This may be the mechanism by which groups adapt their post-natal investment to egg size, but further study is needed to confirm this.

In conclusion, our study found that pre-natal maternal investment, the physical environment and social environment interact to influence the growth rate of superb fairy-wren chicks. Egg volume had lasting effects on nestling mass and structural size. Egg volume and temperature as well as helper presence were important for chick mass gain, and post-natal investment strategies differed between pairs and groups. This study gives a holistic view of how pre-natal maternal investment strategies of a cooperatively breeding species interact with climate and group size to affect chick mass, growth and apparent survival to the next breeding season. Further studies are required to clarify if egg volume has an effect on survival, as some suggestion of an effect was seen in our study but more data would provide further clarity. Future studies would benefit from collecting long-term data on individuals throughout their lifetime to assess whether egg volume has an effect on other traits, such as fecundity and lifespan.

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Supplementary material

Priors and model specifications used for analysis

For Gaussian linear mixed models (specified by 'Family= "gaussian"') and models with count data response (specified by 'Family= "poisson"'), we used a weakly informative prior with an inverse-Gamma distribution (R=list (V=1, nu=0.002), G=list (G1=list (V=1, nu=0.002))) including an additional G1 specification (exactly the same) when there were two random effects.

For binomial models (specified by 'Family= "categorical"') as these models didn't have random effects we used a weakly informative prior to specify the R structure (R=list (V=1, fix=1, nu=0.002)).

Table S1. Sample size (*N*) of clutches, that survived to be measured as chicks, swapped between groups and groups (GG), pairs and pairs (PP) and from group to pair (GP), and pair to group (PG) and those that weren't fostered and raised by a pair (NFP) or a group (NFG).

Swapped between	*N*
GG	2
PP	44
GP	20
PG	21
NFP	21
NFG	16

Table S2. Sample sizes (*N*) of the categorical variables in the models investigating the effect of temperature, helper presence and the cross-fostering treatment on chick mass and growth (results in Table S11).

Response term	Fixed effects		*N*
Day 7 mass	Helper presence	group	18
		pair	55
	egg size reared relative to laid	bigger	33
		same	18
		smaller	22
Day 7 tarsus	Helper presence	group	18
		pair	54
	egg size reared relative to laid	bigger	32
		same	18
		smaller	22
Mass gain	Helper presence	group	16
		pair	53
	egg size reared relative to laid	bigger	30
		same	17
		smaller	22
Tarsus growth	Helper presence	group	16
		pair	50
	egg size reared relative to laid	bigger	29
		same	17
		smaller	20

Table S3. Summary of models assessing the effect of egg volume on chick mass (on day 1 and day 7 of age), skeletal size (tarsus length on day 1 and day 7 of age) and survival to the following breeding season, including the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and *P* value (*p*MCMC). Mother ID is the identity of the mother that laid the clutch.

Response term	Fixed effects		Posterior mean	95% credibility Interval		eff. sample	*p*MCMC
				Lower CI	Upper CI		
Day 1 mass	Intercept		2.12	2.02	2.22	1000	<0.001*
***N*=117**	Egg volume		0.11	0.05	0.17	1000	<0.001*
	Cohort	1819	-0.31	-0.45	-0.17	1000	<0.001*
		1920	-0.85	-1.56	-0.15	1167	0.02*
		2020	-0.35	-0.53	-0.15	1000	<0.001*
Random effect							
N=73	Mother ID		0.004	0.0003	0.01	906.6	
Day 1 tarsus	Intercept		17.27	17.10	17.48	1000	<0.001*
***N*=117**	Egg volume		0.17	0.04	0.27	1000	<0.001*
	Cohort	1819	-0.29	-0.54	-0.01	1000	0.04*
		1920	-1.01	-2.28	0.25	1000	0.11
		2020	-0.49	-0.84	-0.15	1000	0.002*
Random effect							
N=73	Mother ID		0.02	0.0002	0.07	1151	
Day 7 mass	Intercept		7.85	7.64	8.12	1000	<0.001*
***N*=73**	Egg volume		0.19	0.05	0.33	1000	0.02*
	Cohort	1819	-0.09	-0.45	0.22	1000	0.59
		1920	-0.58	-1.70	0.60	1000	0.31
		2020	-0.08	-0.44	0.30	1000	0.67
Random effect							
***N*=54**	Mother ID		0.06	0.0003	0.20	1000	
Day 7 tarsus	Intercept		19.23	18.84	19.54	1000	<0.001*
***N*=72**	Egg volume		0.25	0.03	0.49	859.6	0.04*
	Cohort	1819	-0.44	-0.99	0.07	1156.1	0.10
		1920	-1.42	-3.42	0.43	1000	0.15
		2020	-1.27	-1.91	-0.70	1000	<0.001*
Random effect							
***N*=54**	Mother ID		0.04	0.0002	1.34	1000	
Survival	Intercept		-1.57	-2.77	-0.58	1000	0.002*
***N*=48**	Egg volume		0.54	-0.17	1.42	1142	0.20
	Cohort	1819	0.86	-0.75	2.38	1098	0.28

Significant values are indicated using (*).

Table S4. Summary of the effect of provisioning rate on day 7 chick mass and mass gain (between day 1 and day 7), and chick skeletal size (day 7 tarsus length) and tarsus growth (between day 1 and day 7), including the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and *P* value (*p*MCMC). Mother ID is the identity of the mother that reared the clutch. Significant values are indicated using (*).

Response term	Fixed effects		Posterior mean	95% credibility Interval		eff. sample	*p*MCMC
				Lower CI	Upper CI		
Day 7 mass	Intercept		7.74	7.37	8.10	1000	<0.001
N=67	Provisioning rate		0.03	-0.02	0.07	1000	0.22
	Helpers		-0.27	-0.64	0.10	1000	0.15
	Cohort	1819	-0.10	-0.48	0.25	1000	0.56
		1920	-0.38	-1.56	0.77	977.6	0.53
		2020	0.03	-0.41	0.46	1000	0.94
Random effect							
N=56	Mother ID		0.14	0.0001	0.34	877	
Day 7 tarsus	Intercept		19.26	18.75	19.89	1110.6	<0.001*
N=65	Provisioning rate		-0.01	-0.09	0.06	871.1	0.83
	Helpers		-0.08	-0.71	0.54	1000	0.78
	Cohort	1819	-0.31	-0.88	0.22	1000	0.32
		1920	-1.41	-3.15	0.66	744.6	0.14
		2020	-1.09	-1.82	-0.37	1000	0.006*
Random effect							
N=55	Mother ID		0.49	0.0005	1.03	806.4	
Mass gain	Intercept		5.69	5.41	5.99	788.6	<0.001*
N=67	Provisioning rate		0.03	0.01	0.07	900.4	0.12
	Helpers		-0.29	-0.57	-0.01	1122.4	0.03*
	Cohort	1819	0.22	-0.06	0.51	882.1	0.11
		1920	0.37	-0.61	1.36	873.7	0.47
		2020	0.39	0.03	0.73	837.2	0.03*
Random effect							
N=53	Mother ID		0.04	0.003	0.12	1131	
Tarsus growth	Intercept		2.03	1.68	2.42	1000	<0.001*
N=62	Provisioning rate		0.03	-0.02	0.07	1000	0.30
	Helpers		-0.13	-0.52	0.25	1000	0.52
	Cohort	1819	-0.02	-0.39	0.36	1000	0.95
		1920	-0.64	-1.77	0.68	1000	0.33
		2020	-0.85	-1.26	-0.33	1000	0.002*
Random effect							
N=52	Mother ID		0.06	0.0002	0.22	1000	

Table S5. Summary of models on the effect of temperature (temp) on chick mass gain, skeletal growth (tarsus growth) and survival to the next breeding season. The average maximum temperature in the 6-25 day period before day 7 chick weighed (6-25 days before weighed) and the average maximum temperature during the 0-7 day nestling period (0-7 day nestling period) were tested in separate models. Mother ID is the identity of the mother that reared the clutch. Results include the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and *P* value (*p*MCMC).

Response term	temp variable	Fixed effects		Posterior mean	Lower CI	Upper CI	eff. sample	*p*MCMC
					95% credibility Interval			
Mass gain **N=69**	6-25 days before weighed	Intercept		6.00	-0.21	13.51	1000	0.09
		Egg volume		-0.44	-3.74	2.96	1000	0.81
		Temp	Linear	-0.005	-0.53	0.45	1000	0.99
			Quad	-0.00005	-0.009	0.009	1000	0.99
		Cohort	1819	0.28	0.002	0.61	1000	0.08
			1920	-0.008	-1.16	0.98	1000	1.000
			2020	0.16	-0.55	1.02	1131	0.66
		Egg volume: temp	Linear	0.06	-0.22	0.35	1000	0.66
			Quad	-0.002	-0.007	0.004	1000	0.56
Random effect **N=56**		Mother ID		0.03	0.0003	0.09	1000	
	0-7 day nestling period	Intercept		0.23	-4.80	5.44	1163	0.92
		Egg volume		3.84	0.38	6.77	1000	0.02*
		Temp	Linear	0.42	0.08	0.80	1149	0.02*
			Quad	-0.01	-0.01	-0.001	1000	0.01*
		Cohort	1819	0.33	0.03	0.63	1000	0.04*
			1920	0.15	-0.80	1.26	1000	0.79
			2020	0.51	-0.03	1.01	1000	0.06
		Egg volume: temp	Linear	-0.27	-0.51	-0.03	1000	0.02*
			Quad	0.005	0.0006	0.0092	1000	0.03*
Random effect **N=56**		Mother ID		0.02	0.0003	0.09	1000	
Tarsus growth **N=69**	6-25 days before weighed	Intercept		-0.75	-8.49	5.46	1000	0.88
		Egg volume		0.56	-3.49	4.04	1000	0.79
		Temp	Linear	0.26	-0.22	0.78	1000	0.32
			Quad	-0.01	-0.02	0.003	1000	0.23
		Cohort	1819	-0.08	-0.45	0.31	1000	0.68
			1920	-0.95	-2.03	0.06	1000	0.08
			2020	-0.78	-1.59	0.18	1109	0.07

		Egg volume: temp	Linear	-0.06	-0.38	0.25	1000	0.70
			Quad	0.002	-0.005	0.008	1000	0.61
Random effect **N=54**		Mother ID		0.2206	0.0002	0.4456	1000	
	0-7 day nestling period	Intercept		4.11	-2.43	10.58	1000	0.23
		Egg volume		-2.76	-6.68	1.79	1000	0.20
		Temp	Linear	-0.12	-0.59	0.34	1000	0.61
			Quad	0.002	-0.01	0.01	1000	0.62
		Cohort	1819	-0.11	-0.48	0.27	815	0.53
			1920	-0.86	-2.07	0.31	1000	0.16
			2020	-1.03	-1.66	-0.26	1145	0.002*
		Egg volume: temp	Linear	0.19	-0.09	0.54	1000	0.22
			Quad	-0.003	-0.01	0.002	1000	0.28
Random effect **N=54**		Mother ID		0.14	0.0003	0.32	1000	
Survival **N=48**	6-25 days before weighed	Intercept		-0.43	-50.37	48.83	1000	0.97
		Egg volume		5.97	-52.79	67.81	1119	0.84
		Temp	Linear	-0.02	-3.47	3.637	1000	0.93
			quad	-0.0009	-0.07	0.06	1000	0.98
		Cohort	1819	0.99	-0.55	2.71	1000	0.25
		Egg volume: temp	Linear	-0.37	-4.99	3.87	1115	0.86
			Quad	0.006	-0.07	0.09	1109	0.86
	0-7 day nestling period	Intercept		10.30	-58.79	32.24	1000	0.67
		Egg volume		-4.36	-40.07	32.91	1000	0.83
		Temp	Linear	0.84	-2.27	4.37	1000	0.63
			Quad	-0.02	-0.08	0.04	1000	0.55
		Cohort	1819	1.17	-0.52	3.24	1000	0.22
		Egg volume: temp	Linear	0.18	-2.53	2.63	1000	0.89
			Quad	0.0005	-0.04	0.05	1000	0.98

Significant values are indicated using (*).

Table S6. Summary of models on the effect of the sum of rainfall in the 57-13 day period (rainfall) before chicks were measured at day 7 on chick mass gain, skeletal growth (tarsus growth) and survival. Mother ID was the identity of the female that reared the clutch. Results include the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and *P* value (*p*MCMC).

Response term	Fixed effects		Posterior mean	95% credibility Interval		eff. sample	*p*MCMC
				Lower CI	Upper CI		
Mass gain	Intercept		6.06	5.67	6.39	1200.1	<0.001*
***N*=69**	Egg volume		-0.0008	-0.35	0.30	1000	1.00
	Rainfall		-0.002	-0.005	0.0008	1000	0.14
	Cohort	1819	0.18	-0.13	0.49	895.4	0.23
		1920	-0.09	-1.30	0.95	1077	0.87
		2020	0.19	-0.16	0.54	1000	0.30
	Egg volume: Rainfall		0.0009	-0.002	0.005	1000	0.65
Random effect							
***N*=56**	Mother ID		0.02	0.0002	0.07	1000	
Tarsus	Intercept		2.31	1.85	2.84	1000	<0.001*
growth	Egg volume		-0.10	-0.50	0.24	1000	0.58
***N*=66**	Rainfall		-0.001	-0.005	0.003	1000	0.59
	Cohort	1819	-0.17	-0.62	0.21	1000	0.41
		1920	-0.93	-2.09	0.16	1000	0.13
		2020	-0.90	-1.30	-0.44	1000	<0.001*
	Egg volume: Rainfall		0.002	-0.002	0.006	1000	0.34
Random effect							
***N*=54**	Mother ID		0.15	0.0004	0.36	806.5	
Survival	Intercept		-2.07	-3.88	-0.02	1000	0.03*
***N*=48**	Egg volume		1.08	-0.91	3.02	100	0.27
	Rainfall		0.004	-0.01	0.02	1020	0.63
	Cohort		0.99	-0.73	2.82	1016	0.28
	Egg volume: Rainfall		-0.006	-0.03	0.02	1000	0.55

Significant values are indicated using (*).

Table S7. Summary of models exploring the effect of heat wave (average maximum temperature >35℃) in 2 days prior to day 7 chick measurement on day 7 chick mass, mass gain (between day 1 and day 7), day 7 tarsus length and tarsus change between day 1 and day 7. Mother ID is the identity of the female that reared the clutch. Results include the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and *P* value (*p*MCMC).

Response term	Fixed effects		Posterior mean	Lower CI	Upper CI	eff. sample	*p*MCMC
				95% credibility Interval			
Mass gain	Intercept		5.89	5.71	6.10	1000	<0.001*
N=69	Heat wave		-0.48	-0.89	-0.07	1000	0.02*
	Cohort	1819	0.26	-0.04	0.56	867	0.09
		1920	-0.06	-1.04	1.03	1000	0.94
		2020	0.17	-0.19	0.49	1000	0.32
Random effect							
N=56	Mother ID		0.02	0.0002	0.07	713.1	
	Julian date		0.04	0.0003	0.16	1098	
Day 7 mass	Intercept		7.91	7.69	8.15	1000	<0.001*
N=73	Heat wave		-0.25	-0.74	0.21	1000	0.32
	Cohort	1819	-0.12	-0.44	0.27	1000	0.51
		1920	-0.76	-2.14	0.41	1000	0.24
		2020	0.13	-0.52	0.27	887.8	0.55
Random effect							
N=59	Mother ID		0.06	0.0002	0.20	1000	
	Julian date		0.04	0.0003	0.16	1000	

Significant values are indicated using (*).

Table S8. Summary of model investigating if the maximum temperature (max temp) on the day of the provisioning watch influences provisioning rate. Mother ID is the identity of the female that reared the clutch. Results include the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and *P* value (*p*MCMC).

Response term	Fixed effects	Posterior mean	95% credibility Interval		eff. sample	*p*MCMC
			Lower CI	Upper CI		
Number of feeds	Intercept	3.45	2.90	4.09	1000	<0.001*
N=79	Max temp	-0.02	-0.04	-0.003	1000	0.04*
Random effect						
N=59	Mother ID	0.14	0.0007	0.27	883	

Significant values are indicated using (*).

Table S9. Summary of the model to assess the effect of temperature and helpers on egg volume (N=189). Mother ID (N=91) is the identity of the female that laid the clutch, temperature is the number of days above 25℃ in the 12-2 days before the first egg of the clutch was laid, rainfall is the sum of rainfall in the 59-20 days before the first egg of the clutch was laid and helper indicates if there were helpers present on the territory the eggs were laid in. Results include the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and *P* value (*p*MCMC).

Fixed effects	Posterior mean	95% credibility Interval		eff. sample	*p*MCMC
		Lower CI	Upper CI		
Intercept	-0.34	-0.62	-0.08	1143.7	0.02*
Helper	0.13	-0.27	0.50	860.7	0.55
Temperature	0.05	0.01	0.09	1000	0.03*
Rainfall	-0.08	-0.19	0.02	1115.2	0.14
Helper: temp	-0.01	-0.08	0.04	1000	0.69
Random effect					
Mother ID	0.92	0.55	1.23	1000	

Significant values are indicated using (*).

Table S10. Summary of model looking at the difference in provisioning rate between pairs and groups (helpers [no N=57 or yes N=22]). Results include the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and P value (pMCMC).

| Response term | Fixed effects | | Posterior mean | 95% credibility Interval | | eff. sample | pMCMC |
				Lower CI	Upper CI		
No. feeds per chick	Intercept		5.85	4.73	7.02	1000	<0.001*
chick	Cohort	1819	-0.54	-2.25	0.88	1000	0.50
***N*=79**		1920	-1.14	-7.22	5.71	1000	0.70
		2020	2.29	0.05	4.33	1000	0.04*
	Helpers		0.31	-1.30	1.98	1000	0.73
Random effect							
***N*=59**	Mother ID		1.21	0.0003	5.01	862	

Significant values are indicated using (*).

Table S11. Summary of models exploring the effect of egg volume reared in relation to that laid (egg vol same and egg vol smaller), helper presence (reared by a pair or group) and temperature (the average maximum temperature in the 0-7 day nestling period) on chick day 7 mass, mass gain (between day 1 and day 7), day 7 tarsus length, tarsus growth (between day 1 and day 7) and survival. Mother ID was the identity of the female that reared the brood. This table includes the posterior mean and lower and upper confidence interval (CI), effective sample size (eff. sample), and P value (pMCMC).

Response term	Fixed effects		Posterior mean	Lower CI	Upper CI	eff. sample	pMCMC
				95% credibility Interval			
Day 7 mass	Intercept		6.32	0.34	12.03	1000	0.03*
***N*=69**	Helpers		-0.07	-0.62	0.45	1000	0.81
	Egg vol same		-0.31	-1.15	0.47	1000	0.45
	Egg vol smaller		-0.59	-1.29	0.18	1000	0.13
	Cohort	1819	-0.13	-0.47	0.22	1000	0.48
		1920	-0.79	-1.99	0.46	1000	0.21
		2020	-0.01	-0.64	0.65	1000	0.98
	Temp	quad	-0.003	-0.01	0.005	1000	0.51
		linear	0.13	-0.31	0.51	1000	0.54
	Helpers: egg vol same		0.46	-0.47	1.29	1000	0.33
	Helpers: egg vol smaller		0.44	-0.42	1.28	1000	0.33
Random effect							
***N*=56**	Mother ID		0.09	0.0003	0.26	1000	
	Julian date		0.04	0.0002	0.14	1153	
Day 7 tarsus	Intercept		18.92	10.33	28.42	1230.7	<0.001*
***N*=69**	Helpers		0.50	-0.40	1.22	1000	0.22
	Egg vol same		0.33	-0.69	1.32	1113.7	0.51
	Egg vol smaller		0.05	-1.01	1.19	1000	0.96
	Cohort	1819	-0.41	-0.93	0.20	1000	0.17
		1920	-1.54	-3.45	0.13	1000	0.10
		2020	-1.08	-2.03	-0.11	1000	0.03*
	Temp	Quad	0.0004	-0.01	0.01	1138.4	0.92
		Linear	-0.007	-0.62	0.63	1239.8	0.98
	Helpers: egg vol same		-0.69	-1.79	0.49	1000	0.25
	Helpers: egg vol smaller		-0.79	-2.23	0.34	989.9	0.20
Random effect							
***N*=56**	Mother ID		0.67	0.0008	1.20	1000	
	Julian date		0.08	0.0003	0.34	1000	
Mass gain	Intercept		2.67	-2.37	7.23	1000	0.27
***N*=69**	Helpers		-0.21	-0.66	0.24	738	0.38
	Egg vol same		-0.08	-0.88	0.58	967.2	0.81
	Egg vol smaller		-0.80	-1.41	-0.18	1006.9	0.02*
	Cohort	1819	0.18	-0.11	0.48	1000	0.27
		1920	-0.06	-1.05	1.13	812.7	0.92
		2020	0.53	0.02	1.09	1000	0.05

	Temp	Quad	-0.005	-0.01	0.001	1000	0.11
		Linear	0.25	-0.10	0.57	1000	0.14
	Helpers: egg vol same		0.45	-0.29	1.21	973.7	0.25
	Helpers: egg vol smaller		0.90	0.24	1.60	1000	0.02*
Random effect							
N=56	Mother ID		0.03	0.0003	0.10	1000	
	Julian date		0.03	0.0001	0.11	1000	
Tarsus growth	Intercept		3.01	-3.23	8.14	1000	0.29
N=69	Helpers		0.24	-0.28	0.74	896.1	0.41
	Egg vol same		0.39	-0.35	1.17	1000	0.31
	Egg vol smaller		-0.11	-0.84	0.50	1000	0.71
	Cohort	1819	-0.07	-0.48	0.32	905.5	0.72
		1920	-0.82	-1.96	0.30	1000	0.17
		2020	-0.90	-1.54	-0.21	709.2	0.008*
	Temp	Quad	0.0008	-0.006	0.008	1000	0.85
		Linear	-0.06	-0.46	0.32	1000	0.80
	Helpers: egg vol same		-0.47	-1.35	0.32	1000	0.26
	Helpers: egg vol smaller		-0.15	-1.03	0.69	1000	0.75
Random effect							
N=56	Mother ID		0.26	0.0004	0.45	883.2	
	Julian date		0.06	0.0004	0.20	744.2	

Significant values are indicated using (*).

Chapter 4

How do brood-parasitic cuckoos reconcile conflicting environmental and

host selection pressures on egg size investment?

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How do brood-parasitic cuckoos reconcile conflicting environmental and host selection pressures on egg size investment?

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Brood-parasitic cuckoos lay their eggs in the nests of other birds and thereafter abandon their young to the care of the host. Thus, all maternal investment is restricted to investment in the egg. Optimal investment at this stage is likely to have a large impact on maternal reproductive success. Many bird species optimize the size of their eggs to suit both the prevailing environmental conditions and the number of individuals that will provide care to the chicks. However, relatively few cues are available to avian brood parasites to facilitate optimal investment in their eggs. Moreover, optimization of egg size to suit environmental conditions or the social structure of the host group may be constrained by stronger selection for egg mimicry, which reduces the likelihood of detection and rejection of foreign eggs by the host. We aimed to test how the conflicting selection pressures of (1) selection for large eggs in harsh environmental conditions versus (2) rejection of large eggs by hosts interact to influence the size and shape of Horsfield's bronze-cuckoo, *Chalcites basalis*, eggs. Using a sliding window approach to investigate periods of climate sensitivity, we show that climatic variables did not predict egg size. Nor did cuckoos modify their egg size or shape in relation to the group size of the host (superb fairy-wren, *Malurus cyaneus*). Conversely, host defences did appear to have influenced cuckoo egg morphology: eggs that were relatively short and round had a higher probability of being abandoned by the host than eggs that were long and narrow. This suggests that host defences are the overriding selection pressure on cuckoo egg morphology, and, unlike their hosts, cuckoos do not adapt their eggs to the prevailing climate or host group size.

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In birds, individual investment in egg size is an important determinant of variation in fitness; chicks that hatch from relatively larger eggs gain improved hatching success, higher survival and faster growth rates (for more detail on benefits of large egg size to chicks see Krist, 2011; Mousseau & Fox, 1998; Williams, 1994). However, the extent to which offspring benefit may vary according to environmental and social variables, and mothers are predicted to vary their investment in egg size strategically to maximize these benefits. The 'bigger is better' hypothesis predicts that mothers will be more likely to invest in large eggs in harsh conditions, thereby buffering their young against threats to their survival (Krist, 2011; McGinley, Temme, & Geber, 1987). Conversely, the 'silver spoon' hypothesis predicts that mothers will be more likely to invest in large eggs during favourable conditions, and thereby elevate

offspring condition above what is usually accessible (Grafen, 1988). Females may also modulate egg size according to how much assistance they will have in rearing their young. In cooperatively breeding species, females may produce smaller eggs because additional provisioning by helpers can compensate for the initial lower investment, allowing the female to conserve resources (known as load lightening; Crick, 1992; Hatchwell, 1999; Russell, Langmore, Gardner, & Kilner, 2008; Langmore, Bailey, Heinsohn, Russell, & Kilner, 2016).

The optimization of egg size to environmental conditions relies on mothers being able to predict the environmental and social rearing conditions for their offspring prior to egg laying. This ability has been demonstrated in some sedentary species living in fairly predictable, seasonal environments in socially stable groups (Dixit, English, & Lukas, 2017; Russell, Langmore, Cockburn, Astheimer, & Kilner, 2007; Taborsky, Skubic, & Bruintjes, 2007). However, it is unknown whether migratory species with less predictable rearing conditions are also able to modify the size of their eggs adaptively. One taxon that potentially faces both the greatest challenges and the greatest benefits in predicting the rearing conditions of their

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young is brood-parasitic cuckoos. Many cuckoo species are migratory and begin breeding upon arrival at their breeding site, so they may have little prior information about the preceding climatic conditions at the time they commence breeding (Higgins, 1999; Langmore, Adcock, & Kilner, 2007). Moreover, they have minimal contact with the host individuals that will rear their offspring, so there may be little opportunity to assess host group size prior to egg formation and laying. Yet, despite the challenges of predicting rearing conditions for their young, they are likely to be under strong selection for adaptive modulation of egg size, because their entire investment in their offspring is restricted to maternal investment in eggs (Payne, 1974).

Brood-parasitic cuckoos face an additional constraint in egg size optimization. Many cuckoo hosts reject cuckoo eggs from their nests if they are larger than their own eggs (Langmore, Hunt, & Kilner, 2003; Marchetti, 2000) or if they are the wrong shape (Mason & Rothstein, 1986; Underwood & Sealy, 2006; Zölei, Hauber, Geltsch, & Moskát, 2012; Attard, Medina, Langmore, & Sherratt, 2017; but see Bán et al., 2011), which selects for cuckoo eggs that mimic the morphology of their hosts' eggs. Many hosts discriminate foreign eggs based on visual cues, such as colour and pattern (Spottiswoode & Stevens, 2010). However, in host species with dark, dome-shaped or cavity nests, tactile cues such as egg size and shape may be more important, as there is limited visibility within their nest (Attard et al., 2017; Langmore et al., 2003; Marchetti, 2000; Mason & Rothstein, 1986). Parasitic cuckoos typically parasitize species smaller than themselves, so selection for egg mimicry results in many parasitic cuckoos having small eggs for their body size (Attard et al., 2017; Krüger & Davies, 2002; Payne, 1974; Spottiswoode, Stryjewski, Quader, Colebrook-Robjent, & Sorenson, 2011). Eggs of some cuckoo species have been found to be more similar in breadth than length to those of their hosts (Meshcheryagina, Mashanova, Bachurin, Mitiay, & Golovatin, 2018), perhaps to avoid detection caused by the inconsistencies in egg breadth being detected by the sitter, as a broader egg will protrude higher in the nest cup than a narrow egg (Latter, 1902). Relatively stronger selection on parasitic cuckoos to match the breadth than the length of host eggs might result in a more narrow, elongated egg shape in parasitic cuckoos. Alternatively, overall egg shape mimicry, rather than just egg length or breadth matching, may be more important for egg acceptance by the host (Attard et al., 2017). However, egg shape may also be under selection for other attributes, such as the optimal shape for incubation (Barta & Szekely, 1997).

Here we investigated whether brood-parasitic Horsfield's bronze-cuckoos, *Chalcites basalis*, modulate the size of their eggs according to the prevailing environmental conditions and host group size. The egg size (measured as egg volume) of their primary host, the cooperatively breeding superb fairy-wren, *Malurus cyaneus*, is influenced by both the physical and social environment (Langmore et al., 2016; Russell et al., 2007). Female superb fairy-wrens lay larger eggs during hot and dry conditions, supporting predictions of the 'bigger is better' hypothesis (Langmore et al., 2016). However, this effect is most pronounced in females with helpers, perhaps because these females are in better condition due to the assistance provided by helpers (load lightening, Russell et al., 2007, 2008). The coevolutionary arms race between Horsfield's bronze-cuckoos and superb fairy-wrens has been found to constrain the egg size of the cuckoos, because eggs that are substantially larger than host eggs are abandoned by the host (Langmore et al., 2003). However, it is unknown whether Horsfield's bronze-cuckoos, like their hosts, adjust their egg size adaptively to suit environmental conditions and host group size, or whether such small increases in egg size would be maladaptive due to increased detectability by the host.

We aimed to test how the conflicting selection pressures of (1) selection for large eggs in harsh environmental conditions (hot and dry conditions, Langmore et al., 2016) versus (2) rejection of large eggs by hosts interact to influence the size and shape of cuckoo eggs. First, we investigated whether Horsfield's bronze-cuckoos, like their hosts, adjust their egg size adaptively according to climatic conditions and host group size. We used a 'sliding window' approach (statistical package climwin R, Bailey & van de Pol, 2016) to identify the time period prior to egg laying over which climatic variables most influence cuckoo egg size, and then tested whether climatic variables during this time window interact with host group size to influence cuckoo egg volume. Second, we tested whether the size and shape of cuckoo eggs influences the probability of rejection by their hosts.

METHODS

Study System

Horsfield's bronze-cuckoos are nomadic, parasitic cuckoos that primarily exploit fairy-wrens (Maluridae spp.) in Australia (Brooker & Brooker, 1989; Langmore & Kilner, 2007). In southeastern Australia, they migrate to their host's breeding site in late winter or early spring and promptly begin parasitizing nests. Cuckoos usually lay their egg in the host nest and remove one host egg during the laying period of the host (Brooker, Brooker, & Rowley, 1988). The size, coloration and pattern of the Horsfield's bronze-cuckoo egg is a close match to that of their preferred fairy-wren hosts. However, cuckoo eggs have key visual distinguishing features: they are speckled more evenly over the surface of the egg than the host egg, the shape is more oval (rather than tapered at one end) and the egg is usually approximately 2 mm longer (Langmore et al., 2003). While the cuckoo egg size (mean [range] length x breadth = 18.0 [16.2—19.9] x 12.0 [10.8—13.3] mm) closely matches the host egg size (mean [range] length x breadth = 16.09 [15.1—18.2] x 12.4 [11.1—13.4] mm; Rowley, 1965), it is relatively small for the Horsfield's bronze-cuckoo's body size (mean ± SD: 23.2 ± 2.2 g, Brooker & Brooker, 1996; Dunning, Jr, 2007; Langmore et al., 2003; Langmore & Kilner, 2009). The parasitic chick typically hatches 2 days before the host chicks and evicts the host eggs or chicks to become the sole occupant of the nest.

Superb fairy-wrens are small (mean ± SD: 10.9 ± 0.8 g, Dunning, Jr, 2007), insectivorous passerines, whose nests are preferentially parasitized by Horsfield's bronze-cuckoos at our study site (Langmore & Kilner, 2007). Superb fairy-wrens rarely reject cuckoos at the egg stage (Langmore et al., 2003), perhaps due to poor visibility in their dark, dome-shaped nests (Langmore et al., 2005). Nor do they reject model eggs of the same size but painted a different colour to their own eggs (Langmore et al., 2003). However, they have been found to reject substantially larger model eggs (20 x 15 mm), suggesting that tactile cues such as egg size are important in rejection decisions (Langmore et al., 2003). Superb fairy-wrens rejected large, plastic model eggs by deserting the nest, but they can also use puncture ejection to remove real cuckoo eggs; one female was filmed puncturing and removing the relatively large egg (20 x 15 mm, Langmore et al., 2003) of a fan-tailed cuckoo, *Cacomantis flabelliformis*, from her nest (R. Turner, personal communication 18 October 2019). They are also more likely to desert a nest containing a cuckoo egg if they catch the female cuckoo in the act of parasitism (Langmore et al., 2003). Superb fairy-wrens also reject cuckoo chicks, typically deserting the nestling within 4 days of hatching (Langmore et al., 2003). They renest throughout the season, building as many as eight nests, owing to high predation rates, with a maximum of three clutches being raised to fledging. Clutches typically comprise three to four eggs,

although they can very occasionally comprise as few as two or as many as five eggs. Superb fairy-wrens are co-operative breeders: they can breed in both pairs (just the dominant male and female) or with helpers, which are usually male offspring from previous breeding seasons.

Study Site

The study was conducted in Campbell Park, an open eucalyptus woodland in Canberra in southeastern Australia (149°10′E, 35°16′S; Langmore et al., 2016). Data on cuckoo and host egg size and host group composition were collected for 10 years between 1999 and 2018 (1999, 2000, 2001, 2002, 2006, 2007, 2011, 2015, 2017 and 2018). Superb fairy-wrens were colour-banded for individual identification and all nests in the study site were found and checked for parasitism. As superb fairy-wrens experience high rates of depredation at this site, nests containing cuckoo eggs were caged where possible, which reduces depredation rates from 66% of uncaged nests to 28% of caged nests (Langmore et al., 2003).

Past studies have use different proximate measures for egg size such as egg length, fresh egg mass and egg volume (Aslan & Yavuz, 2010; Krist, 2011). Egg volume was used as a measure of egg size in this study. The length and breadth of cuckoo eggs were measured using Vernier callipers and these measurements were used to calculate egg volume using Hoyt's formula (Hoyt, 1979). Measurements were made by N.E.L. from 1999 to 2015 and C.J.T. in 2017 and 2018. There was no significant difference in measurements between the researchers (variance: $F_{9,7} = 1.03$, $P = 0.99$; equal variance two sample t test: $t_{16} = 0.09$, $P = 0.93$). Egg shape was approximated using a ratio calculated by dividing egg breadth (maximum diameter; Hoyt, 1979) by egg length (the longest plane of the egg, Mason & Rothstein, 1986; Spottiswoode et al., 2011). For this egg shape ratio (which is only one possible measure of egg shape; others include egg asymmetry, Deeming, 2018; Zölei et al., 2012), a value of one represents a perfect sphere with eggs becoming longer and more narrow as values approach zero. Each female cuckoo exploits multiple different superb fairy-wren nests, creating a problem of pseudoreplication in the data set. To control for the use of multiple eggs from each female cuckoo, we attributed eggs to individual females based on either genetic analysis or eggshell pattern. For cuckoo eggs laid between 2000 and 2002, we used microsatellite analysis of sibling relatedness (Langmore et al., 2007). As the genetic analysis confirmed that eggshell patterns reliably identified the eggs of individual female cuckoos, we used this method to assign maternity of eggs thereafter (Langmore et al., 2007).

Our previous work at this study site showed that host females are more likely to desert nests containing a cuckoo egg if they witness the cuckoo parasitizing the nest; relocation of cuckoo eggs to the nests of different females (specifically a researcher adding the cuckoo egg to the nest without removing a host egg) resulted in lower rates of abandonment of nests than those that were naturally parasitized (Langmore et al., 2003). Therefore, we also recorded whether nests were naturally parasitized or cuckoo eggs were fostered to the host nest in this analysis.

Ethical Note

This research was conducted under approval of the Australian National University Animal Ethics Committee Protocol Numbers F. BTZ.61.03, A2012/47, A2016/33, A2017/35 and ACT Government Licence numbers LT1999021, LT200340, LT2004112, LT2005164, LT2006229, LT2011509, LT2012589, LT2013667, LT2014776, LT2015842, LT2016895, LT2017928 and LT20187.

The study used a wild population and required minimal disruption to superb fairy-wren nests to measure superb fairy-wren and Horsfield's bronze-cuckoo eggs. As soon as measurements were complete the researcher moved away from the territory as quickly as possible. Superb fairy-wren nests were checked every 3 days until the commencement of incubation, as this was the least number of approaches possible to still be able to know when the first egg was laid and when the clutch was complete. Before approaching the nest, the researcher scanned the area for potential predators. Nest survival was improved by the addition of a predator-excluding cage at many nests (see above).

Statistical Analysis

All analyses were run using the statistical package R (R Core Team, 2019); linear mixed models (LMM) and generalized linear mixed models (GLMM) were run using the lme4 package (Bates, Mächler, Bolker, & Walker, 2015).

Influence of climatic variables and host group size
Climate Windows. The Australian Bureau of Meteorology (http://www.bom.gov.au/climate/data/) provided daily maximum temperature, minimum temperature, rainfall and humidity data from a weather station located approximately 4 km from the study site. Humidity was measured as average daily vapour pressure (hPa, a measure of the amount of water particles in the air), average daily saturated vapour pressure (hPa, the point at which the air cannot hold any more water particles at the given temperature) and average daily relative humidity (%, the amount of water the air is holding as a ratio of the amount of water the air could hold at the given temperature). For 17 of 7411 (0.18%) days when data were not available from this site the next closest weather station with available data was used (10 days rainfall data from another weather station approximately 4 km from the field site and 3 days minimum temperature, 3 days saturated vapour pressure and 1 day vapour pressure data from another weather station approximately 18 km from the field site). Relative humidity was calculated as an average of the eight measurements taken each day. For 147 of 59 288 (0.25%) measurements, data were not available, so data from the closest weather station were used (139 measurements from another weather station approximately 18 km from the field site, one measurement from another weather station approximately 8 km away and seven measurements from a weather station approximately 147 km away). For three of 59 288 (0.01%) measurements, no substitute data were available and, in these cases, the relative humidity was calculated using the other available values from that day as eight measurements were taken per day. Relative humidity data were substituted or unable to be replaced for a total of 111 of 7411 (1.50%) days.

To investigate whether there were windows of climate sensitivity for cuckoo egg volume, we used the sliding windows approach in the R package climwin (Bailey & Van de Pol, 2015; van de Pol et al., 2016). This method is exploratory and looks for all possible climate windows that may be important for egg volume within a specified period and ranks their relative influence using values of the Akaike information criterion corrected for small sample size (AICc). The period investigated for a relationship between egg volume ($N = 122$) and climatic variables in our model was 150—0 days before egg laying. Eggs may be laid at any time over the 5 month breeding season, so relative windows were used to look at the period before each egg was laid. This means a period for each egg of 150—0 days before laying was analysed for each cuckoo egg, with day 0 being the lay date of the cuckoo egg. Thus, the period analysed was relative to the lay date but consistent in length for each egg. When the precise lay date was uncertain but

could be narrowed down to within 5 days, the median or earliest date of the two median dates was chosen. Cuckoo egg data were excluded when the possible lay date window was more than 5 days ($N = 8$). To account for measurement of multiple eggs laid by individual females, cuckoo ID ($N = 36$) was included in the model as a random effect. It was nested within breeding season ($N = 10$) because cuckoos banded at the study site have never been found to return in subsequent years (Langmore & Kilner, 2007). We tested the relationship between egg volume and six climatic variables: mean maximum and minimum temperature, mean rainfall, mean average daily relative humidity, mean average daily vapour pressure and mean average daily saturated vapour pressure. The cross-validation method was used to reduce the risk of overfitting (explained in van de Pol et al., 2016). All models were run to test for both a linear and a quadratic response between egg volume and climatic variables as this analysis method is exploratory and either option would make biological sense. A potential problem with this exploratory method is that climate windows can be detected by chance due to the large number of randomizations that are run. To determine whether the climate windows that were detected were likely to be from real climate signals rather than chance (type I error, overfitting), they were compared with those detected using the same model but with randomized data to remove any relationship between egg volume and climate variables.

Host Group Size. To test whether the volume of cuckoo eggs laid in the nests of host pairs or groups differed significantly, we created an LMM with restricted maximum likelihood. The fixed effect was helper presence (yes [$N = 32$] or no [$N = 89$]), as most groups had one helper (one helper: $N = 29$; two helpers: $N = 3$; average number of helpers $\pm$ SD $= 1.09 \pm 0.19$) presence of helpers was used rather than number of helpers, and the response variable was cuckoo egg volume ($N = 121$). To investigate whether there were any seasonal trends in egg size, we also included the number of days since 1 September (the earliest start date for superb fairy-wren egg laying) as a fixed effect. The model included a random effect of cuckoo ID nested within year to account for both multiple eggs from some females per year ($N = 36$) and any year effects. When cuckoo ID was unknown the cuckoo egg was given a number within the year the egg was measured ($N = 7$ eggs [6% of all eggs]). This is the most parsimonious approach as it does not assume the eggs are correlated with each other.

Do hosts abandon larger cuckoo eggs?

Several analyses were used to investigate whether superb fairy-wrens use egg shape or size to detect cuckoo eggs and abandon their nests. Hosts might detect larger eggs either by comparing them with the other eggs in the clutch, or by rejecting eggs above a certain absolute threshold in size or shape. Therefore, we performed two different analyses. First, to investigate whether egg volume or shape are used by hosts to detect cuckoo eggs and abandon their nest, we used a GLMM with a binomial distribution and logit link function. The response variable was host response (accept [$N = 64$] or desert [$N = 23$]). The fixed effects were cuckoo egg shape, cuckoo egg volume (both scaled and centred [mean of variable was subtracted from each value and the resulting value was divided by the standard deviation of the variable; Becker, Chambers, & Wilks, 1988] to aid with convergence) and the stage at which the cuckoo parasitized the nest (laying [$N = 82$] or incubating [$N = 5$]). An interaction between egg shape and volume was also tested for. Cuckoo ID was included as a random effect ($N = 26$) to control for both measurement of multiple eggs of individual females and year. A cuckoo egg was regarded as accepted if it was incubated by the female superb fairy-wren (the female is solely responsible for incubation in this species) for a minimum of 3 days

and abandoned if the female did not return to the nest, the eggs were cold and the female superb fairy-wren was still alive.

Second, for four traits (egg volume, breadth, length and shape), we investigated whether the cuckoo egg trait relative to the host egg trait (calculated by taking the average value across all host eggs in the parasitized clutch for each trait and subtracting the relevant cuckoo egg trait value, $N = 44$) affected the host response (deserted [$N = 9$] or accepted [$N = 35$]). Only naturally parasitized nests were used for this analysis. An equal variance two-sample t test was used as there was no significant difference in the variances (volume: $F_{8,34} = 1.243$, $P = 0.611$; breadth: $F_{8,34} = 1.234$, $P = 0.62$; length: $F_{8,34} = 1.225$, $P = 0.629$; shape: $F_{8,34} = 1.374$, $P = 0.486$).

To test whether exposure to the parasitism event interacted with cuckoo egg size or shape in determining whether hosts abandoned cuckoo eggs, we compared rejection rates of naturally laid cuckoo eggs with those that were fostered to a different fairy-wren nest by the researchers. We used a GLMM with a binomial distribution and logit link function. The response variable was host response (accept [$N = 81$] or desert [$N = 26$]). The fixed effects were fostered (yes [$N = 45$] or no [$N = 62$]), egg volume and egg shape (both egg volume and shape were scaled and centred (as described previously) to help with model convergence), and the interaction between egg volume and fostered and between egg shape and fostered. Cuckoo ID ($N = 32$) was included as a random effect.

We investigated whether cuckoo egg shape differed between cuckoo eggs that were accepted versus abandoned when nests were naturally parasitized, and between cuckoo eggs that were accepted versus abandoned when they were relocated to the nest. To do this we used an LMM with cuckoo egg shape as the response and an interaction between host response (accept [$N = 81$] or desert [$N = 26$]) and fostered (yes [$N = 45$] or no [$N = 62$]) as the fixed effect. Cuckoo ID ($N = 32$) was included as a random effect. We then used Tukey pairwise post hoc comparisons to assess whether shape differed significantly between accepted or abandoned eggs that were naturally parasitized or fostered.

RESULTS

Influence of Climatic Variables and Host Group Size

Climate windows

The sliding windows approach did not identify any climate windows that were likely to affect cuckoo egg volume (Table 1). All the best windows detected, with either a linear or a quadratic response, were unlikely to be real signals when compared with climate windows detected with randomized data. Instead, they were likely to have been detected by chance due to the large number of randomizations run in this exploratory method (van de Pol et al., 2016). This indicates that the mean temperature, mean rainfall and mean humidity at the laying site in the 150–0 days before the cuckoos laid their eggs did not affect the volume of the eggs laid.

Host group size

The volume of cuckoo eggs did not vary significantly in relation to host helper presence (LMM: $F_{1,95.03} = 0.05$, $P = 0.83$), indicating cuckoos do not adapt their egg volume to host group size. No seasonal trends in egg volume were detected (LMM: $F_{1,112.05} = 2.44$, $P = 0.12$). However, the random effect cuckoo ID nested in year accounted for a large amount of the variance (5945 ± 77.10) and had a significant effect on cuckoo egg volume ($P < 0.001$), indicating lower within-individual variation than between-individual variation in egg size.

Table 1
Sliding windows analysis of the effect of climate variables on Horsfield's bronze cuckoo egg size

Climate variable	Function	Best window	ΔAICc	Median window	P_c
T_{max}	Linear	86—86	-3.22	105—41	0.41
	Quadratic	62—62	-5.94	105—41	0.33
T_{min}	Linear	48—48	-3.37	107—43	0.34
	Quadratic	48—48	-4.70	109—44	0.47
Rain	Linear	119—104	-2.93	108—42	0.39
	Quadratic	4—3	-5.62	107—42	0.44
RH	Linear	96—96	-2.70	109—43	0.36
	Quadratic	150—114	-5.70	109—44	0.35
VP	Linear	82—82	-2.93	108—42	0.49
	Quadratic	50—49	-4.72	106—41	0.53
SVP	Linear	49—49	-2.64	105—41	0.43
	Quadratic	73—73	-5.13	105—41	0.37

Climate variables investigated were mean maximum temperature (T_{max}), mean minimum temperature (T_{min}), mean rainfall (rain), mean relative humidity (RH), mean vapour pressure (VP) and mean saturated vapour pressure (SVP). Both a linear and a quadratic response for each climate variable was investigated. The table lists the best window detected for each climate variable and its ΔAICc (corrected Akaike information criterion) value, the median window length for a subset of the data that has a 95% chance of containing the best window from all the windows detected and the probability of the best window being a false positive (P_c).

Are Hosts More Likely to Abandon Larger Cuckoo Eggs?

Rejection threshold

Cuckoo egg shape had a significant effect on egg outcome (GLMM: $X^2_1 = 5.44$, $P = 0.02$): cuckoo eggs with a relatively long and thin shape were accepted by the host significantly more often than eggs that were relatively short and wide (Fig. 1). In contrast, absolute egg volume had no effect on egg outcome (GLMM: $X^2_1 = 0.74$, $P = 0.39$) and the interaction between volume and shape was not significant (GLMM: $X^2_1 = 0.20$, $P = 0.65$). In addition, the stage at which the nest was parasitized, either when the superb fairy-wren female was laying or incubating, did not affect whether the cuckoo eggs were accepted or deserted (GLMM: $X^2_1 = 1.94$, $P = 0.16$). There was also no significant effect of cuckoo ID on egg outcome ($P = 0.14$).
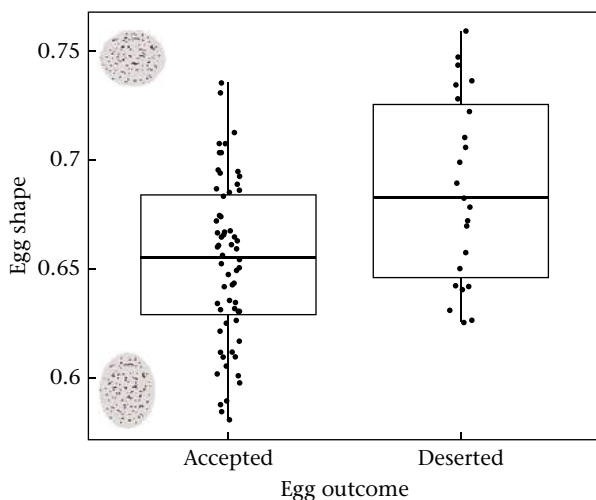
Figure 1. Box plot showing the shape of cuckoo eggs in relation to outcome (whether accepted or deserted by the host). The darker line in each box indicates the median, the upper and lower lines of each box show the minimum and maximum quantiles, the minimum and maximum values in the data spread are represented by the whiskers. The points show the data spread with a slight variation in the width of the point placement so the point density can be more easily observed.

Comparison of superb fairy-wren and cuckoo egg size

The volume or shape of a cuckoo egg relative to the average fairy-wren egg in the parasitized clutch did not affect the probability of nest abandonment (two-sample t test: volume: $t_{42} = 1.30$, $P = 0.20$; shape: $t_{42} = -1.34$, $P = 0.19$). Similarly, neither the breadth nor the length of a cuckoo egg relative to the mean breadth and length of the parasitized superb fairy-wren clutch affected the probability of nest abandonment (two-sample t test: breadth: $t_{42} = 0.61$, $P = 0.55$; length: $t_{42} = 1.20$, $P = 0.06$).

Does Egg Shape Affect Host Response in Foster Nests?

The interaction between exposure to a natural parasitism event and cuckoo egg shape affected whether nests with cuckoo eggs were abandoned (GLMM: $X^2_1 = 4.14$, $P = 0.04$). The shortest and widest eggs that were laid naturally in the host nest were the most likely to be abandoned, indicating that both the presence of the adult cuckoo at the nest and a short, wide egg shape are triggers for nest desertion. However, the interaction between exposure to a parasitism event and cuckoo egg volume did not affect whether nests containing cuckoo eggs were abandoned by the host (GLMM: $X^2_1 = 1.06$, $P = 0.30$). There was no significant effect of cuckoo ID on host response (GLMM: $X^2_1 = 0.21$, $P = 0.64$).

There was no unconscious bias from researchers in terms of the shape of eggs that were fostered versus not fostered (LMM: $X^2_1 = 2.67$, $P = 0.10$). In nests that were naturally parasitized, rejected eggs were significantly shorter and rounder than accepted eggs (Tukey test: $P = 0.004$; Fig. 2). However, in nests where cuckoo eggs were fostered to the nest, shape did not differ significantly between accepted and rejected eggs (Tukey test: $P = 0.98$; Fig. 2).

DISCUSSION

Our results suggest that cuckoo egg size is influenced primarily by selection for egg shape mimicry by the host and is not modulated in relation to the prevailing environmental conditions. Although the host superb fairy-wrens modulate their egg size in relation to both the climate and their group size (Langmore et al., 2016; Russell et al., 2007), the size of cuckoo eggs laid in their nests did not show corresponding variation in relation to these cues. Neither the climate nor host group size affected cuckoo egg volume. By contrast, cuckoo egg shape did appear to be influenced by host selection pressures, because cuckoo eggs that were relatively shorter and rounder were deserted by the host more often. We also found evidence that superb fairy-wrens use a combination of adult cuckoo presence and cuckoo egg shape to detect cuckoo eggs and desert parasitized nests.

Influence of Climatic Variables and Host Group Size

We found no evidence that Horsfield's bronze-cuckoos adapt their egg size to the prevailing climate or to host group size, unlike their host, the superb fairy-wren (Langmore et al., 2016; Russell et al., 2007). There are several possible explanations for this: (1) limited exposure of migratory cuckoos to the environmental conditions at the breeding site prior to the commencement of breeding (Langmore et al., 2007); (2) the environment might have different effects on cuckoos and their hosts (Avilés, Stokke, Moksnes, Røskaft, & Møller, 2007); or (3) selection pressure on egg size from the host may override that from the environment (Aviles et al., 2012). We found evidence for the latter, as cuckoo eggs with a short, round shape were rejected by hosts significantly more than those with a long, narrow shape (see below).
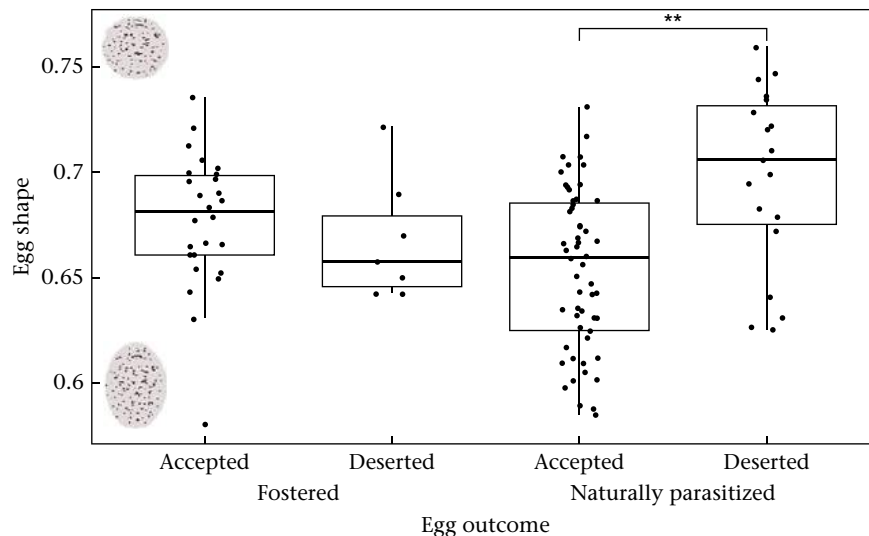
Figure 2. Box plot showing the shape of cuckoo eggs in nests that were naturally parasitized or received a foster cuckoo egg (the egg was relocated by a researcher) in relation to outcome (whether accepted or deserted by the host). The darker line in each box indicates the median, the upper and lower lines of each box show the minimum and maximum quantiles, the minimum and maximum values in the data spread are represented by the whiskers. The points show the data spread with a slight variation in the width of the point placement so the point density can be more easily observed. **$P < 0.01$.

Are Hosts More Likely to Abandon Larger Cuckoo Eggs?

We found no evidence that superb fairy-wrens were more likely to desert eggs of larger volume. However, they did use egg shape to detect and desert nests containing cuckoo eggs. This provides support for the hypothesis that tactile cues are important for egg detection by dome-nesting species, owing to low visibility in their nests (Attard et al., 2017; Langmore et al., 2003; Marchetti, 2000; Mason & Rothstein, 1986).

Our study shows that superb fairy-wrens use overall egg shape rather than relative egg shape to detect cuckoo eggs. This indicates that they do not compare the eggs in their nest to detect cuckoo eggs and suggests that there may be a shape rejection threshold instead. We did not find any evidence that egg length or egg breadth individually were used as cues to detect cuckoo eggs. This is somewhat surprising as Horsfield's bronze-cuckoo eggs have a long, thin shape which we predicted may indicate selection on egg breadth (Latter, 1902; Segura, Di Sallo, Mahler, & Reboreda, 2016). However, it may be that selection for a decreased breadth selects for a corresponding increase in length as compensation, thereby ensuring that the overall size of the egg is not reduced to the detriment of the developing chick. In line with this, a study of eggs of 181 bird species found egg composition to be related to egg shape: eggs with a high proportion of yolk were longer and thinner (Deeming, 2018). If this is true of cuckoo eggs, it could explain their shape. This would be an interesting avenue for future research. Another possibility is that egg shape at the egg poles may be important for egg detection, as cuckoo eggs have two blunt poles while fairy-wrens have one blunt and one tapered pole. However, the discrepancy with cuckoo egg poles not matching those of their host may indicate that this egg trait is not important for egg detection or that it is too costly for the cuckoo to taper one end with a large chick housed inside. This idea requires testing (Polačiková & Grim, 2010; Zölei et al., 2012).

While superb fairy-wrens have been found to reject eggs that are substantially larger than their own (Langmore et al., 2003), we did not find an effect of cuckoo egg volume on nest desertion. The model eggs used to parasitize superb fairy-wrens in our previous study (Langmore et al., 2003) were substantially larger (20 x 15 mm) than average Horsfield's bronze-cuckoo eggs

(18 x 12 mm), and were designed to replicate the eggs of the much larger fan-tailed cuckoo. Rejection of large eggs by the host can explain the small size of the Horsfield's bronze-cuckoo egg relative to its body size (Krüger & Davies, 2002; Langmore et al., 2003), and suggests that the overall size of Horsfield's bronze-cuckoo eggs is now below the egg size rejection threshold of superb fairy-wrens (Davies & Brooke, 1988). Thus, egg size may no longer be a reliable cue for egg rejection, because as egg size becomes more similar between host and parasite, reliability of size as a cue for rejection decreases as the chance of rejection errors increases (Guigueno, Sealy, & Westphal, 2014).

Previous work has shown that hosts are more likely to desert a nest that is parasitized during incubation (as they are more likely to see the parasitism event) than when the egg was placed in the nest by the researcher (Langmore et al., 2003). We found that superb fairy-wrens were more likely to desert naturally parasitized nests when cuckoo eggs were short and round, but when cuckoo eggs were placed in superb fairy-wren nests by researchers there was no significant difference in the shape of eggs that were accepted and deserted. These findings suggest that superb fairy-wrens use a combination of cuckoo presence and cuckoo egg shape to detect and desert cuckoo eggs. Similarly, a combination of cues are used by meadow pipits, *Anthus pratensis*, red-crested cardinals, *Paroaria coronata*, chalk-browed mockingbirds, *Mimus saturninus*, and tawny-flanked prinias, *Prinia subflava*, to detect and reject foreign eggs (de la Colina, Pompilio, Hauber, Reboreda, & Mahler, 2012; Moksnes & Røskaft, 1989; Segura et al., 2016; Spottiswoode & Stevens, 2010). Similar to our findings, meadow pipits use cuckoo presence as a cue to initiate egg detection and, in 50% of cases, cuckoo presence led to nest desertion when a model cuckoo egg was also placed in the nest (Moksnes & Røskaft, 1989). Superb fairy-wrens also use a combination of cues to detect cuckoo nestlings, using the presence of adult cuckoos in the vicinity as well as a lone chick in the nest as cues that they have been parasitized (Langmore, Cockburn, Russell, & Kilner, 2009).

In summary, we suggest that selection pressure from the host is greater than selection pressure from the environment on Horsfield's bronze-cuckoo eggs. Superb fairy-wrens appear to use both the presence of an adult cuckoo near the nest and overall cuckoo egg shape to detect parasitism and desert their nest. Our study

illustrates the importance of looking for multiple cues for parasitism detection by cuckoo hosts. Future studies could explore the possibility of the importance of egg asymmetry as a cue for cuckoo egg detection by superb fairy-wrens.

Data Availability

The data for this study have been archived with Mendeley (Taylor & Langmore, 2020).

Acknowledgments

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Chapter 5

Is breeding phenology changing in response to climate and host breeding
season in the brood parasitic Horsfield's bronze-cuckoo?

This chapter details the analysis of a long-term dataset collected by NEL over 21 years
on phenology of superb fairy-wrens and Horsfield's bronze-cuckoos. NEL and CJT
conceived the idea together, CJT led the refinement of the concept, analysed the data,
wrote the manuscript and both authors contributed to revising the manuscript and
approved the final manuscript.

Is breeding phenology changing in response to climate and host breeding season in the brood parasitic Horsfield's bronze-cuckoo?

Claire J. Taylor and Naomi E. Langmore

Abstract

Climate change is impacting the phenology of species, which are responding at different rates particularly due to differing life history strategies. Migratory brood parasitic cuckoos are at risk of phenological mismatches with their resident hosts, as hosts may be better placed to align their breeding phenology with changes in resources at the breeding site. Horsfield's bronze-cuckoos are migratory and nomadic brood parasites that parasitise the nests of resident superb fairy-wrens. We used a long-term dataset to investigate whether cuckoo arrival to, and departure from, the breeding site has changed in relation to the climate at the breeding site and the breeding phenology of their preferred host, and if any mismatch has impacted the breeding success of cuckoos. Cuckoo arrival date was not influenced by climate at the study site or the start date of the hosts breeding season. In contrast, cuckoos departed earlier when it was either particularly cold and wet or particularly hot and dry, and when their hosts stopped breeding earlier. We found little evidence of a phenological mismatch between cuckoos and their hosts, although trends suggest cuckoo arrival date has become later, and the availability of host nests for parasitism has decreased. In addition, parasitism rate was higher when cuckoos arrived at the study site earlier in relation to the hosts breeding start date. This suggest that there is the potential for a mismatch in the future, with negative consequences for cuckoo breeding success and possibly selection for cuckoos to arrive earlier than the start of their hosts breeding season.

Key words: phenological mismatch, arrival date, departure date

Introduction

Species respond to climate change in many ways and at varying rates, especially across trophic levels and with differing life history strategies (Parmesan and Yohe 2003; Visser et al. 2004; Parmesan 2006; Both et al. 2009). Climate change does not have an equal global effect (IPCC 2007) or even an equal effect on all species in the same area, as species may vary in their phenological response rate to environmental cues (Dewar and Watt 1992; Soler et al. 2014; Thackeray et al. 2016). This means the dynamics within systems are changing and could lead to phenological mismatches in previously closely linked systems such as predator-prey, and parasite-host systems (Visser et al. 1998; Hughes 2003; Parmesan 2006; Jones and Cresswell 2010; Merino and Møller 2010; Kharouba et al. 2018).

Generally, climate change is causing higher spring temperatures, which has resulted in the phenology in temperate areas advancing, such as earlier bud bursts and leaf emergence (Parmesan 2006; IPCC 2007). Advancement in phenology at lower trophic levels may also select for advancement in the breeding phenology of species at higher levels in the ecosystem, such as insects and their insectivorous predators, to match their resources (Cresswell and McCleery 2003; Marra et al. 2005). In addition, higher spring temperatures are not only advancing the timing of insect abundance, but also cause larvae to grow more quickly, resulting in a shorter duration of prey abundance for insectivores (Buse et al. 1999). Climate change has led to many bird species advancing their lay date to align with the earlier peak in abundance of prey to feed their offspring (Lack 1954; Crick et al. 1997; Crick 2004; Dunn and Møller 2014). Some species that have not advanced their lay date, or not to the same extent as their resources, are under increasing selection pressure to advance their breeding (Van Noordwijk et al. 1995; Both and Visser 2001). To adapt to warmer spring temperatures, individuals must

be able to respond to cues from the environment that are indicators of future conditions (Visser et al. 1998; Bonamour et al. 2019).

Resident species may be better placed to adapt to environmental cues and track the advancement of their food resources in relation to breeding than migratory species, due to the year-round climate cues readily available to the resident species (Avilés et al. 2014). For migratory species, these cues are largely inaccessible until they reach their breeding ground. Some cues that migratory species previously relied upon for timing of migration for breeding success in the past may have been decoupled by climate change (e.g. rainfall and food avalability at wintering grounds; Studds and Marra 2011; Bonamour et al. 2019), whereas others might not be influenced by climate change (e.g. endogenous timing cues such as photoperiod; Both and Visser 2001; Walker et al. 2019). Migratory species are being affected by climate change to varying degrees (Jones and Cresswell 2010). The effect of climate change on breeding phenology has been found to differ with length of migration and between migratory and resident species (Halupka and Halupka 2017). Some species have advanced their migratory date and remain in synchrony with their resources at the breeding grounds while others have not, or have to a lesser extent than their resources at the breeding grounds (Both and Visser 2001; Marra et al. 2005). Due to differing responses to climate change, resident and migratory species may experience changes in their interactions at the breeding grounds, such as competition for resources (Ahola et al. 2007) and differing availability of hosts for parasitism (Møller et al. 2013; Avilés et al. 2014).

Migratory, obligate brood parasitic cuckoos are at risk of a mismatch with their hosts as a result of changes in host breeding phenology driven by climate change. Brood parasitic cuckoos dump their eggs in the nests of their hosts, to be raised entirely by the hosts (Davies 2000). Host nest availability in relation to when cuckoos arrive at their breeding grounds may greatly influence their breeding success (Saino et al. 2009; Avilés et al. 2014). Resident hosts are more likely to be exposed to cues about local food availability

and climate than migratory cuckoos, so they are more likely to be able to adjust their timing of breeding in response to these cues (Both and Visser 2001; Møller et al. 2011; Avilés et al. 2014). For example, the migratory, brood-parasitic common cuckoo *Cuculus canorus* is failing to match the advancement in breeding phenology of some of its non-migratory hosts, thus leading to a reduction in the time available for parasitism (Saino et al. 2009). This mismatch is thought to have contributed to the decline in common cuckoo numbers in Europe in recent decades (Saino et al. 2009). Another potential cause for mismatches between cuckoos and their hosts is changes in the duration and number of nesting attempts of the hosts over the breeding season. There is evidence suggesting that the number of nesting attempts in some double brooding species is at risk of decreasing in frequency due to decreased periods of insect abundance associated with earlier warm spring temperatures (Topp and Kirsten 1991; Visser et al. 1998; Husby et al. 2009; Glądalski et al. 2016). Due to advances in timing of host reproduction some cuckoo species now rely on the second brood of the host species (Douglas et al. 2010). For instance common cuckoos in Europe have advanced migration enough to keep up with long-distance migrant hosts, but not short-distance migrant hosts (Saino et al. 2009), so may rely on the second broods of short-distance migrant hosts for parasitism. This poses a major problem for cuckoo breeding success if subsequent broods become less frequent and host breeding season duration decreases. However, spring temperature changes influence both timing of breeding and food availability, and these factors may have different levels of influence on double brooding. Great tits were found to produce more second broods in warmer years due to the comparatively earlier start date of breeding (Glądalski et al. 2016), but this was also dependent to a lesser extent on food availability. In many species, individuals that breed earlier have higher breeding success, indicating selection for earlier breeding (Dunn et al. 2011; Dunn and Møller 2014; Harriman et al. 2017). One reason for higher breeding success with early breeding may be that it allows more time for subsequent broods during the season (Townsend et al. 2013). A meta-analysis of long term data for 54 species found multi-brooded bird species increased the duration of their breeding season over the 45 year study period

(Halupka and Halupka 2017), adding further support to earlier studies finding multi-brooded species were extending their breeding season (Halupka et al. 2008; Møller et al. 2010; Dunn and Møller 2014). This suggests that there may be a greater window of nest availability for cuckoos to target. More research is needed to look at the effect of climate change on the duration of the breeding season in multi-brooded species and what this means for the breeding success of cuckoos that parasitise them.

Mismatches between migratory brood parasitic cuckoos and their hosts have been found in species in the northern hemisphere (Saino et al. 2009; Douglas et al. 2010; Avilés et al. 2014; Yun et al. 2020), but no studies have looked the effect of climate change on these interactions in species in the southern hemisphere. The impact of climate change on cuckoo-host dynamics in the southern hemisphere may differ from the northern hemisphere due to factors such as relatively longer host breeding seasons (Englert Duursma et al. 2017), greater plasticity in the timing of the breeding season of hosts (Englert Duursma et al. 2019), and a greater proportion of species showing partial migration or nomadism in the southern hemisphere rather than intercontinental migration (Higgins 1999; Dingle 2008; Chambers et al. 2013). Investigation of the impact of climate change on cuckoo-host dynamics is particularly vital in Australia, where several species of cuckoos and some of their hosts are among the most severely declining species of insectivorous birds (Gardner and Heinsohn 2007; Cunningham and Olsen 2008; Szabo et al. 2008). Our study investigates the potential phenological mismatch of migratory brood parasitic Horsfield's bronze-cuckoos *Chalcites basalis* to the breeding season of their preferred resident hosts in south-eastern Australia, superb fairy-wrens *Malurus cyaneus* (Brooker and Brooker 1989; Langmore and Kilner 2007). The interactions between the migratory Horsfield's bronze-cuckoos (referred to as cuckoos hereafter), and its multi-brooded, resident host, the superb fairy-wren, provide an ideal system to investigate the potential effects of climate change on phenology and adaptations to climate change in species with different life history strategies. We use a

long-term dataset to investigate whether cuckoo arrival to, and departure from, the breeding site is changing in relation to the climate at the breeding site and the breeding phenology of their preferred hosts. Furthermore, we assess whether the time between cuckoo arrival and the start of the host breeding season has an impact on the breeding success of cuckoos. Specifically, we predict that: i) cuckoo arrival and departure dates and breeding duration will be influenced by temperature and rainfall at the breeding site; ii) changes in breeding phenology in response to climate will differ between migratory cuckoos and resident hosts, iii) an increasing mismatch between the breeding phenology of cuckoos and their hosts will have a negative impact on cuckoo breeding success, and iv) cuckoo breeding success will vary with the number of host nests available for parasitism.

Methods

Study site and species

Superb fairy-wrens are multi-brooded, cooperatively-breeding, insectivorous passerines that are common and resident throughout south-eastern Australia (Cockburn et al. 2016). They lay clutches of 2-5 eggs (usually 3 or 4) between August and February, usually between September and January. Superb fairy-wrens are the primary hosts of obligate brood-parasitic, migratory Horsfield's bronze-cuckoos in south-eastern Australia (Langmore and Kilner 2007). Horsfield's bronze-cuckoos occur from the Malay Peninsula south to the Lesser Sundas and Australia. Their movements vary throughout their distribution, ranging from being resident in northern areas of their range to migratory in the south, and sometimes showing irregular movements suggesting nomadism (Higgins 1999). Their numbers are in decline in Australia (Szabo et al. 2008). They show a serially monogamous breeding system, in which females form temporary partnerships of 2-12 weeks duration with males on exclusive breeding territories, before departing and subsequently being replaced by another female (Langmore et al. 2007).

This study analyses a long-term data set on the breeding biology of Horsfield's bronze-cuckoos and superb fairy-wrens collected over 21 years (1999-2020) in Campbell Park, Canberra in south-eastern Australia (149°10′E, 35°16′S; Langmore *et al.*, 2016). Campbell Park is a 230 ha open eucalypt woodland that is occupied by between 21 and 66 superb fairy-wren groups each year (average = 42). Most superb fairy-wrens were colour banded for identification and in most years all breeding attempts were monitored (incomplete monitoring in 2003-2005, 2007-2010, 2016). Nests were checked every 3 days after nest building commenced to determine when the first host egg was laid and checked for cuckoo eggs on completion of the clutch. We recorded superb fairy-wren annual date of commencement of breeding (first egg laid), the date of the first superb fairy-wren egg laid in all subsequent nests throughout the breeding season (for 13 years), total number of cuckoo eggs laid throughout the breeding season and annual cuckoo parasitism rates (number of superb fairy-wren nests parasitised by Horsfield's bronze-cuckoos divided by the number of superb fairy-wren nests in which eggs were laid during the breeding season).

Horsfield's bronze-cuckoos are a highly vocal species with far-carrying calls (Higgins 1999), so their arrival on the study site is readily detected, as is their subsequent departure. Researchers were present at the study site most days between Mid-August and February and recorded when they first heard or saw cuckoos. For each year, we recorded the cuckoo arrival date (the earliest date on which there was evidence of a Horsfield's bronze-cuckoo at the study site – either a cuckoo was seen or heard, or a cuckoo egg was found in a SFW nest), the initiation of breeding date (first cuckoo egg laid in a fairy-wren nest), the annual cuckoo departure date (the latest date on which there was evidence of cuckoo presence at the study site - either a cuckoo was seen or heard or a cuckoo egg was laid in a SFW nest). In some years cuckoos were not present at the breeding site, so arrival and departure dates could not be recorded for these years (*N*=7 years, departure date was not recorded in one additional year).

At a nearby study site, superb fairy-wrens breeding start date was earlier with higher minimum temperatures in early spring (Lv et al. 2020), however it is unknown how climate affects cuckoo arrival times and breeding duration. To assess the impact of climate on the interaction between species we looked at the extent to which weather impacts the arrival and departure of cuckoos at the breeding site. Daily rainfall, maximum and minimum temperature data were sourced from the Australian Bureau of Meteorology from the nearest available site approximately 4 km from the study site (http://www.bom.gov.au/climate/data/).

Statistical analysis

Cuckoo arrival, departure and duration

To investigate the effect of climate and host nest availability on cuckoo arrival date we used a linear model (LM). The response was the cuckoo arrival date (in Julian days [number of days since 1st January]; N=14 years), with fixed effects of date the first host egg was laid each breeding season, mean minimum temperature for 17 days from the 19th of August to the 4th of September (this time window was previously found to be important for superb fairy-wren breeding season start date, as low minimum temperatures may constrain the start of the hosts breeding season due to higher thermoregulatory cost or lower food availability; Lv et al. 2020) and the sum of winter rainfall (which is likely to influence vegetation and insect abundance at the time cuckoos arrive in late winter/early spring). An interaction between temperature and rainfall was also included.

To investigate if cuckoo departure date is influenced by climate, we used a LM. The response was cuckoo departure date (as a Julian date; N=13 years). We assessed the effect of the sum of rainfall and the mean maximum temperature over December and January (the months when the host typically finishes egg laying), on cuckoo departure date. An interaction between temperature and rainfall was also included.

To investigate if cuckoo departure date is influenced by host nest availability we used a linear regression (LR). The effect of host nest availability on cuckoo departure date was investigated separately to climate because the last host egg date was significantly correlated with rainfall over December and January (Pearson's r (11)=0.71, P=0.007). Cuckoo departure date (as a Julian date; N=13 years) was the response and the date of the last host egg was the fixed effect.

To assess if the duration of cuckoo presence is determined by the duration of the host breeding season we used a LR. The response was the duration of cuckoo presence at the breeding site (N=13 years) and the fixed effect was the duration of the host breeding season. We also assessed if the duration of cuckoo presence was related to host density at the breeding site using an LR. The duration of cuckoo presence at the breeding site (N=13 years) was the response and host density (the number of host territories divided by the size of the study area each year) was the fixed effect.

Phenological Mismatch

To assess if cuckoo arrival date is changing over time, we used a LR with cuckoo arrival date as the response (N=14) and year as the fixed effect. The slope was extracted to assess the relationship between arrival date and year. Similarly, we assessed if the initiation date of host laying is changing over time using an LR with first egg date (N=14 years) as the response and year as the fixed effect. Once again, the slope was used to assess the relationship between lay date and year. Furthermore, following the analysis of Drake and Martin (2018), we tested the degree of covariation in the arrival of cuckoos to the breeding site and initiation date of the host laying using a pair-wise correlation (N=14 years).

To assess if the annual duration of cuckoo presence has changed over the study period, we used an LR with the duration of cuckoo presence (N=13 years) as the response and year as the fixed effect. We also assessed if the duration of the host breeding season has

changed over the study period, using the duration of the host breeding season (N=13 years) as the response and year as the fixed effect.

We assessed the opportunity cuckoos have to parasitize host nests by looking at the proportion of host nests (of the total number of host nests during the season) that are within the time period cuckoos are present at the breeding site (following the analysis of Douglas et al. 2010). We used a generalized linear model (GLM) with a binomial response and logit link function weighted by the number of host nests during the breeding season, to account for the response variable being the proportion of host nests that were within the time period cuckoos were present at the breeding site (N=13 years). The fixed effect was year.

To investigate if the timing between cuckoo arrival and host initiation of lay date is changing, we used a LR. The number of days between the first cuckoo presence and the laying date of the first host egg of the breeding season (N=14) was used as the response variable, and year was the fixed effect.

Cuckoo breeding success

To investigate if the timing between cuckoo arrival and host initiation of lay date affected the parasitism rate, a GLM with a binomial response and logit link function weighted by the number of host nests, to account for the response variable being the proportion of host nests parasitized. The response was the proportion of host nests that were parasitized by cuckoos each year (N=13 years) and the number of days between the first cuckoo presence and the laying date of the first host egg of the breeding season was the fixed effect.

To test if parasitism rate is affected by the host density and rainfall during the breeding season (Langmore and Kilner 2007), we used a GLM with a binomial response and logit link function weighted by the number of host nests, to account for the response variable being the proportion of host nests parasitized. The response was the proportion of host

nests that were parasitized by cuckoos each year (N=13 years) and host density (the number of host territories divided by the size of the study area each year) and rainfall during the breeding season (the sum of rainfall from September-December, rainfall in this period was previously found to interact with territory density and impact parasitism rate; Langmore and Kilner 2007) were the fixed effect. An interaction between host density and rainfall was also included.

To investigate if parasitism rate is affected by the duration of the host's breeding season, we used a GLM with a binomial response and logit link function weighted by the number of host nests, to account for the response variable being the proportion of host nests parasitized. The response was the proportion of host nests that were parasitized by cuckoos each year (N=13 years). The fixed effect was the duration of the host breeding season.

Results

Cuckoo arrival, departure and duration

Arrival of cuckoos at the breeding site (earliest arrival date was 21st of August and the latest arrival date was the 8th of October) was not influenced by the commencement of host breeding (LM: $F_{1,9}$=0.21, P=0.66) or the winter climate (LM: minimum temperature: $F_{1,9}$=0.02, P=0.91; rainfall: $F_{1,9}$=0.23, P=0.64; interaction $F_{1,9}$=1.30, P=0.28; significance of the fixed effects was determined in the same model without the interaction as it was not significant, this did not change the outcome for any of the variables) at the breeding site prior to the breeding season.

The departure date of cuckoos was significantly influenced by the interaction between total rainfall and mean maximum temperature over December and January (LM: $F_{1,9}$=8.75, P=0.02). Cuckoos departed earliest when either mean maximum temperature was high and rainfall was low, or when mean maximum temperature was low and rainfall was high, whereas they departed latest when mean maximum

temperature and rainfall were low (Figure 1). In other words, when conditions were either, particularly wet and cold, or particularly hot and dry cuckoos left the breeding site earlier. Cuckoo departure date also showed a near-significant tendency to be positively related to the end date of the host's breeding season (LR: $F_{1,11}=4.54$, $P=0.06$), indicating that cuckoos tend to depart later when hosts breed for longer (Figure 2).

Duration of cuckoo presence at the breeding site was not significantly influenced by the duration of the host breeding season (LR: $F_{1,11}=0.08$, $P=0.78$) or the host density at the breeding site (LR: $F_{1,11}=0.92$, $P=0.36$).
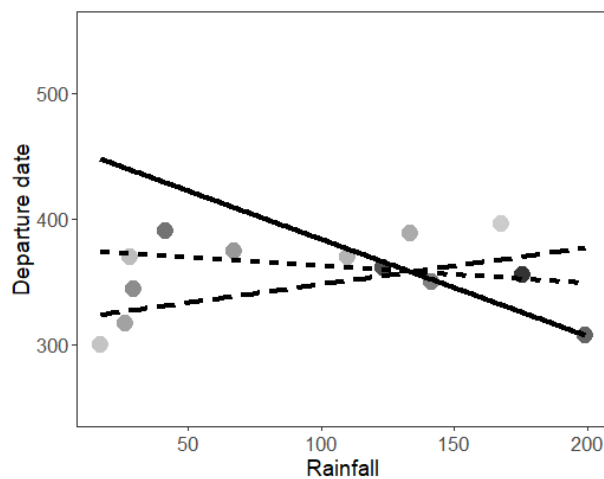
Figure 1. Model predictions of the effect of the interaction between the sum of rainfall and mean maximum temperature during December and January on cuckoo departure date, plotted on the raw data (darkness of point indicates temperature, with the lowest temperatures being darkest and colour progressively lightening as temperature increases). The model predicts that when mean maximum temperature is low (solid line; prediction based on lowest mean maximum temperature in dataset, 24.5℃) cuckoos depart later when there is less rainfall, however when mean maximum temperature is high (dashed line; prediction based on highest mean maximum temperature in dataset, 31.9℃) cuckoos depart later when there is more rainfall. Predictions for the average mean maximum temperature are shown by the dotted line (prediction based on average mean maximum temperature in dataset, 28.92℃).
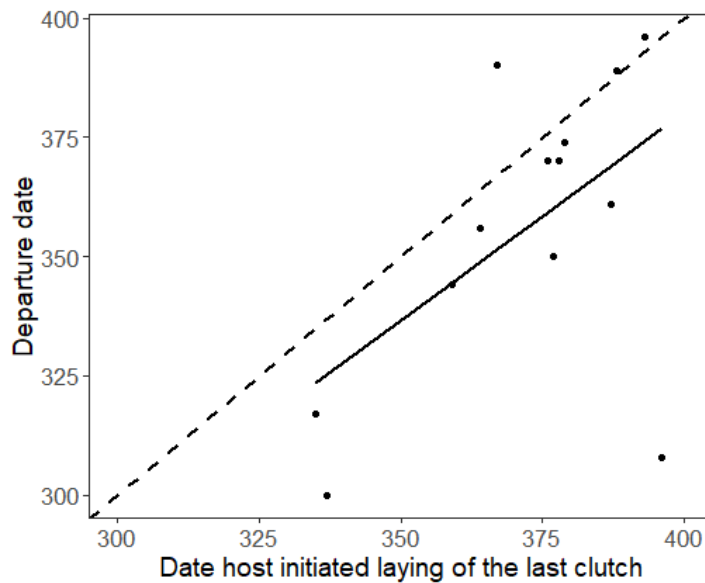
Figure 2. Model predictions of cuckoo departure date in relation to the date the host lays the last egg of the last clutch of the breeding season. The dashed line indicates y=x for comparison.

Phenological mismatch

The number of host nests available whilst cuckoos were present at the breeding site, as a proportion of the total number of nests each year (Figure 4), declined significantly over the study period (GLM: X^2_1=19.74, P<0.001; Figure 3).
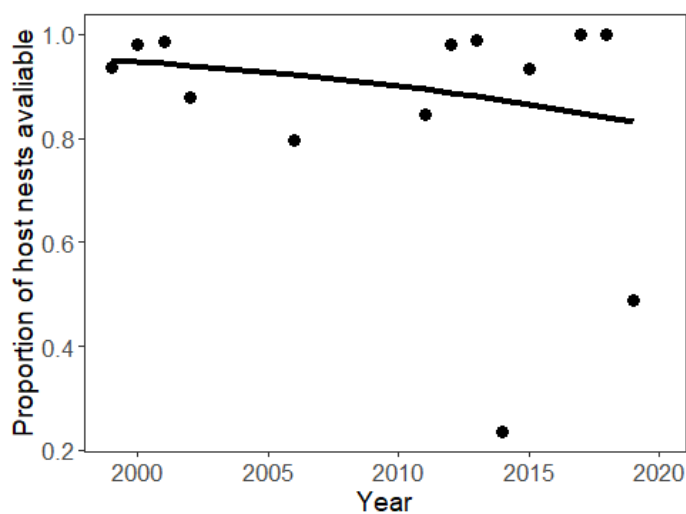

Figure 3. Raw data and model predictions of the proportion of host nests that are available whilst cuckoos are present at the breeding site.
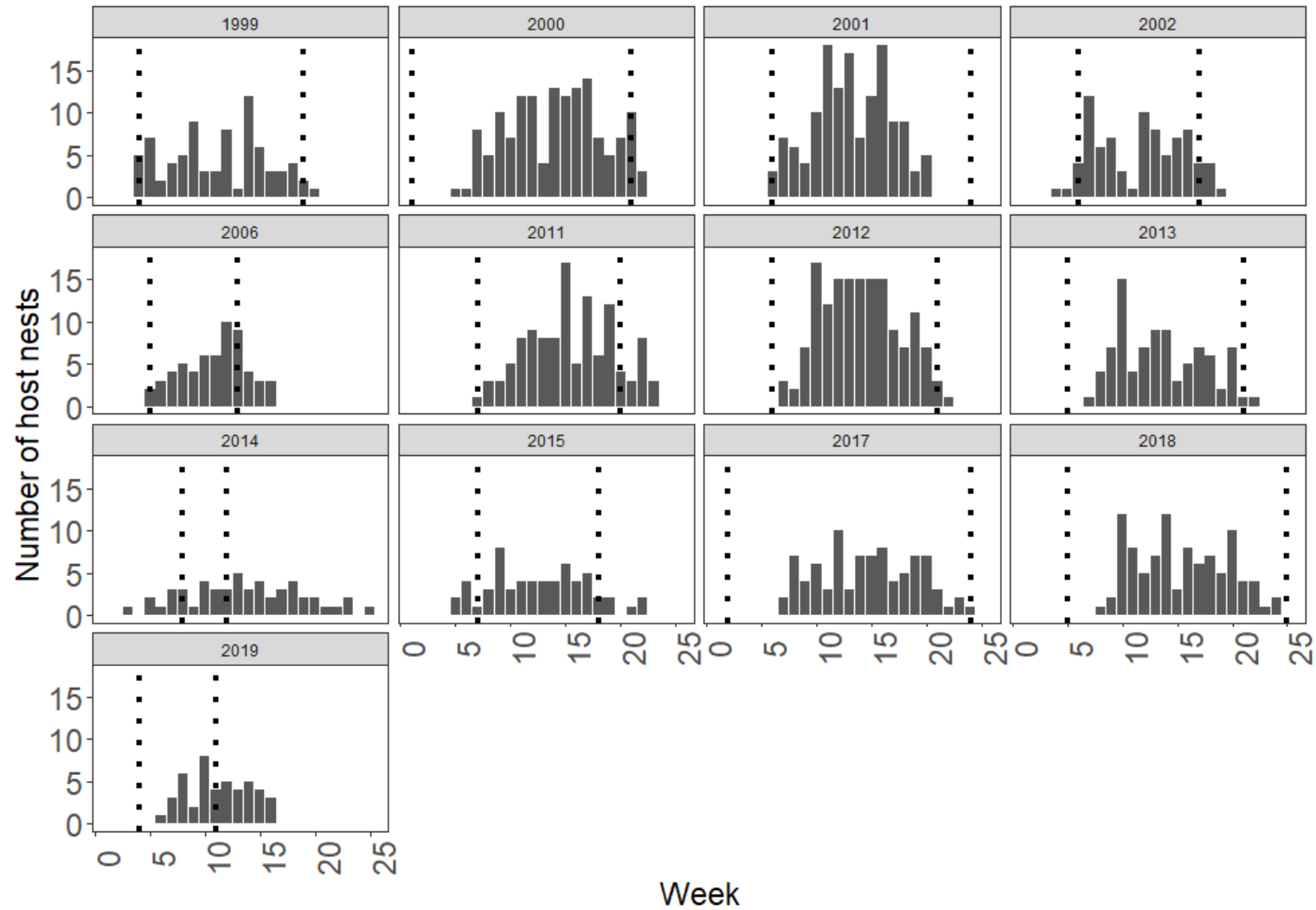
Figure 4. The number of host nests initiated (first egg laid) each week over the breeding season (week 1 begins on the 16th of August each year) and cuckoo arrival and departure date (indicated by the vertical dotted lines) in relation to host nest distribution over the breeding season.

Both cuckoo arrival date and the start of the host breeding season have a positive relationship with year (cuckoo arrival: slope=0.24; host breeding season: slope=0.47), indicating they are both predicted by the model to be occurring later in more recent years than in past years (Figure 5). However, neither cuckoo arrival date (LR: $F_{1,12}$=0.26, P=0.62), nor the start of the host breeding season (LR: $F_{1,12}$=1.48, P=0.25) have changed significantly over the study period. The relationship between the start of the host breeding season and cuckoo arrival at the breeding site is weakly negative (cor=-0.16), but not significantly so (Pearson's r (12)=-0.16, P = 0.58), suggesting that cuckoo arrival date does not co-vary with the start of the host breeding season.
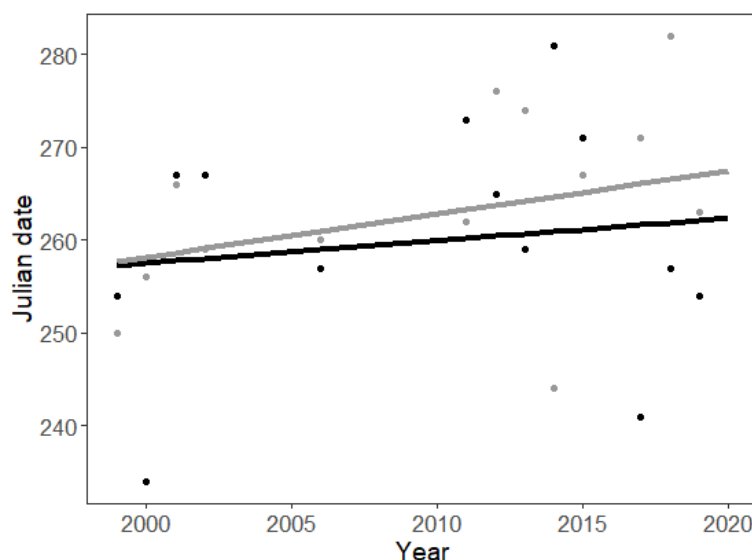
Figure 5. Raw data with model predictions for cuckoo arrival date (black line) and the commencement of host breeding (grey line) over the study period.

The discrepancy between the timing of breeding in the hosts (date host egg first laid) and the timing of arrival of cuckoos at the breeding site (first cuckoo presence detected) has not changed significantly over the study period (LR: $F_{1,12}$=0.11, P=0.74). However, the relationship of the timing between cuckoo and hosts, and year is positive (Figure 6; slope=0.22), indicating that cuckoos may be generally arriving slightly later than they have in the past in relation to host breeding season start date.

Neither the duration of the host breeding season (LR: $F_{1,11}=0$, $P=1.00$), nor the duration of cuckoo presence at the breeding site (LR: $F_{1,11}=0.27$, $P=0.62$) has changed significantly over the study period.
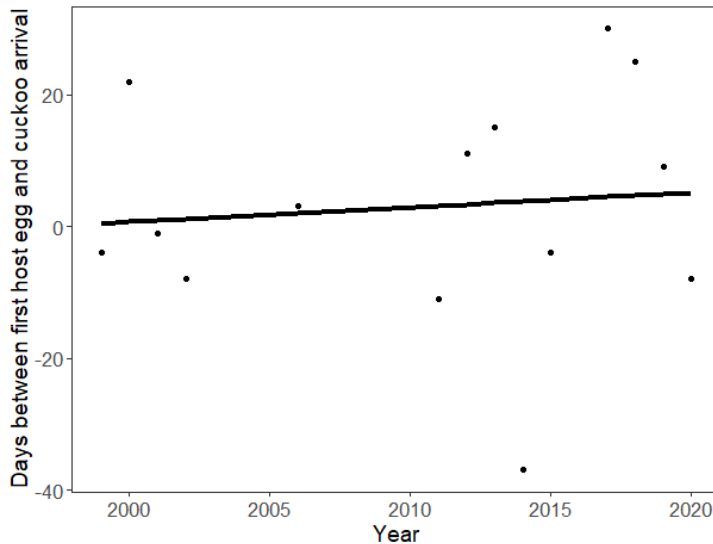

Figure 6. Raw data and model predictions of the number of days between the date the first host egg was laid and the first cuckoo presence was detected each year. Negative values on the y-axis indicate the number of days cuckoos arrived after the host first egg was laid and positive values indicate the number of days cuckoos arrived before the host first egg was laid.

Cuckoo breeding success

Horsfield bronze-cuckoo parasitism rate of superb fairy-wren nests ranged from 0 to 37% (average=8% over all years, and 12% in the years that cuckoos were present at the site). Parasitism rate tended to increase with the duration of the host breeding season, but not significantly so (GLM: $X^2_1=1.73$, $P=0.19$). In addition, there is a positive relationship between rainfall and parasitism, which is stronger when host territory density is high (GLM: $X^2_1=13.09$, $P=<0.001$; Figure 7). The number of days between cuckoo arrival at the breeding site and the start of the hosts breeding season significantly impacted parasitism rate (GLM: $X^2_1=4.63$, $P=0.03$); the parasitism rate increased as cuckoos arrived earlier in relation to the hosts first egg date (Figure 8).
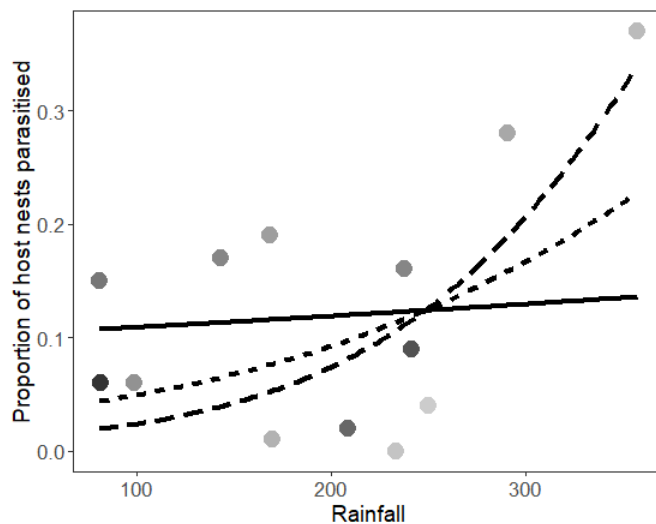
Figure 7. Model predictions of the interaction between the sum of rainfall over the breeding season (September-December) and host density, plotted on the raw data (darkness of point indicates the host density with lowest density being darkest and colour progressively lightening as density increases). The model predicts that the proportion of host nests parasitised increases with rainfall, and increases more steeply when host density is high (dashed line; prediction based on highest host density in dataset, 0.50) or average (dotted line; prediction based on average host density in dataset, 0.36) than when host density is low (solid line; prediction based on lowest host density in dataset, 0.20).
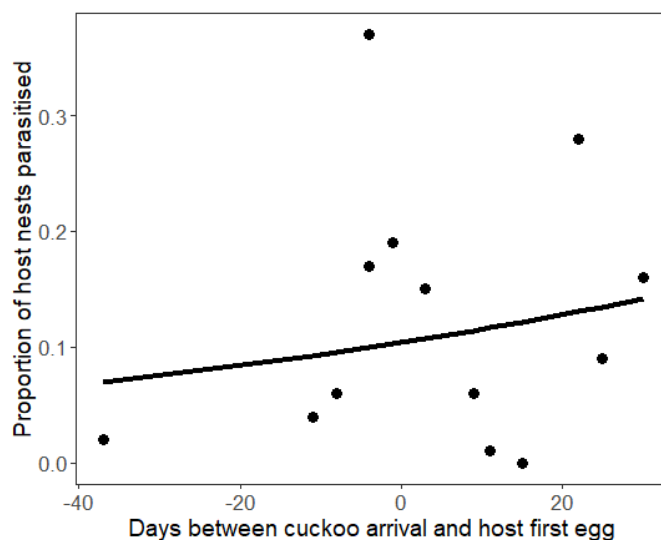

Figure 8. The raw data of the proportion of host nests parasitised, with model predictions showing an increase in parasitism rate when cuckoos arrival date at the study site is earlier in relation to the first egg date of the host. Negative values on the x-axis indicate the number of days cuckoos arrived after the host first egg was laid and positive values indicate the number of days cuckoos arrived before the host first egg was laid.

Discussion

Horsfield's bronze-cuckoo arrival date at the study site was not influenced by climate at the study site or the start date of the host breeding season. In contrast, cuckoo departure date was influenced by both variables; cuckoos departed earlier when it was particularly cold and wet or hot and dry, and when their hosts stopped breeding earlier. The amount of time cuckoos spent at the study site was not affected by the duration of the host's breeding season or host density. We found little evidence of a phenological mismatch between migratory cuckoos and their resident hosts, although trends suggest cuckoo arrival date is becoming later and the availability of host nests for parasitism is decreasing. In addition, parasitism rate was higher when cuckoos arrived at the study site earlier in relation to the commencement of host breeding. These trends suggest that there is the potential for a mismatch in the future with negative consequences for cuckoo breeding success and possibly selection for cuckoos to arrive earlier than the start of their hosts breeding season.

Cuckoo arrival, departure and duration

Climate and the timing of the host breeding season influenced cuckoo departure date from the study site, but not arrival or duration at the study site. Horsfield's bronze-cuckoos are nomadic and have been observed to appear in locations after heavy rainfall periods (Brooker et al. 1979; Eckert 2014). However, we found no evidence that rainfall and temperature at the study site influenced cuckoo arrival date. Cues at the wintering site of migratory Horsfield's bronze-cuckoos may be more important in determining cuckoo arrival to the study site (Saino et al. 2004; Studds and Marra 2011), but there has been little research on the wintering site and movement of Horsfield's bronze-cuckoos during the non-breeding season (Higgins 1999). Alternatively, cuckoo migration may be linked to endogenous timing cues such as photoperiod (day length; Gwinner

1996; Both and Visser 2001; Walker et al. 2019). We found that there was some variation in arrival date between year, with 47 days difference in the earliest and latest arrival dates during the study period. This inconsistency in arrival date might suggest that environmental cues at the wintering site are the more likely cue, but further research is needed to determine what influences cuckoo arrival date.

When conditions were either particularly cold and wet or hot and dry cuckoos departed earlier. More extreme weather conditions are more costly for individuals in terms of thermoregulation (Pendlebury et al. 2004; Salvante et al. 2007) and lead to declines in insect abundance and therefore food availability of insectivores (Woinarski and Cullen 1984; Sánchez-Bayo and Wyckhuys 2019). These climate conditions are likely to be unfavourable for reproduction, which may be why cuckoos depart earlier. Superb fairy-wrens breeding seasons have also been found previously to end earlier in years with more heatwaves and less rainfall (Lv et al. 2020), indicating these conditions are harsh. In line with this, cuckoos tended to stay longer at the study site when hosts bred for longer. We were unable to test if climate or the end of the host breeding season were more influential on cuckoo departure date as the end date of the host breeding season was highly correlated with rainfall.

In contrast, the duration of host breeding season did not affect the duration of the cuckoo presence at the breeding site. Cuckoo arrival was not influenced by host breeding season start date, so the absence of an effect on duration is not unexpected. In addition, host density did not affect the duration of the cuckoo presence at the breeding site. This may be due to the nature of Horsfield's bronze-cuckoos mating system, where females parasitise host nests at one site and then move on to another site and are usually, but not always replaced by another female (Langmore et al. 2007). This inconsistency of female replacement may decouple any potential trend in duration of cuckoo presence and host density at the study site.

Phenological mismatch

Mismatches between cuckoos and their hosts have been found in species in the northern hemisphere (Saino et al. 2009; Douglas et al. 2010; Avilés et al. 2014; Yun et al. 2020). We suggest that Horsfield's bronze-cuckoos may be more robust in relation to phenological mismatches with their host compared to northern hemisphere cuckoos due to their nomadism rather than intercontinental migration (Higgins 1999; Dingle 2008; Chambers et al. 2013), their short-term monogamous or sequentially monogamous life history strategy (Langmore et al. 2007) and relatively longer host breeding seasons (Englert Duursma et al. 2017). While we found little evidence of a current phenological mismatch between cuckoos and their hosts, we found potential for future phenological mismatches and an associated cost of mismatches between cuckoos and their hosts.

Horsfield's bronze-cuckoo arrival date at the study site and the start date of the superb fairy-wren breeding season have not changed significantly over the study period. Cuckoo arrival date at the study site and the start date of the host breeding season both show a tendency to occur later in more recent years. Consistent with our findings, a previous study on superb fairy-wrens also found no evidence of a phenological change in superb fairy-wrens (Lv et al. 2020), despite overall increased temperatures at the study site (Kruuk et al. 2015), due to the climate in the periods identified to influence phenology not significantly changing (Lv et al. 2020). Our study found the rate of phenological change differed between hosts and cuckoos, with that of hosts being almost twice that of cuckoos (Figure 5). Similar to our results, Saino et al. (2009) found that the rates of Sadvancement of common cuckoos was slower than that of their short distance migrant hosts. In our study, despite the difference in the rate of phenological change of cuckoos and their host, the timing of cuckoo arrival at the study site in relation to the start of the host breeding season did not significantly changed over the study period. This absence of a significant change suggests there has been no significant change in overall coordination between cuckoos and their hosts. However, the trend of cuckoo arrival to the study site in relation to the start of the host breeding season is generally positive

indicating cuckoos are arriving later in relation to host start date in more recent years, which has the potential to cause a phenological mismatch in the future (Saino et al. 2009; Møller et al. 2011).

In contrast to many other multi-brooded species (Halupka and Halupka 2017), we found no evidence for a change in duration of breeding season of superb fairy-wrens, which suggests that the window of opportunity available for hosts to target has not changed over the study period. Consistent with this, the duration of Horsfield's bronze-cuckoo presence at the breeding site also did not change over the study period. However, the percentage of host nests that were available whilst cuckoos were present at the breeding site declined significantly over the study period. Brooker and Brooker (2003) found that Horsfield's bronze-cuckoos need at least 12 host territories within their territory to make the breeding site viable and that host density influences parasitism rate (discussed below; Langmore and Kilner 2007). We found the percentage of host nests falling within the time period that cuckoos were at the breeding site was above 80% of all host nests during the breeding season in all but 2 of the 13 years studied (Figure 3). Our findings suggests cuckoos are likely to be able to parasitise subsequent host nests if they arrive at the study site later than the start of the host breeding season (Douglas et al. 2010). However, if the availability of host nests continues to decline in the future, which is possible due to the trend of cuckoos arriving later in relation to the host start of the breeding season, there is potential for a phenological mismatch between cuckoos and hosts.

Cuckoo breeding success

Consistent with our previous work, parasitism rate was positively related to rainfall during the breeding season, with this relationship being stronger when host density was high (Langmore and Kilner 2007). Prior work has found that cuckoo territory size is related to host density, as territory size increased when host density was low, and

cuckoos were absent from the study site when host density was below 0.19 fairy-wren breeding groups/ha (Brooker and Brooker 2003; Langmore et al. 2007; Langmore and Kilner 2007). In addition, years with moderate densities of superb fairy-wrens experienced the highest parasitism rates (Medina and Langmore 2019), due to the trade-off between cuckoo recognition and host defences being greater when host density was high (Langmore et al. 2012; Feeney et al. 2013) and nest availability for parasitism being too low to be economical for cuckoos when host density was very low. In addition, superb fairy-wrens have been found to breed for longer when there is higher rainfall during the breeding season (Lv et al. 2020), suggesting host nests are available for parasitism later in the breeding season when rainfall is higher. Our findings add to the literature on the importance of host density for breeding success of cuckoos (Takasu et al. 1993; Soler et al.1999; Alvarez 2003). As there is evidence that some Horsfield's bronze-cuckoo host species are declining (Gardner and Heinsohn 2007; Cunningham and Olsen 2008) this may cause serious problems for cuckoo breeding success if these declines continue.

Cuckoo brood parasitism rate was higher when cuckoos arrived at the breeding site before hosts began breeding. This finding may suggest that there is selection for cuckoos to arrive earlier in relation to the start of the hosts breeding season as cuckoo breeding success was higher when they begin breeding earlier, a common trend in many species (Dunn et al. 2011; Dunn and Møller 2014; Harriman et al. 2017). There is conflict in our results as to the optimal arrival time for cuckoos in terms of breeding success and the predicted trend. We found that cuckoos have higher breeding success when they arrive earlier in relation to the hosts breeding season but that they have been arriving later in more recent years. These findings may help explain the declining numbers of Horsfield's bronze-cuckoos (Szabo et al. 2008), as the current trend in arrival date is the opposite of that predicted to result in high breeding success. Further research is needed to test whether parasitism rate translates to adult survival in Horsfield's bronze-cuckoos, as

superb fairy-wrens have developed mechanisms to detect and reject cuckoo eggs and chicks (Langmore et al. 2003).

In addition to host nest availability, cuckoos are also at risk of mismatches with their prey as they are caterpillar specialists adapted to eat hairy caterpillars that are unpalatable to many other bird species (Davies 2000; Yom-Tov and Geffen 2005). Further investigation into caterpillar abundance over the breeding season may shed light on the mechanism through which climate impacts Horsfield's bronze-cuckoo departure date and duration at the study site. Future studies should investigate the relationship and potential mismatch in trophic level adaptations to climate change between caterpillar abundance and cuckoos, particularly as there is a strong signal for advancement in phenology of plant species in the southern hemisphere (Chambers et al. 2013).

In conclusion we found little evidence of a phenological mismatch between migratory Horsfield's bronze-cuckoos and their resident superb fairy-wren hosts, although trends suggest that there is the potential for a mismatch in the future if cuckoos continue to arrive later in relation to the host breeding season. In addition, we found that this could have a negative impact on cuckoo breeding success, which may be important in the context of declining Horsfield's bronze-cuckoo numbers.

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Chapter 6

Synthesis and General Conclusions

The evolution of cooperative breeding and obligate brood parasitism have both provided quite a puzzle for biologists, raising many important questions for the understanding of breeding behaviour- Why do individuals help others to raise their offspring, often at the cost of reproducing themselves? Why don't obligatory brood parasites raise their own offspring? What conditions favour the evolution of cooperative breeding and brood parasitism? And what do cooperative breeding and obligate brood parasitism mean for maternal investment?

In cooperative breeding species parents are assisted by other individuals to raise their offspring (Cockburn 2006). Kin selection is the classic explanation of the benefit of helping, as related individuals may gain fitness by aiding in the rearing of offspring they are related to (Hamilton 1964; Hatchwell 2009). Whilst this theory may explain helping in species where helpers are related, this isn't always the case (Riehl 2013), suggesting there must also be additional factors driving the evolution of cooperative breeding. Comparative studies have highlighted the importance of ecological influences on the evolution of cooperative breeding. However, investigating the ecological correlates of cooperative breeding in different species has led to contradictory findings (Lin et al. 2019), as cooperative breeding has been found to be favoured in both harsh and variable environments (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011), and benign (Arnold and Owens 1999) or stable (Gonzalez et al. 2013) environments. In benign and stable environments, high survival rates and habitat saturation result in a surplus of offspring in comparison to territory availability, promoting delayed dispersal of offspring and cooperative behaviour (Arnold and Owens 1999). However in harsh and variable environments, where resources are limited or unpredictable between years, helpers increase the chances of successful reproduction (Emlen 1982; Rubenstein and Lovette 2007). On a global scale, the distribution of cooperative breeders has been found to be predicted by the variability in environments, particularly the variability in rainfall among years (Jetz and Rubenstein 2011). However, Cornwallis et al. (2017) has suggested that the more resilient breeding strategy of cooperative breeding has led to

the colonisation of harsh environments, rather than harsh environments leading to the evolution of cooperative breeding. A lot of studies investigating the relationship between harsh environments and cooperative breeding have used comparative analyses (Rubenstein and Lovette 2007) or are focused on the macro evolutionary scale (Jetz and Rubenstein 2011). However, to test hypothesised benefits of cooperative breeding in harsh environments, developed by looking at trends in the bigger picture, we need to investigate the effect of helpers through experimental studies, as I have done in Chapter 3 of this thesis.

Despite the uncertainty about which ecological conditions favour the evolution of cooperative breeding, in many species there is convincing evidence that helper presence is beneficial (Russell et al. 2003; Ridley 2007; Wiley and Ridley 2016). What form these benefits take depends in part on the maternal investment strategy of the female (Guindre-Parker and Rubenstein 2018). When helpers are present females can provide the same level of care as would be necessary without helpers and increase the overall level of care (additive care) offspring receive (in which case helper presence mostly benefits the offspring) or they can reduce their level of care (load lightening; Crick 1992; Hatchwell 1999; Hammers et al. 2019), which will be made up for by the helpers, and conserve their resources for survival and future breeding attempts (in which case helper presence also benefits the mother). Evidence for load-lightening has previously been found in cooperatively breeding superb fairy-wrens (Russell et al. 2007; Langmore et al. 2016), and our research has used experimental investigation to further explain this investment strategy in relation to the environment (Chapter 3). In addition, we have elucidated the mechanism through which the climatic conditions affect maternal investment in superb fairy-wrens, as our results show that food availability constrained egg investment in drought conditions (Chapter 2). Our findings provide experimental evidence that cooperative breeding is beneficial in variable environments and demonstrate the impact of the environment on cooperative breeders (summarised below).

There is a vast spectrum of types of parental care, ranging from care by only one parent to biparental care, cooperative breeding and brood parasitism (Cockburn 2006). Biparental care is the most common form of care (81% of species), whereas in comparison cooperative breeding is unusual, occurring in 9% of species (Cockburn 2006), and brood parasitism is rare with only 1% of species forfeiting the incubation and rearing of their young (Davies 2000). Within these parental care strategies, maternal investment is particularly important for offspring growth and survival (Williams 1994; Mousseau and Fox 1998; Krist 2011). Maternal investment occurs within the context of the physical environment, and also the social environment in the case of cooperative breeders, which may influence the optimal investment strategy (Russell and Lummaa 2009; Savage et al. 2015). Mothers, with both pre-natal and post-natal care, can invest in their offspring differently at different stages, and investment at one stage may be interrelated to investment at another. In contrast maternal investment of obligate brood parasites is entirely restricted to their eggs (Davies 2000). Due to the complexity of maternal investment, it is important to investigate maternal investment in species with different life history strategies and investigate the different environmental influences on maternal investment, to be able to understand what aids and restricts investment. In this thesis, I have investigated maternal investment in two species with different types of parental care, cooperatively breeding superb fairy-wrens (Chapters 2 and 3) and their obligate brood parasites, Horsfield's bronze-cuckoos (Chapters 4 and 5). This is a particularly interesting study system as the two species experience identical environmental conditions at the breeding site but are different in aspects of their life history, such as parental care. Moreover, while superb fairy-wrens are resident, Horsfield's bronze-cuckoos are nomadic and migratory (Brooker et al. 1979). In addition, superb fairy-wrens and Horsfield's bronze-cuckoos are engaged in a co-evolutionary arms race, which also has the potential to influence maternal investment (Langmore et al. 2003). In theory cooperatively breeding species, in which helpers care for young that

aren't their own, and brood parasitic cuckoos, that don't even care for their own young, seem to be the polar opposites in parental care (Baglione and Canestrari 2017). However, a prior study on these species found that there may be reciprocal selection for parasitism of cooperatively breeding superb fairy-wrens by Horsfield's bronze-cuckoos, as cooperative breeders provide superior rearing conditions for cuckoo chicks, but also have stronger defences against brood parasitism (Feeney et al. 2013). On a larger scale there is a tight correlation in the global distribution of cooperative breeders and obligate brood parasites (Feeney et al. 2013), and a more recent study has suggested there is evidence that cooperative breeding attracts brood parasitism (Wells and Barker 2017). The findings in Chapter 4 of this thesis highlight the difference between these two species in terms of maternal investment strategies; maternal investment in the host was shaped by the environment, whereas maternal investment in the cuckoo was shaped by selection pressure from the host, rather than by the environment. In addition, Chapter 5 reinforces how reliant cuckoos are on their hosts and the tightly-coupled coordination of breeding strategies that has developed through coevolution. Whilst cooperative breeding and obligate brood parasitism may seem to be highly contrasting breeding strategies, this thesis reinforces earlier findings of a tight relationship between cuckoos and their hosts.

Summary of findings

Previous studies on maternal investment in cooperatively breeding superb fairy-wrens found that maternal investment in egg size differs with the physical and social environment (Russell et al. 2008; Langmore et al. 2016). However, the cue or limitation that affected superb fairy-wren egg size had not previously been elucidated. The climate can have direct and indirect effects on maternal investment, as the climate directly influences an individual's thermoregulation costs (Salvante et al. 2007; Visser et al. 2009) and indirectly influences food availability. This is particularly evident in insectivorous species due to the strong association of insects with climate (Denlinger

1980; Porcelli et al. 2017; Sánchez-Bayo and Wyckhuys 2019). We used a food
supplementation experiment during a drought year to disentangle the direct and indirect
effects of climate on an insectivorous bird species and found that the indirect effect of
climate on food availability influenced maternal investment in eggs (Chapter 2). The
separation of the direct and indirect effects of climate allows a better understanding of
how climate change may influence maternal investment in the future. This is particularly
important as droughts are predicted to become more prevalent in the future (Hughes
2003). Our findings indicate this may be problematic for superb fairy-wrens as they
produced smaller eggs without food supplementation in drought conditions (Chapter 2),
but larger eggs were found to buffer chicks against harsh conditions (Chapter 3). If
mothers are unable to produce large eggs in harsh drought conditions, chicks may suffer
growth and survival consequences. Further investigation is required to assess the effect
of food availability on chick growth and survival, particularly when chicks are raised by
groups compared to pairs. Once we established that food availability is a limiting factor
or cue for egg investment in superb fairy-wrens, this raised the question; is egg size
important for superb fairy-wren chick growth and survival?

Many studies have found that maternal investment in egg size has lasting effects on
offspring growth throughout the nestling period (Bolton 1991; Smith and Bruun 1998;
Christians 2002; Krist 2009; Krist 2011), whereas in other species investment at the
post-natal stage (the amount of food provided to chicks) overrides the initial pre-natal
investment (Amundsen and Stokland 1990). We found that in superb fairy-wrens, egg
size influenced nestling growth, and that larger egg size was beneficial in buffering
chicks against harsh climates. Our results illustrate a sophisticated level of fine tuning of
the maternal investment of superb fairy-wrens to the climate and social environment,
and emphasize the benefits of cooperative breeding. Females that bred in groups with
helpers were able to more finely modulate their investment in eggs and nestlings to suit
the prevailing environmental conditions. These findings extend our understanding of the
load lightening investment strategy used by female superb fairy-wren that breed in

groups, and highlight the importance of investigating the interaction of maternal investment at the pre-natal and post-natal stage.

Maternal investment of superb fairy-wrens was impacted by climate through food availability (Chapter 2), which in turn influences offspring growth (Chapter 3). The findings of Chapters 2 and 3 suggest that cooperative breeding may be particularly beneficial in harsh conditions. This is consistent with comparative and macro evolutionary analyses, which have found that the global distribution of cooperatively breeding birds is associated with unpredictable and highly variable climatic conditions (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011) and provides crucial experimental evidence of how the environment influences maternal investment. We found that superb fairy-wren maternal investment in eggs was limited by food availability in drought conditions (Chapter 2), and also that the assistance provided by helpers allowed females to both modulate their investment in eggs according to the prevailing conditions and also to tailor their investment in their chicks according to their prior investment in their eggs, such that offspring growth was maximised (Chapter 3). Future research would benefit from investigating the effect of helper presence and food availability simultaneously.

Understanding maternal investment in brood parasitic cuckoos adds a further challenge, as the cuckoo's only maternal investment is in their eggs, and the post-natal maternal investment is thrust upon the host (Davies 2000). For cuckoo breeding attempts to be successful, cuckoos must deceive the host into accepting the cuckoo's offspring as its own. This life history strategy requires finely tuned coordination of cuckoos to their hosts, as cuckoos are reliant on their hosts to rear their offspring (Davies 2011). Rearing cuckoos is costly for hosts, which has selected for host defences, such as rejection of cuckoo eggs and chicks (Langmore and Kilner 2010). The co-evolutionary arms race between cuckoos and their hosts has selected for cuckoos to mimic the eggs and chicks of their hosts to avoid detection (Langmore and Kilner 2010). Cuckoos are usually larger

than their hosts, which means that they must lay a small egg relative to their body size in order to mimic host egg size. This is in conflict with selection for larger egg size to maximise offspring growth and survival (Krist 2011). We found evidence that selection pressure from hosts was more important than modulation of egg investment to the environmental conditions for Horsfield's bronze-cuckoos (Taylor and Langmore 2020). Cuckoo egg shape mimicry was found to be important to avoid detection and rejection by hosts, as cuckoo eggs that were relatively shorter and rounder were deserted by the hosts, suggesting there is selection pressure from host detection mechanisms for cuckoo eggs to be long and thin. In contrast, climate and host group size did not affect cuckoo egg volume. Furthermore, Chapter 4 demonstrates the importance of looking for multiple cues for egg detection, as hosts only use cuckoo egg shape to detect cuckoo eggs when their nests were naturally parasitised, indicating that adult cuckoo presence is also a cue for cuckoo egg detection.

Another way in which cuckoo breeding success is related to their hosts is the coordination in timing of breeding. Climate change is disrupting previously closely aligned, coevolved systems (Parmesan 2006; Jones and Cresswell 2010; Kharouba et al. 2018), as species, particularly those with different life history strategies, respond at differing rates to the changing environmental conditions (Parmesan and Yohe 2003; Visser et al. 2004; Parmesan 2006; Both et al. 2009). Differing responses of species to climate change has the potential to disrupt the coordination between migratory cuckoos and their resident hosts, as hosts may be better placed to respond to phenological changes in resources at the breeding site. In contrast, environmental cues at the breeding site are largely unavailable to migratory cuckoos until they arrive at the site. In addition, climate change may be decoupling previously relied upon cues for migration such as endogenous cues or climatic cues at the wintering grounds (Studds and Marra 2011; Walker et al. 2019). Alarmingly, we found potential for an increasing phenological mismatch in the future between cuckoos and their hosts, due to non-significant trends showing cuckoos generally arriving later relative to their host breeding season and

decreasing host nest availability for parasitism (Chapter 5). While these trends were non-significant in our study, if they continue on the current trajectory, it is likely this will change in the future. Furthermore, we detected a cost to cuckoo breeding success (lower parasitism rate) when the timing of cuckoo arrival to the study site was later than the start date of the host breeding season. These findings are particularly important in the context of Horsfield's bronze-cuckoo survival and breeding success as some evidence suggests that both cuckoos and their hosts are declining (Gardner and Heinsohn 2007; Cunningham and Olsen 2008; Szabo et al. 2008). Chapter 5 may provide one possible explanation for the decrease in cuckoo numbers.

The findings from Chapters 4 and 5 illustrate that selection from hosts on cuckoos influences cuckoo breeding success, which is further evidence of the tightly coupled, coevolved relationship between cuckoos and hosts. These results are particularly interesting in the context of the differing maternal investment strategies between cuckoos and hosts, and prior evidence of reciprocal selection of cooperatively breeding species by obligate brood parasites. These chapters also highlight the vulnerability of cuckoos to the changing environment, and corresponding changes in host nest availability and host population density.

Future directions

This thesis has contributed to our understanding of how the environment influences maternal investment in the context of cooperative breeding and obligate brood parasitism. However, there is still a lot to investigate. Future studies should:

- study the breeding systems of tropical species. The ecological conditions that select for cooperative breeding are still debated. While the findings that variable environments select for cooperative breeding makes intuitive sense, and there is evidence that cooperative breeding is beneficial in these environments (such as that presented here), there is some suggestion that this may be due to sampling

bias as research is generally occurring in more accessible environments (Cockburn 2020). To either reinforce or dispute, that variable environments are the driving cause of cooperative breeding (rather than or as well as other mechanisms such as habitat saturation), research of the breeding systems of species in more logistically challenging but comparatively temporally stable and benign environments is needed. This is a particularly relevant question in the context of climate change and predictions of species survival in the future climate and how cooperative breeding may play a role.

- focus on egg shape asymmetry as a potential detection mechanism for detecting cuckoo eggs. Chapter 4 found that superb fairy-wrens use overall egg shape to detect cuckoo eggs. Horsfield's bronze-cuckoo eggs have two blunt poles while superb fairy-wren eggs have one blunt and one tapered pole, however we did not explore this difference in egg asymmetry as a cuckoo egg detection mechanism in our study. Egg asymmetry may be one egg shape trait that hosts could use to detect cuckoo eggs (Polačiková and Grim 2010; Zölei et al. 2012). Alternatively, it may be that this egg shape trait is not important for egg mimicry or that it is too costly for cuckoos to taper on end of their eggs due to the large chick inside. Future studies should investigate if inconsistencies between cuckoo and host egg asymmetry are a mechanism hosts use to detect cuckoo eggs, for example by adding model eggs with differing degrees of egg asymmetry to host nests and assessing egg rejection.

- look at the effect of climate change on phenology at different trophic levels in Horsfield's bronze-cuckoos and their specialist caterpillar prey. Horsfield's bronze-cuckoos are not only vulnerable to a phenological mismatch with their preferred host (Chapter 5), but also with their specialist caterpillar prey. Higher spring temperatures in temperate areas, due to climate change, are resulting in advanced phenology such as earlier leaf emergence (Parmesan 2006; IPCC 2007). In turn there is selection for insects to advance their timing along with their food sources. In addition, the warmer temperatures also cause larvae to

grow more quickly, resulting in a shorter duration of prey abundance for insectivores (Buse et al. 1999). As Horsfield's bronze-cuckoos are migratory they may not experience the same phenological advancement as their caterpillar prey at their breeding site, meaning there is a risk of mismatch between the resources they need for breeding and their arrival at the breeding site. This potential phenological mismatch in resources and timing of breeding requires further investigation. In particular it would be useful to compare the peak abundance of caterpillars with cuckoo arrival and nest parasitism rate over the breeding season.

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