Costs and Benefits of Breeding Cooperatively in Fluctuating Environments in African Starlings

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ABSTRACT

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Global climate change is expected to increase the frequency of extreme and unpredictable weather in many parts of the world. As a result, a critical goal for biologists is to predict how organisms may come to cope with increased environmental variability. The key to making these predictions will be to understand how animals currently living in fluctuating environments are able to survive and reproduce under these conditions. Sociality (i.e. group living) and cooperative breeding (i.e. where more than two individuals care for young together) may both facilitate the colonization of highly fluctuating environments. However, the relative benefits of group living and engaging in alloparental care under variable conditions remain unclear. My dissertation examines the fitness consequences of living in one of the world's most unpredictable habitats—the African savanna—in a population of free-living cooperatively breeding superb starlings (*Lamprotornis superbus*). In chapter 1, I examine whether adults benefit from living in large social groups of up to 50 individuals, which are among the largest known for any cooperatively breeding bird. In addition, I test whether group size serves to buffer against harsh environmental conditions. In chapter 2, I examine whether breeders gain reproductive benefits by having alloparents at their nest—I explore the type of reproductive benefits gained (i.e. improved reproductive success versus offspring care load-lightening), as well as whether these benefits occur in both harsh and benign conditions (i.e. temporal variability hypothesis), or are greatest under harsh conditions only (i.e. hard life hypothesis). In chapter 3, I explore whether offspring care load-lightening reduces the cost of reproduction incurred by breeders and alloparents by comparing four physiological mechanisms known to mediate reproductive costs. Lastly, in chapter 4 I test the long-standing assumption that cooperatively breeding species face reduced costs of reproduction by sharing offspring care relative to non-cooperatively breeding species. I compare the oxidative cost of reproduction in superb starlings to greater blue-eared glossy starlings (*L. chalybaeus*), a synoptic non-cooperatively breeding species. Taken together my dissertation findings

demonstrate that group living and alloparental care do not solely buffer against harsh conditions in superb starlings, but instead provide individuals with the flexibility to modify their offspring care behavior according to environmental conditions, to the behavior of other group members, and to their physiological condition prior to breeding—this behavioral flexibility may in turn serve to mitigate fluctuations in the cost of living and breeding in variable environments.

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INTRODUCTION

Global climate change is expected to increase the frequency of extreme and unpredictable weather in many parts of the world (Garcia et al. 2014), which will challenge organisms in two ways: firstly, average environmental conditions that organisms encounter are likely to change, and secondly, organisms may encounter a greater range of environmental conditions than previously (Lawson et al. 2015). As a result, a critical goal for biologists is to predict how organisms may come to cope with increased environmental variability (Boutin and Lane 2014). A promising approach to making these predictions is to examine how animals currently living in fluctuating environments are able to survive and reproduce under these conditions.

Cooperative behavior is thought to facilitate life in fluctuating environmental conditions (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011; Schradin et al. 2012; Lukas and Clutton-Brock 2017; Vincze et al. 2017), as group living, evenly shared parental care, and alloparental care occur more frequently in variable environments. Furthermore, both sociality (i.e. group living) and cooperative breeding (i.e. more than two individuals care for young together) are thought to facilitate the colonization of highly fluctuating environments (Aviles 1999; Cornwallis et al. 2017), suggesting that cooperation may play a direct role in allowing organisms to colonize and cope with fluctuating environmental conditions. However, the underlying mechanism shaping the relationship between fluctuating environmental conditions and social behavior remains unclear. It has long been assumed that cooperation is beneficial in fluctuating environments to allow organisms to cope with the unpredictable occurrence of harsh conditions (i.e. ‘hard life’ hypothesis) (Emlen 1982)—yet, empirical support of this hypothesis is mixed as breeding cooperatively may increase reproductive success under harsh environmental conditions only (consistent with the hard life hypothesis) (Magrath 2001; Canario et al. 2004; Valencia et al. 2006; Covas et al. 2008), across all environmental conditions (Legge 2000; Marshall et al. 2016), or even under favorable conditions only (Koenig et al. 2011). In order to uncover why sociality and cooperative breeding may be beneficial for coping with fluctuating environmental conditions, researchers will need to consider multiple fitness consequences of these cooperative behaviors—including reproductive success, offspring care load-lightening, and adult survival—as well as how each fitness benefit may fluctuate under variable environmental conditions.

The goal of my dissertation is to examine the fitness consequences of group living and cooperative breeding under fluctuating environmental conditions in a population of free-living superb starlings (*Lamprotornis superbus*). Superb starlings inhabit one of the world's most unpredictable environments—the African savanna—where rainfall varies in intensity and duration within and among years (Rubenstein 2016). Significant variation also exists in grass cover across territories, which together with rainfall shapes the availability of insects used to provision nestlings and the preferred prey consumed by adults (Feare and Craig 1998; Rubenstein 2007, 2016). In addition, individuals of this species live in year-round territorial groups of up to 50 individuals, which are among the largest social groups known for any cooperatively breeding bird. Up to 7 breeding pairs will reproduce per breeding season and between 1 and 10 alloparents will assist the breeding pair in caring for young at each nest (Rubenstein 2016). However, over half of all social group members forgo breeding or providing alloparental care within a breeding season, suggesting that social group sizes are significantly larger than necessary for alloparental care alone. My dissertation takes advantage of this feature of the superb starling mating system in order to disentangle the consequences of group living from those of alloparental care in unpredictable environments.

In **Chapter 1**, I examine whether adults benefit from living in social groups and whether group size serves to buffer against harsh environmental conditions. My findings show that group size was positively correlated to adult survival, though differently between the sexes: female survival increased with group size similarly across all conditions, whereas male survival increased with group size in periods of average or above average pre-breeding rainfall. These results suggest that superb starlings gain a net fitness benefit of living in larger social groups, though sociality does not appear to buffer against harsh conditions. Instead, group living may only improve survival under years of favorable pre-breeding rainfall due to potentially increased costs of social conflict in drier seasons in males.

In **Chapter 2**, I examine whether breeders gain reproductive benefits by having alloparents at their nest—specifically, I explore the type of reproductive benefits gained (i.e. improved reproductive success versus offspring care load-lightening), as well as whether these benefits occur in both harsh and benign conditions (i.e. temporal variability hypothesis) (Rubenstein and Lovette 2007), or are greatest under harsh conditions only (i.e. hard life hypothesis) (Emlen 1982; Koenig et al. 2011). Consistent with the temporal variability hypothesis, larger contingents of alloparents increased the number of fledglings while simultaneously allowing mothers to reduce their provisioning rates across all environmental conditions. Conversely, fathers experienced load-lightening in nest guarding only under favorable rainfall conditions. Therefore, breeders did not gain greater fitness benefits from alloparental care under harsh conditions—instead alloparents may provide breeders with the behavioral flexibility necessary to adjust their investment in offspring care in order to invest optimally in current breeding versus self-maintenance (i.e. a tradeoff termed 'cost of reproduction') according to changing environmental conditions.

While superb starlings decreased their investment in offspring care when aided by larger contingents of alloparents, it remains unclear whether behavioral differences in load-lightening represent biologically relevant decreases in offspring care. In **Chapter 3**, I explore whether load-lightening reduces the physiological costs of offspring care by testing whether nestling provisioning or guarding behaviors lead to a cost of reproduction in superb starling breeders and alloparents. Specifically, I compare four physiological mechanisms thought to shape the cost of parental or alloparental care by examining whether glucocorticoids, oxidative stress, immune function and body condition (i) constrain offspring care behavior, (ii) represent a cost of offspring care behavior, or (iii) have carry-over effects on subsequent breeding behavior. My findings suggest that superb starlings modify their investment in offspring care according to their intrinsic state prior to breeding, and therefore face few costs of parental or alloparental care. The flexibility to adjust investment in offspring care according to pre-breeding physiology may represent an important benefit of breeding cooperatively.

The results of Chapters 2 and 3 support the long-standing idea that cooperative breeding may serve to reduce the cost of reproduction (Brown 1978; Crick 1992), though no study has demonstrated that costs of reproduction are reduced in cooperative relative to non-cooperative species. In **Chapter 4**, I test this assumption by demonstrating that the physiological costs of breeding are reduced in cooperative breeders relative to a synoptic non-cooperatively breeding species. Specifically, my results show that individuals of the non-cooperative greater blue-eared glossy starlings (*L. chalybaeus*) incurred an oxidative cost of offspring care during breeding, but that breeders and alloparents of the cooperatively breeding superb starling did not. While rearing young of both species requires a similar total workload, the oxidative cost of reproduction was lower in the cooperative species because the workload was divided among a larger group of individuals, resulting in lower per capita workloads.

Taken together my dissertation findings demonstrate that group living and alloparental care do not solely buffer against harsh conditions in superb starlings, but instead serves to defend adults and nests from predators, and to provide individuals with the flexibility to modify their offspring care behavior according to environmental conditions, to the behavior of other group members, and to their physiological condition prior to breeding—this behavioral flexibility may in turn serve to mitigate fluctuations in the cost of living and breeding in variable environments.

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CHAPTER 1 — FLUCTUATING ENVIRONMENTAL CONDITIONS DRIVE SEX-SPECIFIC SURVIVAL BENEFITS OF GROUP LIVING

Sarah Guindre-Parker & Dustin R. Rubenstein

ABSTRACT

Group living is a precursor for the evolution of cooperative breeding—where more than two individuals care for young together—and may even shape the evolution of alloparental care via group augmentation. Yet, the fitness consequences of group living have been difficult to study in cooperatively breeding species, where it remains challenging to disentangle effects of group living from those of alloparental care. Furthermore, both group living and alloparental care may increase the ability of organisms to colonize harsh environments, though the relative importance of each remains unknown. We use a long-term dataset collected in cooperatively breeding superb starlings (*Lamprotornis superbus*) to determine the fitness consequences of group living, as social groups are significantly larger than, and uncorrelated to, the number of alloparents present in each group. In addition, we examine whether group size serves to buffer against harsh environmental conditions. Survival models showed that group size was positively correlated to adult survival, though differently between the sexes: female survival increased with group size similarly across all environmental conditions, whereas male survival increased with group size in periods of average or above average pre-breeding rainfall. These results suggest that superb starlings gain a net fitness benefit of living in larger social groups, which occurs independently of the benefits of having more alloparents at the nest. Rather than buffering against harsh conditions, our results suggest that increased costs of social conflict may mask the benefit of group size in drier seasons in males. Our findings support the group augmentation hypothesis, and show that fluctuating environmental conditions play an important role in shaping the fitness benefits of group living.

INTRODUCTION

Social animals face automatic costs of group living, including increased competition for resources and mates, but no automatic benefit (Alexander 1974). As a result, group living is only expected to arise when individuals gain fitness benefits that outweigh the automatic costs of sociality (Alexander 1974). Such potential benefits are diverse, but can be summarized into four general categories: group living may (i) reduce predation risk, (ii) improve foraging, (iii) increase access to mates, or (iv) improve health or condition (reviewed in Krause and Ruxton 2002). The degree to which individuals of social species benefit from group living is thought to increase with group size (Paquet et al. 2016), though the social costs of group living may also increase in larger groups (Bilde et al. 2007; Shen et al. 2014). As a result, the relative costs and benefits of sociality are thought to shape group size (Molvar and Bowyer 1994; Silva et al. 1994), an important component of an individual's social environment that ultimately affects fitness (Avilés and Tufiño 1998; Shreeves and Field 2002). Understanding the evolution of sociality—including the fitness consequences of group living—is important in order to understand the evolution of mating and breeding systems (Ebensperger 2001; Groenewoud et al. 2016). For example, cooperative breeding where three or more individuals care for young together is thought to evolve in two steps: group living must first arise, followed by the evolution of alloparental care (Ligon and Burt 2004).

Group living may confer direct fitness benefits to cooperatively breeding species, and may even shape the evolution of alloparental care as suggested by the group augmentation hypothesis—if larger group sizes confer fitness benefits, current group members are more likely to recruit new members by caring for young that are not their own but that are within their social group (Kokko et al. 2001; Kingma et al. 2014). While theoretical models have supported the group augmentation hypothesis (Kokko et al. 2001), little empirical evidence exists for group augmentation in free-living cooperatively breeding species because it remains challenging to disentangle this hypothesis from others benefits of alloparental care (Kingma et al. 2014). For example, the majority of avian cooperative breeders live in family groups (Riehl 2013) where it can be impossible to parse out the fitness consequences of kin selection from group augmentation. Nonetheless, many cooperative breeders do live with at least some non-relatives (Groenewoud et al. 2016), including approximately 45% of cooperatively breeding birds (Riehl 2013): in these species the role of direct fitness benefits in driving the evolution of group living and alloparental care should outweigh the role of indirect benefits (Clutton-Brock 2002). Therefore, in species with (i) low within-group relatedness, (ii) where group size is significantly larger than the number of individuals that engage in alloparental care, and (iii) where social groups continue to interact closely during non-breeding periods, potential advantages of sociality may be particularly important in shaping the evolution of group living and cooperative breeding. To our knowledge few studies have explored the possibility that group living confers additional fitness benefits independently of those gained from alloparental care, as group size and the number of alloparents present within a group are often correlated and difficult to examine separately (e.g. Groenewoud et al. 2016).

Identifying the drivers of variation in group size and the fitness consequences of sociality has been further complicated by variability in the costs and benefits of group living: both the costs and benefits of living in social groups may vary with ecological conditions among populations inhabiting different geographic areas (Farabaugh et al. 1992; Baglione et al. 2002*a*, 2002*b*; Kocher et al. 2014), or within a single population through time (Brown et al. 2016). Environmental conditions are thus important drivers optimal group size (Markham et al. 2015; Brown et al. 2016), though the role of fluctuating environmental conditions in shaping the fitness consequences of group living remains unclear. Conversely, comparative studies in birds (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011) and mammals (Lukas and Clutton-Brock 2017) have shown that cooperative breeders are more likely to occur in unpredictable environments, and two hypotheses aim to explain this biogeographic pattern: (i) the temporal variability hypothesis suggests that having alloparents help rear young may provide fitness benefits under both harsh and benign conditions (Rubenstein and Lovette 2007), while (ii) the hard life hypothesis suggests that benefits of alloparental care only occur under harsh conditions (Emlen 1982; Koenig et al. 2011, 2016). To date, similar hypotheses that focus more broadly on the fitness consequences of group living in fluctuating environmental conditions have not been proposed. Yet, both group living and cooperative breeding may allow organisms to more easily colonize harsh environments relative to solitary or non-cooperatively breeding ones, respectively (Aviles 1999; Cornwallis et al. 2017)—together, these studies suggest that group living plays a role in the likelihood that organisms will colonize harsh habitats, including in cooperative breeders. Group living may therefore provide fitness benefits in fluctuating environments independently of those gained from alloparental care, though to our knowledge this possibility has never been explored.

Here we use a long-term dataset collected in plural cooperatively breeding superb starlings (*Lamprotornis superbus*) to test whether fitness varies with group size in fluctuating environmental conditions. This study system allows us to examine the fitness consequences of group living independently of the benefit of having alloparents at the nest, because the number of alloparents at a nest is not correlated to social group size (Rubenstein 2006; Guindre-Parker and Rubenstein 2017). Superb starlings defend year-round territories and live in large social groups of up to 50 individuals (mean ± SE = 22.2 ± 0.65), which are among the largest of any cooperatively breeding bird (Rubenstein 2016). Non-breeding group members either become alloparents at one of the active nests (mean proportion of a social group ± SE = 13 ± 0.7%) or forgo breeding or providing alloparental care altogether ('non-breeder/non-alloparents', mean proportion of a social group ± SE = 53 ± 1.7%). Therefore, superb starling social groups are considerably larger than required for alloparental care alone, though it remains unclear why individuals of this species live in such large social groups given that only a fraction of group members contribute to offspring care. Despite half of all group members forgoing parental or alloparental care behaviors, the cumulative nest guarding and provisioning performed at a nest enhances breeder fitness in two ways: nests with more numerous alloparents are more likely to fledge a greater number of young (Rubenstein 2007*a*; Guindre-Parker and Rubenstein 2017), and breeders aided by larger contingents of alloparents experience load-lightening by reducing their investment in offspring care (Guindre-Parker and Rubenstein 2017). Load-lightening varies with environmental conditions in a sex-specific manner, however, as females reduce their offspring care similarly under all environmental conditions whereas males only reduce their investment in paternal care during seasons of high breeding rainfall (Guindre-Parker and Rubenstein 2017). Fluctuating environmental conditions therefore shape the benefit of alloparental care in male superb starlings, though it remains unclear whether environmental conditions also shape the fitness consequences of group living in this species.

Superb starlings live in one of the world's most variable habitats—the African savanna (Rubenstein 2016)—where the intensity and duration of rainfall varies within and among years, and drives variation in social group size. Previous work has shown that social groups increase in size following periods of increased pre-breeding rainfall (Rubenstein 2006, 2011), though spatial variation in group size is not related to food availability within each territory (Rubenstein 2011). Variability in superb starling social group size is most likely driven by immigration rather than within-group reproductive success, because annual fledging success is extremely low and larger groups are not more likely to fledge young (Rubenstein 2011, 2016). Larger group sizes do confer a reproductive benefit, however, as group size reduces variance in reproductive success most likely through improved group defense from nest predators (Rubenstein 2006, 2011). Yet, since superb starling social groups accept immigrants of both sexes and within-group relatedness is low (Rubenstein 2016), direct benefits of group living are also expected to occur in order to maintain such large social groups (Clutton-Brock 2002). To date, no study has investigated the fitness consequences of group living for adult superb starlings, so here we examine whether adult survival rates vary with social group size and whether the net fitness consequence of group living varies with fluctuating environmental conditions. Specifically, we predict that (i) adult superb starlings in larger groups will have higher survival, and (ii) group size will buffer against fluctuating environmental conditions, where the effect of group size on survival will be greatest under poor environmental conditions.

MATERIALS AND METHODS

Study system

A population of superb starlings in seven social groups was monitored continuously from 2001 to 2015 at the Mpala Research Centre, Kenya (0°17’N, 37°52’E). In our study population, superb starlings breed twice per year during the short (October-November) and long (March-June) rainy seasons, during which 0 to 7 breeding pairs will reproduce in each group (short rain mean ± SE = 1.5 ± 0.11; long rain mean ± SE = 2.3 ± 0.14). Individuals are uniquely marked with a numbered metal band and a combination of three colored bands. Birds born within this population are banded as nestling, while immigrants are captured and marked via routine trapping during the dry season, when most immigration takes place (Rubenstein 2016). Superb starling females tend to disperse from their natal group, while males tend to be the philopatric and are more likely to remain on their natal territory (Rubenstein 2006). Despite this, females can remain on their natal territories (though they are unlikely to breed) (Rubenstein 2006) and males frequently disperse and breed in a non-natal social group, as approximately half of the males in our study population are immigrants (Rubenstein 2016).

In our study population, 49% of individuals were banded at the nest and are therefore of know age. Birds captured outside of the nest were aged according to their iris color (see Appendix 1.1)—we assumed immigrants with a brown and white iris were 1 year of age, while immigrants with a pure white iris were a minimum of 2 years of age. Individuals in this study population typically immigrate between 1 and 3 years of age, though it can be difficult to age immigrants accurately beyond 2 years—while the majority of females in our study population disperse from their natal group by 3 years of age (i.e. 76%) (Rubenstein 2006), some do delay dispersal beyond this period and it would be impossible to differentiate older immigrants from younger ones based on iris color. Immigrant age therefore represents a minimum age rather than an exact one.

We used focal nest observations performed during the long and short breeding seasons to identify breeders and alloparents at active nests (mean cumulative observation length per nest ± SE = 5.6 ± 0.15 hours), while we used routine census observations and trapping data collected during the non-breeding period (directly preceding the rainy seasons) to identify non-breeder/non-alloparents. Group size was calculated using a combination of all observations to determine the total number of individuals alive within the group, as calculated previously for this species (Pollack and Rubenstein 2015).

Environmental conditions

Environmental conditions during the breeding season fluctuate among years (i.e. breeding rainfall), and territories (i.e. grass cover). Both rainfall and grass cover shape the availability of insects, which are the preferred source of food delivered to nestlings and consumed by adults (Feare and Craig 1998; Rubenstein 2006). As a result, rainfall and grass cover are likely to impact superb starling reproduction and survival. In addition, pre-breeding rainfall (i.e. rainfall that occurs during the dry season) may also play a role in shaping fitness in superb starlings since it is during this period that breeding roles are determined (Rubenstein 2007*b*) and when the majority of immigration takes place (Rubenstein 2016).

We measured variability in precipitation by quantifying pre-breeding and breeding rainfall in each year using an automated Hydrological Services TB3 Tipping Bucket Rain Gauge (Rubenstein 2011). We calculated breeding rainfall in the long (March to June) and short rains (October to November), as well as the pre-breeding rainfall that fell during the dry season immediately preceding the long (December to February) and short rains (July to September). Territory quality was defined as grass cover, calculated as the long-term average of the proportion of dropped pins that touched vegetation from monthly vegetation transects performed between 2008 and 2015 (Rubenstein 2007*c*).

Survival Models

We used Cox proportional-hazards regression models to determine whether group size or environmental conditions shaped survival in male and female superb starlings—this type of survival model is commonly used in wildlife studies (Fox et al. 2006; Saino et al. 2011; Wolfe et al. 2016), likely because it can handle both time-independent and -dependent predictors and does not require the specification of a probability distribution for survival times (Fox and Weisberg 2002). We performed our analyses on a subset of individuals in the population (N = 92 for males, N = 107 for females), only including birds that had bred at least once in their social group. Doing so ensured that (i) we would no include vagrant birds (i.e. floaters) in our analyses that were not true group members, and (ii) we would not confound death and dispersal, as individuals do not disperse once they have recruited into a group and made a breeding attempt. We assumed that individuals that had not been observed for 5 subsequent breeding seasons were no longer alive—their death was recorded as the end of the period when they were last observed. Our results were robust to this assumption, as they did not change if we extended the length of subsequent breeding seasons required before assuming an individual had died (e.g. 6 or 7 seasons; see sensitivity analyses below). As we were not able to determine the age of adults that were present at the beginning of our banding efforts in 2001, we excluded these individuals from survival analyses (though they were included in group size estimates)—this represented 29% of adult males and 18% of adult females that have bred at least once in our study population. Therefore, the only censoring in our analyses occurred when individuals were still alive at the end of the study period.

We built separate proportional-hazards models for males and females, each including the following predictor variables: pre-breeding rainfall, breeding rainfall, grass cover, and group size. In addition, we included interactions between group size and each of the three environmental predictors. All continuous variables were standardized prior to analyses and we checked that these variables were not linearly related to one another (all VIF<2 excluding interaction terms). Models also included individual identity via the cluster term in order to account for the non-independence of observations collected for the same individual across seasons. Lastly, we checked that our dataset did not violate the proportional hazard assumption. Proportional-hazards models were performed using the ‘survival’ package (v2.40-1) in R (v3.2.4; R Core Team 2016).

In addition to this model (hereafter ‘original model’), we performed sensitivity analyses to test whether our assumptions about death and the age of immigrants influenced our findings. We re-ran our original model, modifying the following assumptions one at a time: (i) individuals that had not been observed for 6 consecutive breeding seasons were considered to be dead, (ii) individuals that had not been observed for 7 consecutive breeding seasons were considered to be dead, and (iii) individuals banded as immigrants with a white iris are a minimum of 3 years of age. We present the coefficient estimates of all four models, though we only include test statistics, tests of significance and hazard ratios for our original model (as in Johnson et al. 2004).

RESULTS

We found that female survival was unrelated to pre-breeding rainfall, to breeding rainfall, or to territory grass cover (Table 1.1). However, female survival increased significantly in larger social groups (Figure 1.1), where a larger proportion of females were alive after 12 years in larger relative to smaller social groups. Yet, there was no significant interaction between group size and any of the three environmental variables included in our model (Table 1.1), suggesting female survival was positively correlated to group size independently of fluctuations in environmental conditions.

Similarly to females, we found that male survival was unrelated to pre-breeding rainfall, to breeding rainfall, or to territory grass cover (Table 1.1). Male survival was also positively correlated with group size, and with interactions between pre-breeding rainfall and group size as well as grass cover and group size. Briefly, male survival increased with group size, though only in breeding seasons following periods of average or above average pre-breeding rainfall (Figure 1.2). Similarly, male survival was greatest in large groups but only on favorable territories where territory grass cover was average or above average (see Appendix 1.2). Therefore, male survival increased with group size only under average or above average environmental conditions.

Sensitivity analyses of our Cox proportional-hazards regression models revealed that our assumptions about the age of immigrants or our classification of individuals as dead did not bias our findings. In 3 of 4 models for females, survival rates was positively correlated with group size (Figure 1.3A)—the only model where group size was not a significant predictor of female survival assumed that females were dead following an absence of 7 consecutive breeding seasons rather than 5 or 6 seasons—modifying this assumption likely reduced the statistical power of the Cox proportional-hazards regression model, making it more challenging to detect a potential effect of group size on female survival. Nevertheless, this model indicated that group size was associated with an increase in survival (as in the other 3 models), though this was only a marginally significant effect ($P = 0.08$). Similarly, all 4 models for males supported our conclusion that survival is correlated to an interaction between pre-breeding rainfall and group size (Figure 1.3B). However, only 2 of the 4 models (including the original model) found a significant effect of group size and an interaction between grass cover and group size on male survival. We interpret these latter two effects with caution, as we recognize that these results may vary with assumptions about our definition of death or the age of immigrants. Therefore, these sensitivity analyses support our findings that (i) female survival is positively correlated with group size across all environmental conditions, and (ii) male survival is positively correlated with group size, though only under average or above average seasons of pre-breeding rainfall.

DISCUSSION

We investigated whether adult survival in male and female superb starlings varied with social group size, and whether larger social groups buffered against the potentially negative impact of harsh environmental conditions on adult fitness. Our results showed that adult survival and group size were positively correlated, though differently in males and females: female survival was highest in larger groups across all environmental conditions, whereas male survival was highest in larger groups in periods of average or above average pre-breeding rainfall. Thus, our study provides evidence that group living may have a positive effect on adult survival independently of alloparental care in a cooperative breeder, though in a sex-specific manner. Our results also indicate that larger groups do not increase survival under harsh conditions for either of the sexes, but instead increase male survival in seasons of high pre-breeding rainfall. This study is correlative in nature, and while it is possible that increased adult survival contributes to increasing social group size (i.e. older individuals remain in social group for longer), this is unlikely to be the case in superb starlings where the average age of group members is actually negatively correlated to group size (see Appendix 1.3). It is more likely that social group size increases when younger immigrants join the group—therefore, larger social groups most likely confer direct benefits to group members, resulting in increased adult survival rates.

We found empirical support for the group augmentation hypothesis (Kokko et al. 2001; Kingma et al. 2014), where adult survival increased with group size, suggesting that positive selection on group size may favor the formation of larger groups in superb starlings. Social groups may increase in size in one of two ways: through increasing the number of young fledged remaining on their natal group, or through the recruitment of immigrant birds. The fitness benefit associated with living in a larger group could help to explain why non-breeder/non-alloparent birds contribute to nest defense despite the potential cost associated with mobbing a predator (Tórrez et al. 2012). By defending a nest within their social group from a predation attempt, non-breeder/non-alloparent group members may increase the likelihood that the nest will successfully fledge young—if these young are males and remain on their natal territory as adults, the size of their social group will increase. However, there is limited evidence that group size impacts fledging success in superb starlings, as larger social group do not improve reproductive success despite reducing variance in reproductive success (Rubenstein 2011). A likely alternative mechanism for increasing group size in superb starlings is via increasing immigrant recruitment, as the majority of male and female group members were not born in the social group where they bred (immigrants represent 58.7% of male and 93.5% of female group members that breed at least once and were included in our analyses). Previous work has found evidence that superb starlings recruit immigrants in at least one context: immigrant females form kin coalitions via co-immigration or when a newly immigrated female subsequently recruits a younger relative to join her social group (Pollack and Rubenstein 2015). By increasing the number of young successfully fledged or accepting or actively recruiting immigrants into their social group, superb starlings stand to benefit from increased survival and longer lifespans. Similarly, immigrant birds may preferentially join larger groups, where they stand to gain a greater fitness benefit compared to joining a smaller social group.

While our study demonstrated that group size increased female and male survival, the potential mechanisms underlying these findings remain less clear. Group living could increase the survival of group members via reduced predation risk, improved foraging, or improved heath or condition (Krause and Ruxton 2002). The most likely possibility is that larger social groups benefit from reduced predation risk, as group size is important in nest defense in superb starlings (Rubenstein 2006) as well as other cooperatively breeding species (Arnold 2000)—indeed, the number of individuals that responded to a nest predator model was greater in large relative to small social groups in superb starlings (Rubenstein 2006). Over one third of individuals responding to the predator model were neither breeders nor alloparents guarding or provisioning nestlings (Rubenstein 2006), suggesting that all group members regardless of their breeding role play an important part in defending nests against predators (Rubenstein 2016). If a threshold number of individuals were required to successfully deter a nest or adult predator, living in a larger social groups may increase the likelihood that this threshold is met (Rubenstein 2006). Superb starling predator mobbing behavior may also extend beyond nest defense to encounters between adults and predators, as mobbing behavior toward a nest predator (snake) was similar to attacks on a predator that may target both nestlings and adults (hawk) (Rubenstein 2006). Likewise, mobbing behavior is repeatable across ecological contexts in other species, including towards adult or nest predators (Strnad et al. 2012), or during the breeding or non-breeding season (Nijman 2004). It is also possible that group living may reduced predation risk differently across fluctuating environmental conditions, which would explain why group size only increased male survivorship under conditions of average or above average pre-breeding rainfall. For example, predator-mobbing intensity in the pied flycatcher (*Ficedula hypoleuca*) not only increased with the availability of neighboring conspecifics (i.e. number of neighbors), but this relationship was shaped by habitat quality (Krama et al. 2012). Pied flycatcher mobbing was greatest as the number of neighbors increased in a high quality habitat relative to a disturbed habitat

(Krama et al. 2012). A similar interaction between social and ecological environmental conditions could shape mobbing behavior superb starlings. Alternatively, larger superb starling social groups may shape fitness via improving the health or condition of group members, though this possibility has not been explored. It is less likely that group living increases survival via improved foraging in this species, because environmental conditions that shape the availability of insects—including rainfall and territory grass cover—were not correlated with survival in either males or females. Similarly, superb starlings often forage beyond territory boundaries and group size is unrelated to territory quality, suggesting that larger groups do not have improved access to food (Rubenstein 2011).

While our results suggest that superb starlings gain a net fitness benefit of living in larger groups, potential reproductive conflict also increases as groups get larger, which could impose costs on group members (Rubenstein and Shen 2009; but see Shen et al. 2014). One way that dominant individuals may reduce the cost of social conflict they incur is by allowing others within their social group to breed (Rubenstein et al. 2016), resulting in plural breeding. Vertebrate groups—particularly those like superb starlings that accept unrelated immigrant members and cause kin relatedness to become low (Rubenstein 2016)—may only increase in size if more individuals are allowed to breed (Rubenstein et al. 2016). Therefore, while positive selection on group size in superb starlings likely favored the evolution of larger social groups that accept both male and female immigrant members, increasing social conflict in larger groups likely shaped the evolution of plural cooperative breeding in this species. Plural cooperative breeding may also be more likely to occur in fluctuating environments because the strength of social conflict can vary across environmental conditions (Shen et al. 2012). Group conflict and the associated cost to dominant individuals may be relaxed under harsh environmental conditions (Shen et al. 2012), which would subsequently allow for larger social groups to form as an evolutionarily stable strategy in fluctuating environments. The opposite is true in superb starlings, where social conflict and aggressive interactions increased in drier years and decreased under conditions of high rainfall (Rubenstein 2007*b*). Thus, increased fitness costs from social conflict in harsher dry years could mask the fitness benefits of group living in superb starlings, though our results suggest this may only be the case in males. It is possible for the sexes to respond differently to social conflict, as females may be more likely to resolve conflicts using threats while males use aggression, as observed in other plural breeding species (Cant and Young 2013). Identifying the mechanisms that shape the costs and benefits of group living in superb starlings will be necessary to reconcile the sex-specific differences we observed in the fitness consequences of group living in this species.

In conclusion, this research has highlighted a net fitness advantage of group living, which likely occurs independently of the benefit of having alloparental care in a cooperatively breeding bird. Social group size is correlated with increased survival in superb starlings of both sexes, though environmental conditions influence this relationship in a sex-specific manner: group size was unrelated to male survival in years of low pre-breeding rainfall, while group size was associated with increased female survival similarly across environmental conditions. Similarly to research in colonial cliff swallows (Brown et al. 2016), this study supports that fluctuating environmental conditions play an important role in shaping the fitness consequences of group living. Future studies on the evolution of group living in cooperatively breeding species as well as other social mating or breeding systems will therefore need to examine whether the benefits and/or costs of sociality vary with environmental conditions.

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TABLES

Table 1.1: The results of cox proportional-hazards regression models for (A) female and (B) male superb starlings. For each predictor, we present the coefficient estimate and standard error, *Z*-statistic, *P*-value, and the hazard ratio with 95% confidence intervals. A negative estimate or a hazard ratio of <1 indicate that a predictor is positively correlated with survival, while a positive estimate or hazard ratio of >1 indicate a negative correlation

	Estimate ± SE	***Z***	***P***	**Hazard Ratio (95% CI)**
(A) FEMALES				
Pre-breeding rain	0.02 ± 0.27	0.08	0.94	1.01 (*0.65 – 1.59*)
Breeding rain	-0.24 ± 0.24	-1.21	0.23	0.78 (*0.53 – 1.16*)
Grass cover	0.58 ± 0.43	1.77	0.08	1.79 (*0.94 – 3.41*)
Group size	-0.96 ± 0.46	-2.22	0.03*	0.38 (*0.16 – 0.89*)
Pre-breeding rain * group size	-0.33 ± 0.35	-1.09	0.28	0.72 (*0.39 – 1.31*)
Breeding rain * group size	0.21 ± 0.31	0.75	0.45	1.24 (*0.70 – 2.20*)
Grass cover * group size	0.12 ± 0.51	0.26	0.80	1.12 (*0.46 – 2.72*)
(B) MALES				
Pre-breeding rain	-0.54 ± 0.33	-1.74	0.08	0.58 (*0.32 – 1.07*)
Breeding rain	-0.36 ± 0.32	-1.27	0.20	0.70 (*0.40 – 1.21*)
Grass cover	-0.27 ± 0.26	-1.01	0.28	0.76 (*0.46 – 1.24*)
Group size	-0.84 ± 0.40	-2.59	<0.001*	0.43 (*0.22 – 0.81*)
Pre-breeding rain * group size	-1.21 ± 0.41	-3.30	<0.001*	0.29 (*0.15 – 0.61*)
Breeding rain * group size	-0.34 ± 0.40	-1.00	0.32	0.71 (*0.36 – 1.38*)
Grass cover * group size	-0.65 ± 0.35	-2.27	0.02*	0.52 (*0.29 – 0.91*)

FIGURES

Figure 1: Superb starling female survival increased with group size. The thick survival curves represent survival model predictions with other parameters set to their mean values while the small vertical hatches indicate a death event. Survival curves are color-coded for group sizes that are above average (i.e. 1 standard deviation above average; black), (ii) average (i.e. population wide; dark grey) or (iii) below average (i.e. 1 standard deviation below average; pale grey). Thin dotted lines represent 95% confidence intervals.

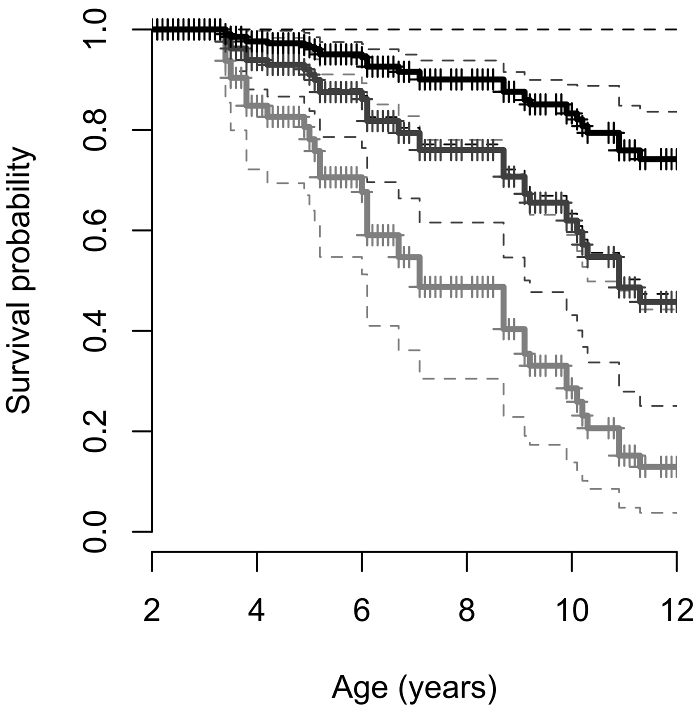


Figure 1.2: Superb starling male survival was correlated with an interaction between the group size and pre-breeding rainfall. When pre-breeding rainfall was (A) below average (i.e. 1 standard deviation below average) group size did not to increase male survival. However when pre-breeding rainfall was (B) average or (C) above average (i.e. 1 standard deviation above average), group size significantly increased male survival. The thick survival curves represent survival model predictions with other parameters set to their mean values while the small vertical hatches indicate a death event. Line colors represent group size – (i) above average (i.e. 1 standard deviation above average; black), (ii) average (dark grey) or (iii) below average (i.e. 1 standard deviation below average; pale grey). Thin dotted lines represent 95% confidence intervals.

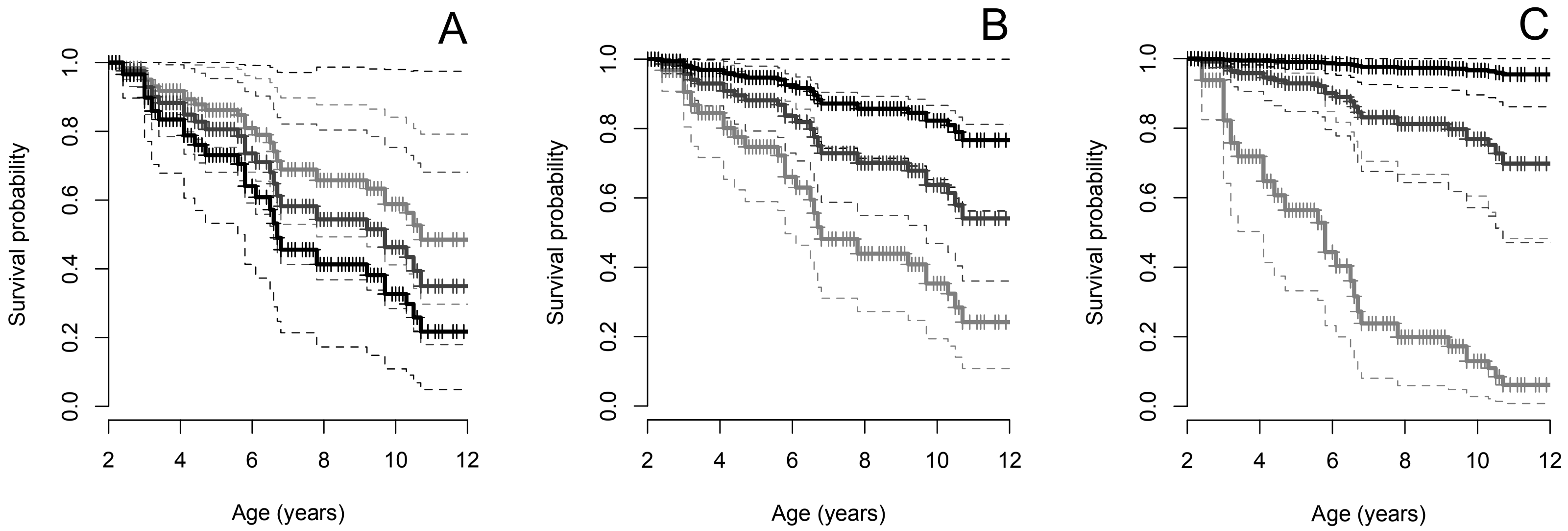
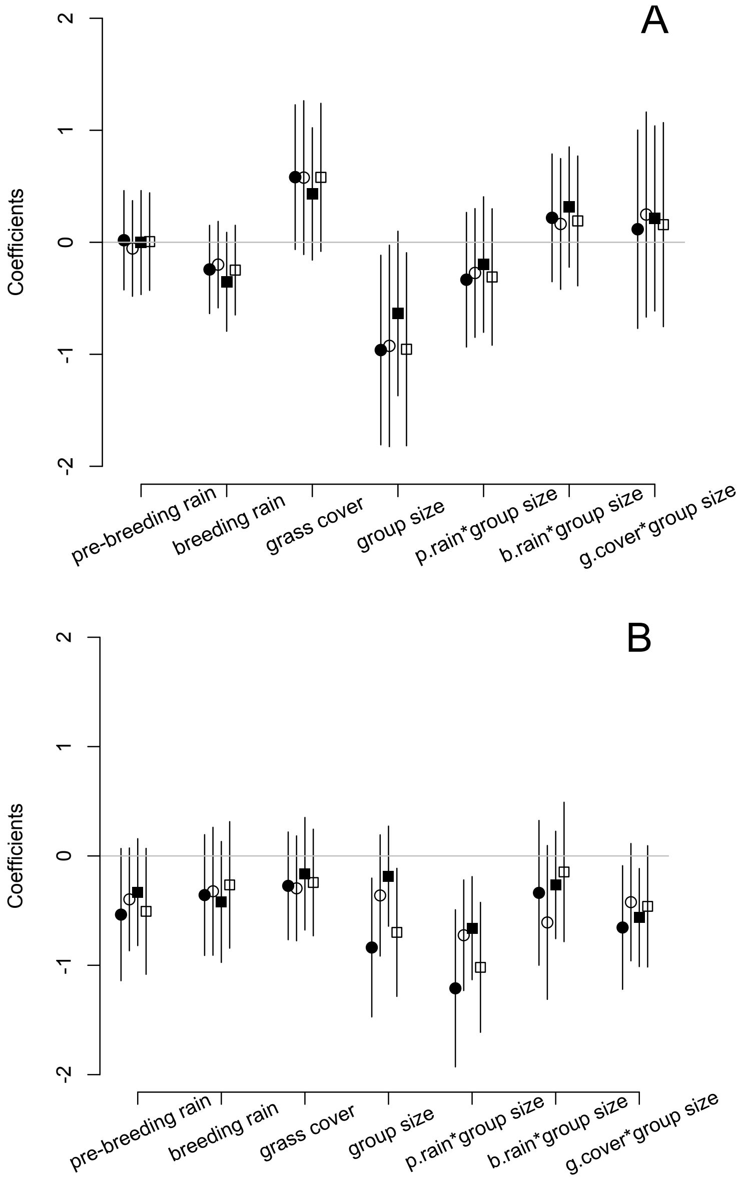


Figure 1.3: Coefficient plots from sensitivity analyses revealed that (A) group size increased female survival in all but one model (assuming death following a 7 season absence). Conversely, (B) male survival increased due to an interactive effect of pre-breeding rainfall and group size. Symbols indicate coefficient estimates from various models with their 95% confidence intervals. Closed circles represent coefficients from our original model, open circles represent coefficients from a model which assumed death following an absence of 6 seasons, closed squares represent coefficients from a model which assumed death following an absence of 7 season, and open squares represent coefficients from a model which assumed adult immigrants were 3 years old. Environmental conditions are abbreviated as follows: pre-breeding rainfall – p.rain, breeding rainfall – b.rain, and grass cover – g.cover.



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CHAPTER 2 — MULTIPLE FITNESS BENEFITS OF ALLOPARENTAL CARE IN FLUCTUATING ENVIRONMENTS

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ABSTRACT

Although cooperatively breeding vertebrates occur disproportionately in unpredictable environments, the behavioral mechanism underlying this pattern remains unclear. Organisms may breed cooperatively in environments where conditions fluctuate because alloparents are necessary to increase breeder fitness across all conditions (temporal variability hypothesis) or only under harsh conditions (hard life hypothesis). Empirical studies of these hypotheses have been equivocal because they failed to simultaneously consider whether alloparents provide current reproductive benefits or future load-lightening benefits (reduced parental care that favors future fitness). To distinguish between the hard life and temporal variability hypotheses, we investigated whether the number of alloparents at a nest increased reproductive success or load-lightening in superb starlings (*Lamprotornis superbus*), and if these benefits varied across environmental conditions. Consistent with the temporal variability hypothesis, larger contingents of alloparents increased the number of fledglings while simultaneously allowing mothers to reduce their provisioning rates across all environmental conditions. Conversely, fathers experienced load-lightening in nest guarding only under favorable rainfall conditions. We suggest that breeding cooperatively may give individuals the flexibility to trade-off current reproductive benefits against future load-lightening benefits according to fluctuating environmental conditions. Cooperative breeding in unpredictable environments may thus be a plastic strategy to mitigate variability in the cost of offspring care. Our results highlight the importance of considering how offspring care decisions vary across both breeding roles and fluctuating environmental conditions.

INTRODUCTION

Understanding how organisms cope with and adapt to climate variability is important in the face of global warming (Devictor et al. 2008; Karell et al. 2011; Huey et al. 2012), which is resulting in increased environmental variability across many regions of the world (Garcia et al. 2014). One behavioral coping strategy that may allow populations to adapt to fluctuating environments is breeding cooperatively, where breeders receive help from one or more alloparents in caring for young (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011). Broad-scale comparative analyses have demonstrated that cooperatively breeding bird (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011) and mammal species (Lukas and Clutton-Brock 2017) occur more frequently in unpredictable environments where annual rainfall tends to be low and highly variable through time. But what is the underlying behavioral mechanism to explain these biogeographic patterns? In fluctuating environments, having alloparents to help rear young may provide fitness benefits under both harsh and benign conditions (‘temporal variability hypothesis’, Rubenstein and Lovette 2007), or only under harsh conditions (‘hard life hypothesis’, Emlen 1982; Koenig et al. 2011, 2016). Although these hypotheses are similar, they make contrasting predictions about the environmental conditions under which alloparents should provide the greatest fitness benefits to breeding individuals. Specifically, the hard life hypothesis predicts that alloparents will increase the fitness of breeders most significantly under harsh conditions, whereas the temporal variability hypothesis predicts that alloparents will provide a fitness benefit across all environmental conditions in these unpredictable climates.

Previous efforts examining the benefits of cooperative breeding behavior have focused primarily upon the reproductive benefits of having a greater number of alloparents. Typically, the presence of alloparents can increase the number or quality of young produced annually by enhancing the quality of offspring care provided (Canario et al. 2004; Koenig and Walters 2011), or the number of breeding attempts undertaken by a breeding pair (Woxvold and Magrath 2005). However, alloparents may also provide future fitness benefits by allowing breeders to lower their current investment in offspring care, which can increase their own body condition (Russell et al. 2003) or survival (McGowan et al. 2003; Meade et al. 2010; Paquet et al. 2015). When alloparents provide offspring care, breeders may choose to respond by either (1) *decreasing* the amount of care they provide themselves, thereby reducing the cost of reproduction that they incur (termed compensatory care, or 'load-lightening'. Crick 1992; Hatchwell 1999), or (2) *maintaining* their contribution to offspring care, thereby increasing the cumulative care that breeders and alloparents provide to the young (termed 'additive care', Hatchwell 1999). The care decision rules that breeders use in response to alloparental care may therefore mask the current reproductive benefits of having alloparents. For example, alloparents may not increase reproductive success at a nest if breeders opt to lighten their load in favor of future fitness benefits such as increased survival. To fully understand the fitness benefits of receiving alloparental care in unpredictable environments, it is therefore important to simultaneously consider current reproductive benefits and potential future benefits associated with load-lightening.

Empirical tests of the hard life and temporal variability hypotheses have primarily focused upon the reproductive benefits of having alloparents, which has generated mixed results. For example, alloparents have been shown to increase reproductive success under harsh environmental conditions only (consistent with the hard life hypothesis) (Magrath 2001; Canario et al. 2004; Valencia et al. 2006; Covas et al. 2008), across all environmental conditions (consistent with the temporal variability hypothesis) (Legge 2000; Marshall et al. 2016), or even under favorable conditions only (Koenig et al. 2011). Part of this inconsistency in how alloparental care influences fitness in fluctuating environments may exist because environmental conditions could contribute to shaping breeder care strategies, yet to our knowledge no study has investigated whether breeder care decision rules change across environmental conditions. Breeders are expected to use load-lightening when the relative costs of offspring care are elevated (Johnstone 2011), which occurs most frequently when environmental conditions are harsh (Erikstad et al. 1998). Therefore, alloparental care may lead to load-lightening benefits for breeders under unfavorable environmental conditions, as would be predicted by the hard life hypothesis. However, load-lightening could also be favored across all environmental conditions if reproduction is consistently costly in the unpredictable environments in which cooperative breeders are typically found. Thus, although environmental conditions appear to influence the incidence and biogeographic distribution of cooperative breeding behavior in birds, the underlying fitness benefits—either reproductive or load-lightening—of breeding cooperatively in fluctuating environments remain poorly understood.

Here we examine how variability in rainfall and territory quality shape the current and future fitness benefits of having alloparents in the cooperatively breeding superb starling, *Lamprotornis superbus*. Superb starlings inhabit one of the world's most unpredictable environments—the African savanna—where rainfall varies in intensity and duration within and among years (Rubenstein 2016). Significant variation also exists in grass cover across territories, which together with rainfall shapes the availability of insects used to provision young (Rubenstein 2007a, 2016). To identify the fitness benefits of receiving alloparental care in fluctuating and unpredictable environments, we test whether alloparents increase the number of nestlings fledged and/or load-lightening (1) across all environmental conditions (as predicted by the temporal variability hypothesis), or (2) only under harsh environmental conditions (as predicted by the hard life hypothesis). Ultimately, this research will improve our understanding of the behavioral mechanism through which fluctuating environmental conditions can alter the fitness benefits of breeding cooperatively, thereby helping to explain why cooperatively breeding species occur more frequently in unpredictable environments.

MATERIALS AND METHODS

Study system

Superb starlings are plural cooperative breeders that live in large social groups of up to 50 individuals, including multiple breeding pairs (typically three or four, Rubenstein 2016). A population of uniquely marked superb starlings in seven social groups was monitored continuously from 2002 to 2015 at the Mpala Research Centre, Kenya (0°17'N, 37°52'E). In this population, superb starlings breed twice per year, during the short (October-November) and long rains (March-June) (Rubenstein 2011). The number of alloparents at a nest is variable (range 1-14; Rubenstein 2016) because some group members choose to forgo both breeding and alloparental care in a given breeding season (termed non-breeder/non-alloparents). As a result, the number of alloparents at a particular nest can vary considerably and independently of group size (see Appendix 2.1). Alloparents range in age from juveniles to adults, can be of either sex, and can be related or unrelated to the breeding individuals (Rubenstein 2007b).

Measuring environmental variation

We measured variability in precipitation by quantifying pre-breeding and breeding rainfall for each breeding season using an automated Hydrological Services TB3 Tipping Bucket Rain Gauge (Rubenstein 2011). We calculated breeding rainfall as the sum of daily rainfall from March to June for the long rains and from October to November for the short rains. We also calculated pre-breeding rainfall, which occurs during the dry seasons immediately preceding each rainy season, from July to September for the short rains and from December to February for the long rains. Both pre-breeding and breeding rainfall play important yet distinct roles in shaping superb starling behavior and reproductive success. That is, pre-breeding rainfall influences the adoption of different breeding roles (Rubenstein 2007c), whereas breeding rainfall drives the availability of insects available for feeding young during breeding (Rubenstein 2016).

We also examined how variability in territory quality could affect reproductive or load-lightening benefits. Territory quality was defined as the amount of grass cover on a territory, calculated as the long-term average of the proportion of dropped pins that touched vegetation from monthly vegetation transects performed between 2008 and 2015 (Rubenstein 2007a). Like breeding rainfall, territory grass cover is positively correlated to the availability of insects used to provision young (Rubenstein 2016).

Monitoring reproduction

We monitored 716 nesting attempts over the course of this study by visiting nests every 1 to 3 days to determine clutch size and the fate of each nestling until fledging (Rubenstein 2007d). The two primary causes of fledging failure in superb starlings are nestling starvation and predation (Rubenstein 2016). Starvation was identified when chicks were found dead in or near the nest, or when one previously underweight nestling disappeared, whereas predation usually left visible damage to the nest and often resulted in the entire clutch disappearing at once. Although nest predators are quite diverse (e.g. birds, baboons, snakes, squirrels, genets, and mice, Rubenstein 2006) and vary in the type of damage they cause, the most commons signs of nest predation included tearing of the nest, blood in the nest, or nestling bands found near the nest. We assumed that nestlings were depredated when previously heavy nestlings went missing suddenly and earlier than the typical fledging age. Predation events were further differentiated from fledging via observations in the subsequent year, as juvenile superb starlings remain with their parents in their natal groups for 2 years post-fledging (Rubenstein 2016). In the rare cases where we could not determine what happened to one or more nestlings, they were simply classified as missing and excluded from our analyses (<2.5% of cases from >1,000 hatchlings).

Measuring parental care behavior

Active nests were observed for 2 hrs approximately twice per week, which is comparable to other behavioral studies of cooperative breeding birds (Brouwer et al. 2014; Cram et al. 2015; Dias et al. 2015). All nests included in this study were observed for a minimum of 2 hrs, but the average nest was observed for nearly 6 hrs (mean ± SE = 5.6 ± 0.15 hrs). The identity of each bird arriving within 20 m of the nest was determined with a spotting scope, and the time of arrival and departure from the nest area was recorded. We also recorded entry and exit times from the nest, and whether birds delivered food into the nest. When food was brought to the nest, we recorded the size of food items delivered to young (relative to bill length). However, we found that mothers, fathers, and alloparents delivered similar sized food items to nestlings (mean ± SE: fathers 1.2 ± 0.09; mothers 1.1 ± 0.06, alloparents 1.2 ± 0.09), making provisioning rate a stronger indicator of offspring care than prey item size (as in Browning et al. 2012). We used these observations to calculate two behaviors that reflect investment in offspring care: nest guarding and offspring provisioning (Rubenstein 2006). Guarding was defined as the proportion of time an individual spent within 20 m of the nest (but not inside) relative to the length of the observation period. Time spent perched near the nest constitutes guarding in superb starlings because (1) perched individuals frequently make alarm calls when predators approach (Rubenstein 2016), and (2) alloparents rapidly attack predator models placed near the nest, performing approximately half of the attacks on the models (Rubenstein 2006). Offspring provisioning rate was defined as the number of trips per hour where an individual delivered food into the nest.

Defining breeding roles

At each nest, we measured the guarding or provisioning rate of the mother, father, and the average alloparent (averaged from all alloparents at the nest). We chose to average alloparent behavior regardless of their relatedness to breeders, age, or sex because previous work in this species found that these factors account for few differences in alloparental care behavior (Rubenstein 2006). Breeders at each nest were identified by a combination of behavioral and genetic analyses. Briefly, the mother was identified as the bird incubating at a nest, while the social father was identified as the male closely following the incubating female (Rubenstein 2007b). We also confirmed parentage using microsatellite markers (Rubenstein 2005) from nestlings and adults using DNA extracted from blood samples (Rubenstein 2007a, 2007b). Conversely, all additional individuals provisioning offspring and/or spending time guarding the nest were classified as alloparents. We then counted the number of alloparents observed at each nest to assess whether individuals of each breeding role adjusted their behavior based upon the size of the alloparent contingent. A preliminary investigation revealed that we were more likely to detect a greater number of alloparents at nests observed for a longer cumulative period of time (see Appendix 2.2). To account for this, we calculated the residuals of the number of alloparents on observation time and used this as an index of the number of alloparents present at a nest (see see Appendix 2.2). Larger positive residuals indicate that a nest had a larger contingent of alloparents than expected given the length of time we spent observing that nest, while more negative values indicated a smaller contingent of alloparents than expected based on observation length.

Statistical Analyses

Reproductive benefits of alloparental care: We built four generalized linear mixed models (GLMMs) to examine how (1) clutch size, (2) the number of fledglings, (3) the number of hatchlings starved, and (4) the number of hatchlings depredated related to the number of alloparents across environmental conditions. Each model include pre-breeding rainfall, breeding rainfall, grass cover, and the residual number of alloparents as fixed effects in addition to the interaction between the number of alloparents and each of the three environmental variables. We also included a random effect of nest ID in each model to control for re-nesting events. We assumed a poisson error distribution (log link function) to model clutch size, and a negative binomial error distribution (logit link function) with zero-inflation to model the number of fledglings, starved nestlings, and depredated nestlings because these variables were highly skewed towards zero (due to the high occurrence of nesting failure) and were overdispersed.

Load-lightening benefits of alloparental care: We built four GLMMs for each of the two offspring care behaviors (guarding vs. provisioning) examining how environmental conditions or the relative number of alloparents at a nest altered the behavior of (1) the group cumulatively, (2) fathers, (3) mothers, or (4) alloparents. The number of minutes spent nest guarding and the number of provisioning trips an individual performed were modeled using a negative binomial error distribution with zero-inflation (count data skewed towards 0 and overdispersed), as well as an offset accounting for the cumulative length of focal observations performed at each nest (Bolker et al. 2017). Each model included pre-breeding rainfall, breeding rainfall, grass cover, and the number of alloparents present as fixed effects, the interaction between the number of alloparents and the three environmental variables as fixed effects, and the ID of the mother and father as independent random effects.

All GLMMs were run in the package “glmmADMB” v0.8.3.3 in R (v3.2.4; R Core Team 2016). We originally included two additional fixed effects in all models—breeding season and social unit—but removed these variables because they were redundant with rainfall and grass cover, respectively. We then tested for multicollinearity among our remaining fixed effects and found that our predictor variables were not linearly related to one another (all VIF<2). Each continuous fixed effect was standardized before analyses (i.e. converted to z-score) to improve model convergence (Bolker et al. 2009). We checked the normality and homogeneity of GLMM residuals, which were suitable.

RESULTS

Reproductive benefits

Clutch size did not vary with pre-breeding or breeding rainfall, grass cover, the relative number of alloparents at a nest, or any interaction between these variables (Table 2.1). Instead, mothers consistently laid approximately three eggs (mean ± SE = 3.12 ± 0.03) regardless of environmental or social conditions. Similarly, environmental conditions had no effect on the number of nestlings that fledged successfully. However, having a higher number of alloparents providing offspring care at a nest significantly increased the number of young fledged (Table 2.1). Consistent with the temporal variability hypothesis, we found that this reproductive benefit of having alloparents at a nest did not vary across environmental conditions (i.e. interactions between pre-breeding rainfall, breeding rainfall, or grass cover and the number of alloparents were not significant in our model) (Table 2.1).

Next we examined the mechanisms underlying differences in fledging success. The number of nestlings that starved or were depredated at a nest was unrelated to pre-breeding rainfall, breeding rainfall, or grass cover. The number of starved nestlings was also unrelated to the number of alloparents at a nest and the interaction between the number of alloparents and environmental conditions (Table 2.1). However, the number of nestling depredated decreased significantly with a larger contingent of alloparents, an effect that was consistent across all environmental conditions as interactions between environmental variables and the number of alloparents were not significant (Table 2.1). Nests with relatively more alloparents are therefore less at risk of predation. Thus, alloparents provide reproductive benefits to breeding superb starlings by reducing nestling depredation events 2.1).

We also found that the cumulative guarding and offspring provisioning performed at a nest by all breeders and alloparents increased with breeding rainfall, as well as with the size of the alloparent contingent at that nest (Table 2.2), providing a potential behavioral mechanism through which alloparents increase the number of nestlings fledged 2.2). Cumulative nest guarding increased with the relative number of alloparents significantly more during harsh years with below average breeding rainfall (<230mm; Figure 2.3), although this increase remained positive when breeding rainfall was above average (≥230mm). Conversely, the cumulative provisioning rate at a nest increased with the number of alloparents but only on low quality territories with below average grass cover (≤63%); on high quality territories with above average grass cover (>63%), the provisioning rate did not change with the number of alloparents present 2.4). Together, these results indicate that nests with more alloparents benefit from increased cumulative offspring care and significantly more so under unfavorable conditions, either with low breeding rainfall or low grass cover.

Load-lightening benefits

To determine whether individuals of different breeding roles experience load-lightening benefits and whether these benefits differ across environmental conditions, we examined the behavior of mothers, fathers, and alloparents separately. First, we found that mothers spent a greater proportion of their time guarding the nest and provisioning young at a higher rate as breeding rainfall increased (Table 2.3). However, mothers did not alter their parental care behaviors with pre-breeding rainfall or grass cover (Table 2.3). We also found that mothers did not experience load-lightening in their nest guarding behavior 2.2), suggesting that maternal nest guarding decisions are not affected by the presence of alloparents. However, mothers did experience load-lightening in their provisioning rates, as they performed less offspring provisioning as the number of alloparents increased; Figure 2.2). Importantly, this provisioning load-lightening occurred independently of environmental conditions, as we found no significant interactions between environmental variables and the number of alloparents at a nest. Therefore, mothers experienced load-lightening benefits of having alloparents at the nest by reducing their provisioning rates similarly across all rainfall or grass cover conditions.

In contrast, fathers did not alter their offspring care behavior according to any of the three environmental conditions examined in this study (Table 2.4). Fathers did experience load-lightening in their nest guarding behavior, but only in years of above average breeding rainfall; Figure 2.3). When breeding rainfall was below average, fathers surprisingly increased their nest guarding as the number of alloparents increased at their nest. These results neither support the hard life hypothesis nor the temporal variability hypothesis, as fathers only experienced load-lightening under favorable environmental conditions. Fathers also did not experience load-lightening in their nestling provisioning rates. Instead, paternal provisioning rates increased as the number of alloparents present increased; Figure 2.4), particularly on low quality territories with below average grass cover. Fathers on high quality territories with above average grass cover still increased their offspring provisioning rates as the number of alloparents increased, but not as quickly as those on low quality territories.

Like mothers, the average alloparent increased its nest guarding and nestling provisioning rates as breeding rainfall increased (Table 2.5). However, pre-breeding rainfall and grass cover were unrelated to alloparent offspring care behaviors (Table 2.5). Rather than showing load-lightening, however, alloparents increased the nest guarding and offspring provisioning they performed when there were relatively more alloparents at a nest. The positive relationship between an alloparent's nest guarding behavior and the number of alloparents increased more rapidly in harsh years with below average breeding rainfall; Figure 2.3). This relationship remained positive during benign years with above average rainfall, but the slope of this relationship was not as steep. Conversely, the positive relationship between an alloparent's provisioning rate and the number of alloparents at a nest was consistent across all environmental conditions (interactions between the number of alloparents and environmental variables were not significant; Table 2.5).

DISCUSSION

This study tested two competing hypotheses for why cooperatively breeding species are found more frequently in unpredictable environments—the hard life hypothesis, which argues that alloparents provide reproductive or load-lightening benefits only under harsh conditions, and the temporal variability hypothesis, which argues that alloparents provide reproductive or load-lightening benefits across all environmental conditions. We found support for the temporal variability hypothesis, as superb starling alloparents provided current reproductive benefits equally in harsh and benign pre-breeding and breeding conditions, as well as on high and low quality territories. This is similar to results found in banded mongooses (*Mungos mungo*) and laughing kookaburras (*Dacelo novaeguinieae*)—two species found in similarly unpredictable environments—as alloparents increased reproductive success independently of rainfall and territory quality, respectively (Legge 2000; Marshall et al. 2016). Conversely, several studies have found that alloparents increase reproductive success only during unfavorable (i.e. hard life hypothesis) (Canario et al. 2004; Covas et al. 2008) or favorable environmental conditions (Koenig et al. 2011). Future work examining how and why reproductive and load-lightening fitness benefits of alloparental care change across environmental conditions will be necessary to reconcile current discrepancies in the literature.

To our knowledge, this study is the first to simultaneously explore whether the reproductive and load-lightening benefits of cooperative breeding change across environmental conditions. We found that in addition to reproductive benefits, superb starling mothers experienced load-lightening benefits of having a larger contingent of alloparents at their nests across all environmental conditions, a finding that is also consistent with the temporal variability hypothesis. Previous work in plural breeding chestnut-crowned babbler (*Pomatostomus ruficeps*) found that breeders can simultaneously gain reproductive and load-lightening fitness benefits of having alloparental care (Russell 2016). Specifically, babbler mothers only partially decreased their provisioning rate with every additional alloparent present, which allowed for the cumulative provisioning at the nest to increase with the number of alloparents while simultaneously providing load-lightening for the mother. Therefore, both chestnut-crowned babbler and superb starling mothers face a two-fold advantage to recruiting alloparents, as alloparents increase their current reproductive success while also reducing potential costs of parental care in favor of the mother's own condition or survival. The reproductive and load-lightening fitness benefits of cooperation have also been examined in the acorn woodpecker (*Melanerpes formicivorus*), where breeders do not gain load-lightening benefits and instead maintain provisioning rates regardless of group size (Koenig and Walters 2011). Breeders and alloparents in this species both increased their provisioning rates under favorable environmental conditions (Koenig and Walters 2011), and therefore reproductive success also increased under favorable conditions (Koenig et al. 2011). These studies (Koenig and Walters 2011; Koenig et al. 2011; Russell 2016) along with our own highlight interspecific differences in whether breeders favor reproductive or load-lightening fitness benefits when aided by alloparents. Theoretical models suggest that group size, relatedness, and the cost of care will affect whether current reproductive versus future load-lightening benefits are favored (Carranza et al. 2008; Johnstone 2011), but future theoretical work should consider the impact of environmental variability on this trade-off.

Our results did not support the hard life hypothesis because we found no evidence that the reproductive or load-lightening benefits of having more alloparents were elevated only under harsh conditions. Instead, we found two novel patterns of offspring care behavior that are not currently predicted by either the hard life or temporal variability hypotheses. First, we found that fathers experienced load-lightening in their nest guarding behavior only under favorable rainfall conditions. Second, we found that under unfavorable conditions, fathers and alloparents increased their nest guarding and provisioning rates with the relative number of alloparents present. To our knowledge, no other study has found evidence for load-lightening under favorable conditions only. Since superb starling fathers perform less offspring care relative to mothers (Rubenstein 2016), they may not incur as high a cost of reproduction relative to breeding females. Therefore, it is possible that fathers gain fewer fitness benefits by trading off current versus future fitness benefits than do mothers (Johnstone 2011). Our second surprising finding—that fathers and alloparents may *increase* offspring care with the number of alloparents—has been documented in only one other study. Breeders of the azure-winged magpie (*Cyanopica cyanus*) increased their investment in offspring care behavior when joined by alloparents (Valencia et al. 2006). Much like superb starlings, nest failure in the azure-winged magpie is primarily driven by predation and not by offspring starvation (Valencia et al. 2006). Valencia and colleagues (Valencia et al. 2006) therefore suggested that a larger contingent of alloparents may increase the value of the current brood for the breeders, particularly in species where alloparents provide protection against predators (Carranza et al. 2008). In turn, fathers and alloparents may be more motivated to invest in offspring care when a brood is most likely to succeed (i.e. when more alloparents are present). Why fathers and alloparents would respond to changes in brood value associated with the size of the alloparent contingent at a nest while mothers invest similarly across broods requires further study, particularly because mothers can manipulate brood value based upon the number of alloparents present in other species (Russell et al. 2007).

Finally, an alternative to this model of differential brood value by breeding role is that fathers and alloparents socially reinforce each other's contributions to offspring care, either passively or actively. Passively, individuals may flock or follow conspecifics during feeding trips or guarding bouts, which could lead to an increase in the frequency of provisioning trips or time spent guarding in larger groups. Superb starlings often form foraging flocks during the breeding and non-breeding season (Feare and Craig 1998), and they typically travel to and from the nest in small groups (Guindre-Parker and Rubenstein, personal observations), which lends support to this idea. Since harsh conditions also favor flocking in some bird species (Gardner 2004), superb starlings may remain closer together under unfavorable conditions, which would be consistent with our result that fathers and alloparents increase their offspring care behaviors most under harsh conditions. Alternatively, active social reinforcement could entail enforcing alloparent contributions to offspring care via aggressive behaviors or punishment by group members (Clutton-Brock and Parker 1995), such that larger groups may enforce offspring care to a greater degree. It remains unclear whether alloparents are punished for performing too little offspring care in superb starlings, and whether offspring care behavior is more strongly reinforced under harsher conditions.

In conclusion, our results support the temporal variability hypothesis, as alloparents provide (1) current reproductive benefits to breeders and (2) future load-lightening benefits to mothers, something that occurred across all environmental conditions. Conversely, we found little evidence for the hard life hypothesis, and instead uncovered a new pattern, namely that superb starling fathers experienced greater load-lightening benefits of alloparental care under favorable rather than unfavorable conditions. Importantly, our study demonstrates that reproductive and load-lightening benefits of cooperative breeding differ across breeding roles and may vary across environmental conditions. Therefore, studies examining the fitness benefits of breeding cooperatively that do not account for differences in breeding role or variation in environmental conditions may not capture the complexities of offspring care decision rules that shape alloparental behavior in cooperative breeders. We suggest that breeding cooperatively in fluctuating environments may provide organisms with the behavioral flexibility necessary to adjust their investment in offspring care relative to the behavior of others in their social group or to environmental conditions in order to invest optimally in current breeding versus self-maintenance. This result is consistent with the ‘bet-hedging hypothesis’, which argues that cooperative breeding behavior evolves to reduce environmentally-driven fecundity variance (Rubenstein 2011). It is also consistent with studies of maternal egg investment in superb fairy-wrens where maternal investment in eggs is dependent upon the size of the alloparent contingent at the nest (Russell et al. 2007). Thus, the flexibility to trade-off investment in current reproduction and future survival or reproduction via offspring investment strategies may represent a potential mechanism underlying patterns of environmentally-driven reproductive variance, as well as explain why cooperatively breeding species occur more frequently in unpredictable environments. Ultimately, considering how offspring care decision rules vary across both breeding roles and fluctuating environmental conditions will provide new insights into how and why cooperative breeding behavior is linked to ecology.

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TABLES

Table 2.1: Parameter estimates and 95% Wald confidence intervals for four GLMMs examining how environmental conditions, the relative number of alloparents at a nest, or the interaction between these variables shape (A) clutch size, (B) the number of nestlings that fledged, (C) the number of nestlings that starved, or (D) the number of nestlings depredated. Asterisks highlight significant variables.

	Estimate ± SE	***Z***	***P***	**95% Wald CI**	
(A) Dependent variable: Clutch size					
Intercept	1.16 ± 0.05	25.0	<0.001*	1.07	1.25
Pre-breeding rain	-0.03 ± 0.05	-0.58	0.56	-0.13	0.07
Breeding rain	0.03 ± 0.05	0.60	0.55	-0.07	0.13
Grass cover	0.01 ± 0.05	0.19	0.85	-0.08	0.10
No. alloparents	-0.07 ± 0.09	-0.84	0.40	-0.24	0.10
Pre-breeding rain * No. alloparents	-0.04 ± 0.08	-0.50	0.62	-0.19	0.11
Breeding rain * No. alloparents	-0.02 ± 0.08	-0.24	0.81	-0.18	0.14
Grass cover * No. alloparents	-0.02 ± 0.08	-0.23	0.82	-0.17	0.14
(B) Dependent variable: Number of nestlings fledged					
Intercept	0.41 ± 0.12	3.30	<0.001*	0.17	0.65
Pre-breeding rain	-0.13 ± 0.12	-1.07	0.28	-0.36	0.11
Breeding rain	0.13 ± 0.10	1.21	0.23	-0.08	0.33
Grass cover	0.03 ± 0.11	0.30	0.76	-0.19	0.26
No. alloparents	0.82 ± 0.22	3.71	<0.001*	0.39	1.25
Pre-breeding rain * No. alloparents	0.11 ± 0.21	0.53	0.60	-0.30	0.52
Breeding rain * No. alloparents	0.32 ± 0.20	1.60	0.11	-0.07	0.71
Grass cover * No. alloparents	-0.20 ± 0.23	-0.90	0.37	-0.65	0.24
(C) Dependent variable: Number of nestlings starved					
Intercept	-2.72 ± 0.61	-4.45	<0.001*	-3.92	-1.52
Pre-breeding rain	0.09 ± 0.31	0.30	0.77	-0.52	0.70
Breeding rain	-0.17 ± 0.31	-0.55	0.58	-0.80	0.43
Grass cover	0.13 ± 0.30	0.43	0.67	-0.46	0.72
No. alloparents	0.46 ± 0.56	0.82	0.41	-0.64	1.60
Pre-breeding rain * No. alloparents	0.16 ± 0.50	0.32	0.75	-0.81	1.13
Breeding rain * No. alloparents	-0.52 ± 0.54	-0.97	0.33	-1.57	0.53
Grass cover * No. alloparents	-0.48 ± 0.59	-0.83	0.41	-1.63	0.67
(D) Dependent variable: Number of nestlings depredated					
Intercept	0.88 ± 0.10	9.08	<0.001*	0.69	1.06
Pre-breeding rain	0.02 ± 0.10	0.24	0.81	-0.18	0.22
Breeding rain	0.06 ± 0.11	0.54	0.59	-0.15	0.27
Grass cover	0.01 ± 0.08	0.19	0.85	-0.13	0.16
No. alloparents	-0.28 ± 0.15	-1.94	0.053	-0.58	0.003
Pre-breeding rain * No. alloparents	-0.002 ± 0.13	-0.02	0.99	-0.26	0.25
Breeding rain * No. alloparents	-0.05 ± 0.14	-0.34	0.73	-0.32	0.22
Grass cover * No. alloparents	0.003 ± 0.11	0.03	0.98	-0.21	0.22

Table 2.2: Parameter estimates and 95% Wald confidence intervals for two GLMMs examining how environmental conditions, the number of alloparents at a nest, or the interaction between these variables shape (A) time spent nest guarding and (B) the number of provisioning trips performed by all group members cumulatively. Asterisks highlight significant variables.

	Estimate ± SE	***Z***	***P***	**95% Wald CI**	
(A) Dependent variable: Nest guarding					
Intercept	-0.54 ± 0.06	-9.00	<0.001*	-0.66	-0.42
Pre-breeding rain	0.04 ± 0.06	0.69	0.49	-0.07	0.15
Breeding rain	0.19 ± 0.05	3.51	<0.001*	0.08	0.30
Grass cover	0.04 ± 0.06	0.62	0.54	-0.08	0.15
No. alloparents	0.35 ± 0.09	3.73	<0.001*	0.17	0.53
Pre-breeding rain * No. alloparents	0.07 ± 0.09	0.84	0.40	-0.10	0.25
Breeding rain * No. alloparents	-0.18 ± 0.09	-1.99	0.046*	-0.36	-0.003
Grass cover * No. alloparents	-0.04 ± 0.08	0.45	0.65	-0.20	0.13
(B) Dependent variable: Provisioning trips					
Intercept	0.47 ± 0.10	4.66	<0.001*	0.27	0.66
0.38Pre-breeding rain	0.01 ± 0.07	0.13	0.90	-0.13	0.15
Breeding rain	0.15 ± 0.07	2.31	0.02*	0.02	0.28
Grass cover	0.04 ± 0.09	0.45	0.65	-0.14	0.22
No. alloparents	0.26 ± 0.13	1.99	0.047*	0.004	0.52
Pre-breeding rain * No. alloparents	-0.08 ± 0.13	-0.60	0.55	-0.32	0.17
Breeding rain * No. alloparents	0.18 ± 0.12	1.54	0.12	-0.05	0.42
Grass cover * No. alloparents	-0.33 ± 0.13	-2.52	0.01*	-0.59	-0.07

Table 2.3: Parameter estimates and 95% Wald confidence intervals for two GLMMs examining how environmental conditions, the number of alloparents at a nest, or the interaction between these variables shape (A) time spent nest guarding and (B) the number of provisioning trips performed by mothers. Asterisks highlight significant variables.

	Estimate ± SE	***Z***	***P***	**95% Wald CI**	
(A) Dependent variable: Nest guarding					
Intercept	-1.52 ± 0.06	-25.9	<0.001*	-1.64	-1.41
Pre-breeding rain	-0.04 ± 0.07	-0.63	0.53	-0.18	0.09
Breeding rain	0.24 ± 0.07	3.47	<0.001*	0.10	0.37
Grass cover	-0.005 ± 0.05	-0.09	0.93	-0.11	0.10
No. alloparents	0.02 ± 0.11	0.13	0.89	-0.21	0.24
Pre-breeding rain * No. alloparents	-0.05 ± 0.11	-0.43	0.66	-0.27	0.17
Breeding rain * No. alloparents	-0.16 ± 0.10	-1.50	0.13	-0.26	0.05
Grass cover * No. alloparents	0.01 ± 0.09	0.10	0.92	-0.17	0.19
(B) Dependent variable: Provisioning trips					
Intercept	-0.12 ± 0.10	-1.22	0.22	-0.32	0.08
Pre-breeding rain	0.09 ± 0.08	1.17	0.24	-0.06	0.24
Breeding rain	0.12 ± 0.06	2.16	0.031*	0.01	0.23
Grass cover	0.02 ± 0.09	0.24	0.81	-0.15	0.19
No. alloparents	-0.38 ± 0.13	-2.89	0.004*	-0.63	-0.12
Pre-breeding rain * No. alloparents	-0.004 ± 0.13	-0.03	0.98	-0.26	0.25
Breeding rain * No. alloparents	0.09 ± 0.11	0.84	0.40	-0.13	0.32
Grass cover * No. alloparents	-0.13 ± 0.12	-1.07	0.28	-0.37	0.11

Table 2.4: Parameter estimates and 95% Wald confidence intervals for two GLMMs examining how environmental conditions, the number of alloparents at a nest, or the interaction between these variables shape (A) time spent nest guarding and (B) the number of provisioning trips performed by fathers. Asterisks highlight significant variables.

	Estimate ± SE	***Z***	***P***	**95% Wald CI**	
(A) Dependent variable: Nest guarding					
Intercept	-2.00 ± 0.15	-13.6	<0.001*	-2.29	-1.71
Pre-breeding rain	0.18 ± 0.11	1.62	0.11	-0.04	0.39
Breeding rain	0.18 ± 0.10	1.86	0.06	-0.01	0.37
Grass cover	0.11 ± 0.11	0.93	0.35	-0.12	0.33
No. alloparents	0.01 ± 0.15	0.04	0.97	-0.29	0.30
Pre-breeding rain * No. alloparents	0.07 ± 0.18	0.39	0.70	-0.28	0.41
Breeding rain * No. alloparents	-0.40 ± 0.16	-2.55	0.01*	-0.71	-0.09
Grass cover * No. alloparents	0.16 ± 0.13	1.22	0.22	-0.10	0.43
(B) Dependent variable: Provisioning trips					
Intercept	-1.58 ± 0.22	-7.15	<0.001*	-2.01	-1.15
Pre-breeding rain	-0.12 ± 0.17	-0.71	0.48	-0.45	0.21
Breeding rain	0.13 ± 0.14	0.92	0.36	-0.15	0.41
Grass cover	0.19 ± 0.20	0.94	0.35	-0.21	0.59
No. alloparents	1.11 ± 0.24	4.53	<0.001*	0.63	1.59
Pre-breeding rain * No. alloparents	0.21 ± 0.28	0.76	0.45	-0.33	0.76
Breeding rain * No. alloparents	0.18 ± 0.34	0.53	0.60	-0.49	0.84
Grass cover * No. alloparents	-1.01 ± 0.25	-4.07	<0.001*	-1.49	-0.52

Table 2.5: Parameter estimates and 95% Wald confidence intervals for two GLMMs examining how environmental conditions, the number of alloparents at a nest, or the interaction between these variables shape (A) time spent nest guarding and (B) the number of provisioning trips performed by alloparents. Asterisks highlight significant variables.

	Estimate ± SE	***Z***	***P***	**95% Wald CI**	
(A) Dependent variable: Nest guarding					
Intercept	-2.23 ± 0.11	-20.8	<0.001*	-2.44	-2.02
Pre-breeding rain	-0.01 ± 0.09	-0.10	0.92	-0.20	0.18
Breeding rain	0.27 ± 0.09	3.00	0.003*	0.09	0.45
Grass cover	-0.09 ± 0.10	-0.87	0.38	-0.29	0.11
No. alloparents	0.63 ± 0.18	3.53	<0.001*	0.28	0.98
Pre-breeding rain * No. alloparents	-0.07 ± 0.16	-0.45	0.65	-0.38	0.24
Breeding rain * No. alloparents	-0.33 ± 0.17	-1.98	0.047*	-0.66	-0.004
Grass cover * No. alloparents	-0.21 ± 0.18	-1.16	0.24	-0.57	0.15
(B) Dependent variable: Provisioning trips					
Intercept	-2.17 ± 0.21	-10.34	<0.001*	-2.58	-1.76
Pre-breeding rain	0.10 ± 0.19	0.54	0.59	-0.26	0.46
Breeding rain	0.45 ± 0.17	2.57	0.01*	0.11	0.79
Grass cover	-0.05 ± 0.17	-0.31	0.75	-0.39	0.28
No. alloparents	1.22 ± 0.28	4.35	<0.001*	0.67	1.77
Pre-breeding rain * No. alloparents	-0.01 ± 0.33	-0.02	0.98	-0.64	0.63
Breeding rain * No. alloparents	-0.29 ± 0.32	-0.89	0.37	-0.92	0.35
Grass cover * No. alloparents	-0.37 ± 0.27	-1.34	0.18	-0.90	0.17

FIGURES

Figure 1: The number of offspring fledged increases with the number of alloparents providing care at a nest (solid line). The number of alloparents was unrelated to the number of nestlings that succumbed to starvation (dotted line), but alloparents did decrease the number of nestlings that were depredated (dashed line). Lines represent GLMM model predictions, where other parameters are set to their mean values.

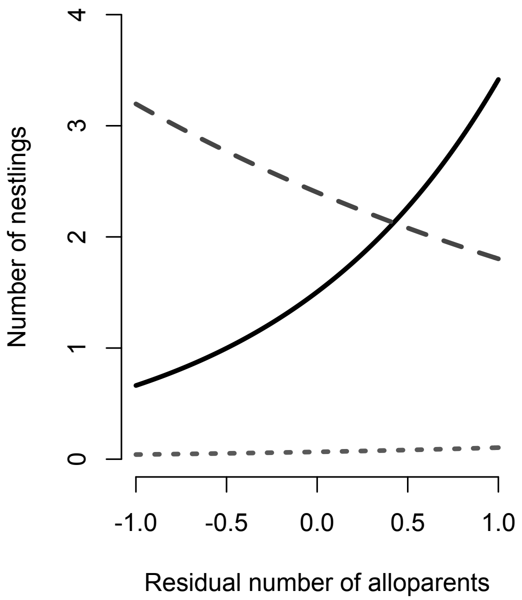


Figure 2.2: (A) Cumulative nest guarding (solid grey line) increased with the number of alloparents present at a nest. Mothers (dotted black line) and fathers (solid black line) did not alter their nest guarding behavior as the number of alloparents increased, but individual alloparents (dashed black line) were more likely to perform greater nest guarding when there were more alloparents present. Similarly, (B) the cumulative provisioning rate by all individuals increased with the number of alloparents present. Mothers experienced load-lightening since they provisioned at a lower rate when there were more alloparents at a nest. Conversely, fathers and alloparents increased their provisioning rates when the number of alloparents at a nest increased. Lines represent GLMM model predictions, where other parameters are set to their mean values.

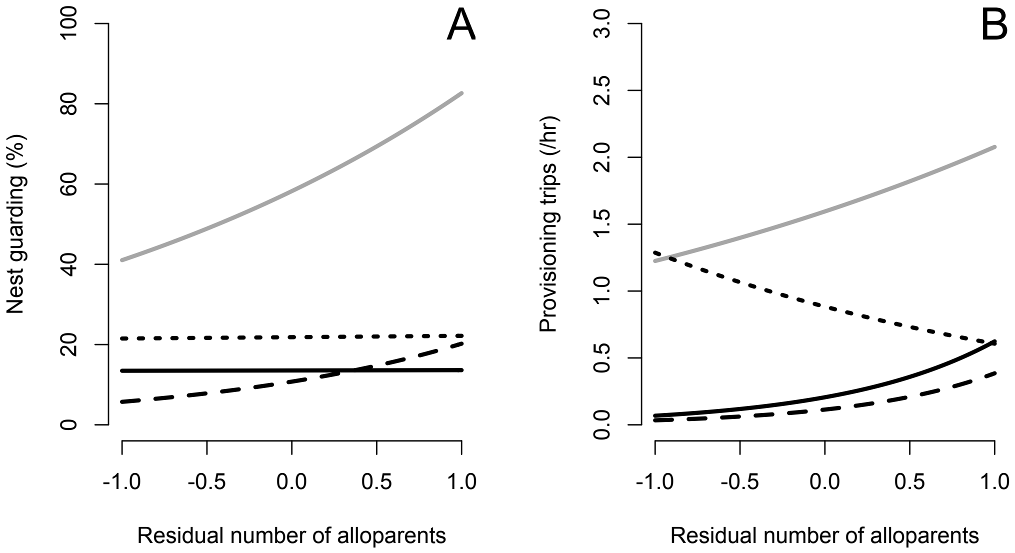


Figure 2.3: (A) Fathers experienced load-lightening only under above average breeding rainfall (solid line) while they increased nest guarding as the number of alloparents increased when breeding rainfall was below average (dashed line). (B) Individual alloparents increased their nest guarding behavior with the number of alloparents at a nest, but significantly more when breeding rainfall was below average rainfall. Similarly, (C) cumulative guarding at a nest increased with the number of alloparents, and significantly more in seasons of below average rainfall. Lines represent GLMM model predictions, where other parameters are set to their mean values.

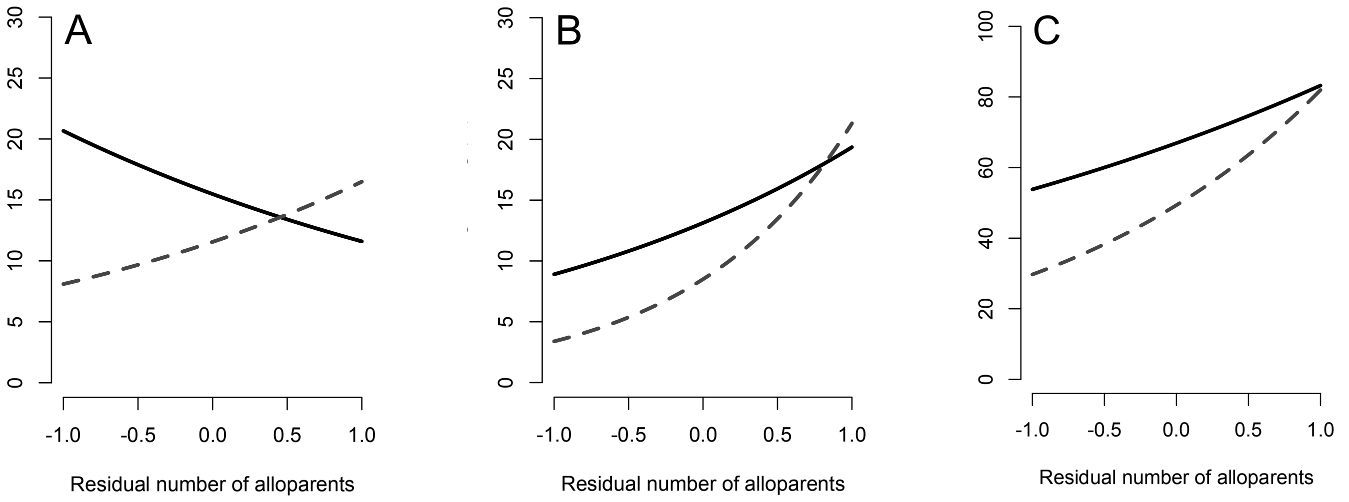
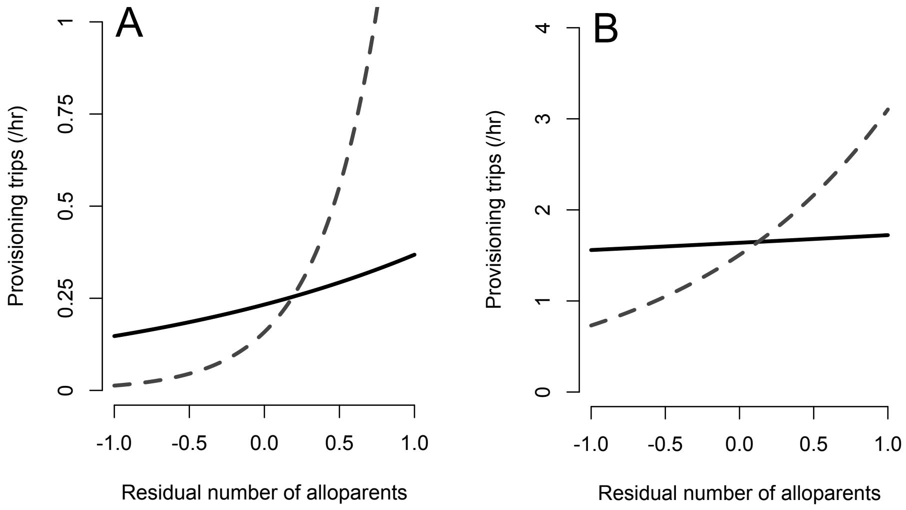


Figure 2.4: The provisioning behavior of (A) fathers and (B) all individuals at a nest (i.e. cumulative behavior) increased with the residual number of alloparents present, and significantly more so on territories with below average grass cover (dashed line) compared to territories with above average grass cover (solid line). Lines represent GLMM model predictions, where other parameters are set to their mean values.



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CHAPTER 3 — PHYSIOLOGICAL CONSTRAINTS, COSTS AND CARRY-OVER EFFECTS OF REPRODUCTION IN A COOPERATIVELY BREEDING BIRD

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ABSTRACT

The cost of reproduction is a life-history trade-off where investment in current reproduction via costly parental care decreases subsequent fitness. While this trade-off is thought to occur ubiquitously across species, there is equivocal evidence that parental care behaviors are costly. A major challenge of studying the cost of parental care has been a lack of consensus over which physiological mechanisms underlie this trade-off. Our study compares four physiological mechanisms thought to shape the cost of parental care by examining whether glucocorticoids, oxidative stress, immune function and body condition (i) constrain offspring care behavior, (ii) represent a cost of offspring care behavior, or (iii) have carry-over effects on subsequent fitness. We use a 4-year correlative dataset collected in cooperatively breeding superb starlings (*Lamprotornis superbus*), where parental and alloparental care vary widely from one individual to the next. Our results showed that baseline corticosterone, antioxidant capacity, and female body condition prior to chick rearing constrained investment in offspring care. Conversely, a single trait represented a cost of offspring care and shaped the likelihood of breeding in subsequent seasons (reactive oxygen metabolites and bacteria killing ability, respectively). Our results suggest that superb starlings modify their investment in offspring care according to their intrinsic state prior to breeding, and therefore face few costs of parental or alloparental care. This flexibility to adjust investment in offspring care according to pre-breeding physiology may represent an important benefit of breeding cooperatively. Importantly, our study also demonstrates that multiple physiological mechanisms may shape constraints or costs of parental care, and these should be considered simultaneously in future studies.

INTRODUCTION

Sexually reproducing organisms are faced with a fundamental decision—to invest valuable resources and energy in reproduction or in their own survival. The negative impact that current reproduction can have on subsequent survival or reproduction represents a fundamental life history trade-off called the ‘cost of reproduction’ (Williams 1966; Stearns 1992). Evidence of this critical life history trade-off comes from a range of organisms, including humans (Lycett et al. 2000), small mammals (Speakman 2008), birds (Descamps et al. 2009), fish (van den Berghe 1992), insects (Salmon et al. 2001; Kotiaho and Simmons 2003), and plants (Obeso 2002). The cost of reproduction is thought to occur because providing parental care is difficult or energetically expensive, yet critical for the successful rearing of young (Gross 2005; Linker and Székely 2005). Although theory suggests that the cost of reproduction an organism incurs should be proportional to their investment in offspring care, this does not always appear to be the case (Santos and Nakagawa 2012; Elliott et al. 2014). Despite numerous empirical studies examining the costs of parental care, it remains unclear why parental care effort reduces future survival or reproduction in some species but not in others (Harshman and Zera 2007; Williams and Fowler 2015).

One of the primary challenges associated with studying future survival or reproductive costs associated with parental care is that we lack a general understanding of the mechanism by which parental care can inhibit future fitness (Harshman and Zera 2007; Speakman 2008; Williams and Fowler 2015). Multiple potential physiological mechanisms underlying the cost of reproduction have been identified (outlined in Table 3.1)—in order for a physiological trait to mediate the cost of parental care, reproductive effort must cause changes in the physiological trait, which in turn must have carry-over effects that alter survival or future reproductive success. However, organisms may adjust their investment in reproduction according to their pre-breeding condition (Descamps et al. 2011; Stier et al. 2012; Ouyang et al. 2013), so it is also important to consider whether physiological condition prior to breeding may constrain subsequent investment in offspring care behaviors. Here, we differentiate between a constraint and a cost as follows: a physiological constraint represents a favourable physiological trait prior to offspring rearing that increases investment in parental care, whereas a cost is a decrease in physiological condition that results proportionally to investment in a parental care. It is possible for a single trait to act as both a constraint and a cost of parental care, though a physiological cost may be difficult to detect if the same trait also acts as a constraint (Stier et al. 2012).

Birds have proven to be useful models for examining the mechanisms underlying the cost of reproduction because it is relatively easy to quantify or manipulate brood size at the egg stage in order to measure or alter an individual's breeding workload (reviewed in Santos and Nakagawa 2012; Williams and Fowler 2015). The four leading physiological mechanisms thought to mediate the cost of reproduction in birds include glucocorticoid hormones, oxidative stress, immune function and body mass or fat reserves (Alonso-alvarez and Velando 2012), all of which act as constraints or costs of reproduction and have carry-over effects on future fitness (Table 3.1). Despite a variety of empirical studies in birds examining these mechanisms, the relative importance of each remains equivocal because numerous studies have failed to find support for the relationships between these traits and investment in parental care and/or subsequent survival or reproduction (Harshman and Zera 2007; Speakman 2008; Williams and Fowler 2015). To understand why evidence for the mechanisms underlying the cost of reproduction has been equivocal, we must examine why the role of each trait in shaping the cost of reproduction remains debated.

Glucocorticoid hormones (e.g. cortisol and corticosterone; hereafter CORT) are often thought of as 'stress hormones' because titers are increased in response to acute stressors (Breuner et al. 2008), yet it is baseline CORT that mostly likely mediates a cost of reproduction because of the role it plays in mediating homeostasis (McEwen and Wingfield 2003; Love et al. 2014). Life history stages or environmental changes necessitating elevated energy expenditure are expected to result in increased baseline CORT (McEwen and Wingfield 2010)—short increases in baseline CORT occurring in the appropriate life history or environmental context may be advantageous (Romero 2002; Landys et al. 2006), though chronically elevated CORT can be detrimental (Suorsa et al. 2003; Breuner et al. 2008). Therefore, CORT generally increases with offspring care workload (Bonier et al. 2009b, 2011; Crossin et al. 2013) and elevated CORT can decrease survival (Suorsa et al. 2003; Breuner et al. 2008). However, the relationship between CORT and fitness remains debated (Bonier et al. 2009a) as many studies fail to find a relationship between baseline CORT and fitness or find a relationship in the opposite direction than the one predicted above (reviwed in Bonier et al. 2009a). Recent work suggests that the relationship between CORT and fitness is difficult to generalize because it is highly context dependent (Love et al.

2014; Madliger and Love 2016)—the relationships between baseline CORT and fitness may be sex-specific (Angelier et al. 2010), may differ between first and second broods (Love et al. 2014), or may differ with habitat quality (Madliger and Love 2016). Furthermore, it remains difficult to understand the relationship between baseline CORT and parental care behavior, because manipulating baseline CORT is known to cause changes in parental behavior (Crossin et al. 2012; Ouyang et al. 2013), but manipulating parental care behavior is also known to cause changes in baseline CORT (Madliger and Love 2016).

Oxidative stress, the imbalance between harmful reactive oxygen metabolites and neutralizing antioxidants, has been suggested as a physiological driver of the cost of reproduction (Alonso-Alvarez et al. 2004; Bergeron et al. 2011; Metcalfe and Monaghan 2013; Speakman and Garratt 2014). Briefly, individuals providing greater offspring care may face elevated metabolic activity which results in the production of reactive oxygen metabolites or lowers antioxidant defenses (Guindre-Parker et al. 2013; Cram et al. 2015). Thus, individuals face reduced survivorship as oxidative damage accumulates (Cadenas and Davies 2000; Saino et al. 2011). However, many studies have failed to find a relationship between oxidative stress and survival (Robert et al. 2007; Montgomery et al. 2012) or parental care (Ołdakowski et al. 2012; Metcalfe and Monaghan 2013). Similarly, the oxidative cost of reproduction may occur only in specific tissues (Schmidt et al. 2014; Xu et al. 2014), or oxidative damage repair mechanisms may limit the degree to which oxidative damage affects future fitness (Davies 2000).

Since immune responses represent a vital barrier to pathogens, yet are energetically demanding to produce (Hanssen et al. 2004; Rubenstein et al. 2008), individuals may become immunosuppressed if they reallocate energy to costly parental care (Hanssen et al. 2005; Cox et al. 2010). If individuals performing parental care are immunocompromised, they may be more susceptible to pathogens and have a higher risk of mortality. However, the vertebrate immune system is complex and even within an individual, trade-offs among different branches of the immune system can provide conflicting support for whether parental care leads to immunosuppression or not (Hegemann et al. 2013). Furthermore, baseline investment in immunity may not always be representative of immune responsiveness (Vermeulen et al. 2016), and may not affect fitness in the absence of an infection (Krams et al. 2013).

Finally, investment in parental care may deplete body condition and muscle or fat reserves

(Velando and Alonso-Alvarez 2003; Canestrari et al. 2007), tissues that are important for juvenile and adult survival (Haramis et al. 1986; Cox and Cresswell 2014; Naef-Daenzer and Gruebler 2016). However, adaptive mass loss during reproduction may actually be advantageous if performing provisioning trips with extra weight is too energetically demanding (Freed 1981). Similarly, the relationship between body condition and survival may not be consistent across species (Milenkaya et al. 2015). Thus, while there is empirical evidence linking each of these four potential physiological mechanisms to parental effort and reduced subsequent fitness, it remains unclear which mechanism—if any—most strongly mediates the cost of reproduction because studies comparing and contrasting all four mechanisms simultaneously within the same system are lacking.

Here we examine the four primary physiological mechanisms that potentially mediate the cost of avian reproduction. We perform this study in the plural cooperatively breeding superb starling, *Lamprotornis superbus*, where up to 14 individuals aid in offspring care (Rubenstein 2006). Although it is widely assumed that cooperative breeding behavior in vertebrates results in part from costly parental care (Emlen 1982), the costs of reproduction in social species have only been explored in a few studies (Canestrari et al. 2007; Shen et al. 2011; Cram et al. 2015; Guindre-Parker and Rubenstein 2017a). Yet, cooperatively breeding species are ideal systems to explore how natural variation in offspring care shapes physiological costs of offspring care (Costantini 2016a), because care involves a combination of parental and alloparental forms. As a result, investment in offspring care in cooperative breeders varies among individuals according to breeding roles (e.g. breeders versus alloparents) (Mumme et al. 1990; Rubenstein 2006), as well as within breeding roles according to a variety of additional factors including sex (Brouwer et al. 2014), relatedness to the offspring (Browning et al. 2012), individual condition (van de Crommenacker et al. 2011), and environmental conditions (Wiley and Ridley 2016).

We examine the four leading physiological mechanisms of the cost of parental care and consider whether these physiological traits (i) act as constraints on offspring care, (ii) represent a cost of offspring care, and (iii) have carry-over effects that shape subsequent fitness. We predict that if these physiological traits during incubation constrain subsequent offspring care behavior, individuals in better physiological condition prior to breeding (i.e. lower baseline CORT, lower oxidative stress or higher antioxidants, higher immune responsiveness, or high body condition) will provide a greater degree of offspring care during chick rearing. If these physiological traits represent costs of reproduction, we predict that individual providing greater offspring care will face a greater within-individual increase in baseline CORT, increase in oxidative stress or decrease in antioxidants, decrease in immune responsiveness or decrease in body condition from incubation to chick rearing. Lastly, we predict that if these physiological traits have carry-over effects, individuals in better physiological condition during chick rearing (i.e. lower baseline CORT, lower oxidative stress or higher antioxidants, higher immune responsiveness, or high body condition) will be most likely to breed in the subsequent short or long rain breeding seasons. Because these physiological systems are not mutually exclusive, we predict that multiple physiological traits will simultaneously represent constraints or costs of parental care, and have carry-over effects on future breeding behavior. In addition to testing these hypotheses, we discuss the role that costly parental care may have played in shaping cooperative breeding behavior in superb starlings.

METHODS

Study system

A population of superb starlings consisting of nine social groups was monitored continuously from 2001 to 2016 at the Mpala Research Centre, Kenya (0°17'N, 37°52'E). Each individual was uniquely marked with a numbered metal band and a combination of three colored bands. In this population, superb starlings breed twice per year, during the short (October-November) and long (March-June) rainy seasons (Rubenstein 2011). Superb starlings are plural cooperative breeders that live in large social groups of up to 50 individuals that defend year-round territories (Rubenstein 2016). Every breeding season, between 0 and 7 breeding pairs per group (mean ± SE = 1.9 ± 0.09) will undertake one or more nesting attempts. Non-breeding individuals in the group either act as alloparents at one of the active nests within their social group, or will neither contribute to breeding or alloparental care (termed ‘non-breeder/non-alloparents’). As a result, some group members perform no offspring care at all, while others invest over half of their time in performing offspring care (e.g. investment in nest guarding ranges from 0 to 55% of an individual’s time, mean ± SE = 14 ± 2%).

Sampling birds

We trapped individuals during the long-rains from 2013 to 2016 using food-baited ground-traps or mist-nets around focal nests, and collected a small blood sample from the brachial vein within 3 minutes of capture. We used this blood sample to measure baseline CORT, oxidative stress and immune function. Blood samples were centrifuged in the field and plasma was stored frozen until analysis (up to 6 months) (Rubenstein et al. 2008).

Individuals were captured during two breeding stages: (i) *incubation*, defined as post-clutch completion but before hatching to exclude potential costs of egg laying or nest building; and (ii) *chick rearing*, when nestlings were between 4 and 16 days of age. We re-sampled individuals across both stages whenever possible, but high nest predation rates made it difficult to recapture every individual during chick rearing. Therefore, we sampled 69 individuals during incubation and 48 individuals during chick rearing, of which 23 individuals were sampled during both periods.

Measuring offspring care behavior

We observed active nests with a spotting scope for a minimum of 2 hours per observation period (mean ± SE = 4.4 ± 0.4). During focal observations we monitored the identity of each bird that came within 20 m of the nest as well as those that delivered food to chicks (Rubenstein 2007a). We calculated three behaviors that reflect investment in offspring care: (i) *nest guarding*, defined as the proportion of time an individual spent within 20 m of the nest—but not inside—relative to the length of the observation period; (ii) *provisioning rate*, defined as the number of trips per hour where an individual delivered food into the nest; and (iii) *average size of prey*, defined as the average size of the food delivered to the nest relative to bill size. These parental and alloparental care behaviors were chosen because they increase reproductive success in superb starlings (Rubenstein 2006; Guindre-Parker and Rubenstein 2017b), and are costly to perform in other species of birds (van den Berghe 1992; Markman et al. 1995; van de Crommenacker et al. 2011; Cram et al. 2015).

We examined carry-over effects by determining whether physiology influenced the likelihood that an individual would breed in the following short rain breeding season (i.e. the very next breeding season starting in November of the same year) or the following long rain breeding season (i.e. two breeding seasons later, a year from the current season). We note, however, that there are typically fewer breeding pairs in the short rain breeding season compared to the long rain season (short rain mean ± SE = 1.5 ± 0.11; long rain mean ± SE = 2.3 ± 0.14; two sample t-test $t = 4.3$, $P < 0.001$). We surveyed territories in subsequent breeding seasons as part of routine long-term data collection, and identified active nests and performed focal observations at these nests to identify the breeding pair (Rubenstein 2006). We were not able to determine whether physiology led to carry-over effects on annual mortality because the vast majority of individuals in our study were re-sighted in the following year (i.e. 88%). Furthermore, short-term re-sighting probabilities are not very meaningful in this long-lived bird as it is common for an individual to go unobserved for a breeding season in our study population only to return later (Guindre-Parker and Rubenstein, unpublished data).

Physiological analyses

Glucocorticoids

We measured baseline CORT using a commercially available enzyme immunoassay kit according to the manufacturer's directions (Enzo Life Sciences corticosterone kit). Briefly, plasma was thawed and 1% steroid displacement buffer was added for a minimum of 5 min before diluting samples 1:10 in assay buffer provided in the kit. This protocol was validated for superb starlings, where 4 dilutions of the same plasma sample yielded similar hormone concentrations (mean = 6.9% difference between dilutions, for dilutions of 1:6, 1:8, 1:11 and 1:15). Each plate included a standard curve (range = 32 – 20,000 pg/ml) and two blank controls (CORT-free assay buffer). Each year, we ran samples within 6 months of collection by randomizing the position of samples on the plate, including all samples from the same individual within a year on a single plate. Diluted samples, standards, and blanks, were run in duplicate. We read the plate using a plate reader (Bio-Rad model 680 XR) at 405 nm and CORT titers were calculated from the absorbance relative to that of the standard curve and expressed in ng/ml. The intra-assay and inter-assay coefficients of variation were 3.1% and 5.0%, respectively.

Oxidative Stress

To quantify oxidative stress in both species, we assessed reactive oxygen metabolites (ROM) and total antioxidant capacity (OXY) from plasma samples. We chose to measure ROM because they are more stable derivatives of reactive oxygen species and represent a marker of early oxidative damage (Guindre-Parker et al. 2013; Costantini 2016b). Although ROM is an indirect index of oxidative damage, it has been shown to correlate with increased rates of telomere loss (Geiger et al. 2011; Hau et al. 2015) and reduced survival in free-living birds (Geiger et al. 2011; Costantini and Dell'Omo 2015). We measured ROM using a commercially available kit (dROM test, Diacron International, Italy) according to standard protocols with slight modifications (Costantini et al. 2008; Baldo et al. 2015) that have been validated for this species (Guindre-Parker and Rubenstein 2017a). Briefly, we diluted 10 µL of plasma with 400 µL of a 1:100 mixture of the alkyl-amine solution and acetate buffer. Similarly to previous studies (Costantini et al. 2011; Guindre-Parker et al. 2013), we found that a lipid precipitate formed at the bottom of the wells. Therefore, instead of running the assay in a 96-well plate, we ran the assay in 1.5mL eppendorf tubes before pipetting the liquid layer into a 96-well plate. We incubated reagents and plasma in eppendorf tubes for 75 min at 37°C and centrifuged the tubes at 10,000 rpm for 30 sec to isolate the precipitate at the bottom of the tube. We then pipetted 190 µL of the liquid into duplicate wells of a flat-bottomed 96-well plate (Costantini et al. 2011; Guindre-Parker et al. 2013). On each plate, we ran a standard curve of H_2O_2 (a strong oxidant; range = 0.16-5.12 mg H_2O_2/dL). Absorbance was read at a wavelength of 490 nm and concentrations of reactive oxygen metabolites are given in mg H_2O_2/dL.

We also quantified antioxidant defenses by measuring the general capacity of plasma antioxidants (OXY) to neutralize a strong oxidant (hypochlorous acid, or HOCl). We measured OXY using a commercial kit (OXY test, Diacron International, Italy) that has been validated for this species (Guindre-Parker and Rubenstein 2017a). Briefly, we diluted plasma samples to 1:100 with deionized water and added 5 µL of diluted samples in triplicate to wells of a flat-bottomed 96-well plate with 200 µL of the HOCl solution (Costantini et al. 2008, 2011; Guindre-Parker et al. 2013). The plate was shaken at 450 rpm for 10 sec and incubated at 37°C for 10 min. On each plate, we included a standard curve of HOCl (range = 0.43 - 6.8 µmol HOCl/mL). We then added 2µL of the color-changing chromogen solution to each well (N,N-diethylparaphenilendiamine) and read the plate at 490 nm after shaking for 30 sec. Concentrations of antioxidants are expressed in µmol of HOCl/mL.

For both oxidative stress assays, multiple samples from a single individual were run on the same plate, but positions within a plate were randomized. The mean intra-assay and inter-assay coefficients of variation for dROM were 5.9% and 6.8%, and for OXY were 5.4% and 7.9%, respectively.

Immune Function: Bacteria Killing Ability

We measured the ability of plasma samples to kill a standardized bacterial strain to assess constitutive innate immune function (Tieleman et al. 2005; Matson et al. 2006). This test represents a broad and integrative measure of immunity—including antibodies, acute phase proteins, and complement activity (Tieleman et al. 2005)—that involves exposing plasma to a quality-controlled strain of *Escherichia coli* (Lyfo disk derived from control strain ATCC 8739). Preliminary analyses revealed that bacteria killing ability (BKA) is not repeatable in plasma samples frozen more than a few months or thawed multiple times (Guindre-Parker, *unpublished data*), so we only performed BKA assays in the field from 2014-2016 within 2 weeks of sample collection (as in Ezenwa et al. 2012). We diluted plasma (1:10) in CO_2-independent media (Gibco product number 18045), added approximately 100 colony-forming units of *E. coli* (mean ± SE = 86 ± 5.5) and incubated each sample on agar for 12 hours in triplicate (Rubenstein et al. 2008). Bacteria killing ability was then averaged across triplicates, as the number of the bacteria colonies killed by the plasma divided by a positive control (bacteria only on agar). We always included a negative control to ensure that agar plates were not contaminated with bacteria colonies independently, and the mean intra-assay coefficient of variation was 13.4%.

Body Condition

We measured body condition by taking the residuals of a linear regression of mass on tarsus length (Rubenstein 2007b), a reliable index of condition that controls for variation in skeletal size (Jakob et al. 1996). However, for within-individual analyses, we simply calculated the change in body mass because tarsus length is highly repeatable in adult birds (Potti and Merino 1994).

Statistical analysis

We examined whether baseline CORT, ROM, OXY, BKA or body condition during incubation constrained subsequent offspring care behaviors using general Linearized Mixed Models (LMM) with each physiological trait as a dependent variable and the following predictor variables: nest guarding, provisioning rate and average size of prey. We square root transformed baseline CORT and BKA to normalize LMM residuals; the other dependent variables did not require a transformation. Next, we examined whether within-individual changes in baseline CORT, ROM, OXY, BKA or body mass from incubation to chick rearing were correlated to an individual's investment in offspring care behavior using LMMs with nest guarding, provisioning rate, and average size of prey as predictor variables. No transformations were necessary in our analyses of within-individual change in physiology. Finally, we examined whether physiology during chick rearing shaped the likelihood of breeding during the subsequent short or long rain breeding seasons using LMMs with an individual's breeding status (yes/no) in the short and long rain breeding seasons as predictors. We square root transformed baseline CORT and BKA to normalize LMM residuals. All LMMs included a random effect of individual ID nested within group ID and year to control for individuals that were resampled across seasons. Preliminary analyses revealed that CORT, ROM, OXY and BKA did not differ between the sexes in either of the two breeding stages (two sample t-test, $P > 0.1$), supporting previous findings in superb starlings (Rubenstein 2007b; Rubenstein et al. 2008; Rubenstein and Shen 2009; Guindre-Parker and Rubenstein 2017a). As a result, we combined the sexes in our LMMs where CORT, ROM, OXY, and BKA were the dependent variables. Conversely, our preliminary analyses indicated that body condition differed between the sexes during incubation and chick rearing ($P < 0.05$), though within-individual change in body mass over the course of breeding did not ($P > 0.1$)—therefore, we built separate LMMs for males and females to examine whether body condition during incubation constrained offspring care and whether body condition during chick rearing altered the likelihood of breeding in subsequent seasons. We checked that predictor variables were not linearly related to one another (all VIF<2). All LMMs were run in the package 'nlme' 3.1-130 in R (v. 3.2.4; R Core Team 2016).

RESULTS

First, we investigated whether multiple physiological mechanisms constrained investment in offspring care behaviors. We found that individuals with higher baseline CORT during incubation performed greater nest guarding during the period of offspring care 3.1A), thought baseline CORT did not constrain nestling provisioning rates or the size of prey items delivered to nestlings (Table 3.2). Similarly, ROM did not constrain nest guarding, provisioning rates or prey size (Table 3.2). In contrast, incubation OXY was positively correlated to the size of prey items delivered to nestlings, suggesting that only individuals with elevated antioxidants during incubation are able to deliver larger prey to nestlings; Figure 3.1B). This correlation remained statistically significant if we excluded individuals that did not provision young (i.e. delivered prey size of 0) from the model (revised model coefficient ± SE = 1.55 ± 0.45, $t = 3.43$, $P = 0.006$; Figure 3.1B). However, incubation OXY was not correlated to provisioning rates or nest guarding (Table 3.2). BKA did not shape investment in offspring care (Table 3.2). Finally, body condition during incubation was negatively related to the size of prey items delivered to the nest, though only in females; Figure 3.1C)—however, this relationship was no longer statistically significant if we excluded individuals that did not provision young (i.e. delivered prey size of 0, revised model coefficient ± SE = -3.87 ± 4.23, $t = -0.91$, $P = 0.41$; Figure 3.1C). Body condition did not constrain nest guarding behavior or nestling provisioning rates in either sex (Table 3.2). Thus, multiple physiological mechanisms during incubation—baseline CORT, OXY and female body condition—appear to constrain investment in nest guarding and the size of prey delivered to nestlings in superb starlings.

Next, we examined whether changes in physiology from incubation to chick rearing reflected parental or alloparental care effort. We found that the within-individual change in baseline CORT was unrelated to the degree of nest guarding, provisioning rate, or prey item size (Table 3.3). However, the change in ROM was negatively correlated to the average size of prey items delivered to nestlings; Figure 3.2A) such that individuals delivering larger prey to nestlings had a greater decrease in ROM from incubation to chick rearing relative to ones that delivered smaller prey (Table 3.3). If we excluded individuals that did not provision young (i.e. delivered prey size of 0), however, this relationship was no longer statistically significant (revised model coefficient ± SE = -0.94 ± 0.33, $t = -2.8$, $P = 0.12$; Figure 3.2A). Similarly, changes in ROM were not related to nestling provisioning rates or to nest guarding behavior (Table 3.3). Lastly, within-individual changes in OXY, BKA, and body mass were unrelated to investment in offspring care behaviors examined in our study (Table 3.3). Therefore, only ROM appears to represent a physiological cost of parental or alloparental care in superb starlings, though this depends on whether individuals that do not provision young are included in our analyses.

Finally, we determined whether physiological state at the end of reproduction (i.e. during chick rearing) had carry-over effects that could influence the likelihood of breeding in future seasons (i.e. subsequent short or long rain breeding seasons). We found that baseline CORT during chick rearing was not related to the likelihood of breeding in the following year (Table 3.4). Similarly, neither ROM nor OXY affected the probability that an individual would breed in the future (Table 3.4). However, we did find that individuals with higher BKA during chick rearing had an increased likelihood of breeding in the following short rainy season; Figure 3.2B). However, this carry-over effect was short lived, as elevated chick rearing BKA did not predict the likelihood of breeding two seasons later in the following long rain breeding season (Table 3.4). Finally, body condition was unrelated to future breeding status in the short or long rain breeding seasons for males or females (Table 3.4).

DISCUSSION

We examined four physiological mechanisms that have been shown to constrain parental care, mediate the cost of parental care and have carry-over effects that shape subsequent fitness in birds. We found that several physiological traits including baseline CORT, antioxidants, and female body condition constrained offspring care behavior because these physiological traits during incubation were correlated to subsequent investment in offspring care during chick rearing. We originally predicted that individuals in better physiological conditions during incubation would perform greater offspring care, though our results did not always support this prediction. In support of our prediction, we found that individuals with greater antioxidant capacity during incubation provisioned young with larger prey, which is similar to previous findings that antioxidants can constrain investment in parental care (Pike et al. 2007) and clutch size (Blount et al. 2004; Bize et al. 2008). Conversely and unlike our prediction, we found that incubation baseline CORT was positively correlated with nest guarding during chick rearing, suggesting that birds with elevated CORT prior to hatching provide a greater degree of offspring care. While chronically elevated baseline CORT may be unfavorable and reduce survival (Goutte et al. 2010), elevated baseline CORT during brief periods of high energetic demand may be advantageous—our results add to a growing body of evidence that elevated baseline CORT prior to reproduction may actually prepare individuals for the energetic demands associated with parental care (Ouyang et al. 2013; Hennin et al. 2014; Love et al. 2014; Bowers et al. 2016; Dupoue et al. 2016). Similarly, we found that females in lower body condition during incubation subsequently provisioned young with smaller prey items, suggesting that body condition constrains offspring care in the opposite direction as we had predicted. It is possible that individuals in lower body condition are lighter, and benefit from expending less energy during foraging relative to larger or heavier individuals (Freed 1981; Tinbergen and Dietz 1994). This may be particularly likely for a non-migratory tropical bird, were individuals do not rely on their fat and muscle reserves for energetically demanding migration post-breeding (Holberton et al. 2005; Guy Morrison et al. 2007). Alternatively, females in lower body condition during incubation may keep larger prey items for themselves and provision young with smaller prey.

Next, our study examined whether individuals that invested in offspring care to a greater degree paid physiological costs in the form of reduced condition from incubation to chick rearing. We found a single example of a physiological cost, though in the opposite direction as we would predict: rather than facing greater oxidative damage from finding and delivering larger prey items to nestlings, individuals actually decreased in reactive oxygen metabolites from incubation to chick rearing when they delivered larger prey to the nest. A possible explanation for this surprising result may be that individuals finding larger prey, both for nestlings and for themselves, may have higher quality diets—diet quality may in turn alter reactive oxygen metabolites (Costantini 2010). Alternatively, delivering smaller prey to nestlings may result in individuals having to compensate via other aspects of provisioning behavior, such as a higher provisioning rate (Wiebe and Slagsvold 2015). However, this is unlikely to be the case in our study because birds provisioning nestlings with larger prey did not provision young more frequently (estimate ± SE = 0.08 ± 0.08, $R^2 = 0.04$, $P = 0.35$, $N = 27$). It is possible that we failed to detect other costs of reproduction in our study of superb starlings because a cost of reproduction may not be detectable without manipulating breeding effort via brood augmentation or reduction (Metcalfe and Monaghan 2013), particularly in systems where individuals adjust their investment in reproduction according to their pre-breeding condition. Nevertheless, it remains informative to know whether free-living animals are able to avoid a cost of offspring care by adjusting their investment in current reproduction according to their pre-breeding state (Costantini 2016a). Similarly, our study compared individuals across their natural range of variation in parental and alloparental care investment (i.e. ranging from individuals naturally providing significant offspring care to none at all), an approach that has recently been suggested as the most powerful comparison to explore the physiological underpinnings of the cost of reproduction in cooperative breeders (Speakman and Garratt 2014; Costantini 2016a). Alternatively, the timescale for measuring a physiological cost of reproduction may extend beyond the breeding season (Bonnet et al. 1999), or a cost of reproduction may occur over multiple breeding attempts rather than a single brood (Love et al. 2014). Indeed, superb starlings are multi-brooded within a breeding season (Rubenstein 2016), though we were not able to reliably capture individuals post-breeding or to study individuals across multiple breeding attempts. Despite these potential limitations, previous work in a non-cooperative synoptic species—the greater blue-eared glossy starling, *Lamprotornis chalybaeus*—did detect significant oxidative costs of reproduction in male and female breeders using an identical field sampling protocol, while the same cost was absent in cooperatively breeding superb starlings (Guindre-Parker and Rubenstein 2017a). This suggests that we are able to detect oxidative costs of parental care using our study design, and that such a cost may indeed be absent in superb starlings. Furthermore, we simultaneously tested four physiological mechanisms known to shape the cost of parental care in other species (Table 3.1), which provide independent support that superb starlings face little to no cost of parental or alloparental care.

Rather than face physiological costs of offspring care, superb starlings primarily appear to adjust their parental or alloparental care behavior according to their intrinsic state prior to chick rearing. Breeders and alloparents alike appear to have flexible offspring care decision rules, as our results demonstrated that physiological condition prior to breeding shaped offspring care behavior. Superb starling investment in offspring care is thus highly flexible and varies with breeding rainfall and the size of the alloparent contingent present at a nest (Guindre-Parker and Rubenstein 2017b), as well as with intrinsic condition prior to breeding. In light of this flexibility, it is not surprising that superb starlings face little cost of providing offspring care. One important benefit of breeding cooperatively may be to provide individuals with the flexibility to adjust their investment in parental or alloparental care (Valencia et al.

2003; Guindre-Parker and Rubenstein 2017b). In contrast, species with uni- or bi-parental care may have less flexibility to modify their investment in offspring care according to intrinsic or extrinsic conditions, as one parent may not be able to compensate for reduced investment in offspring care by its partner (Schwagmeyer et al. 2002). Individuals of cooperatively breeding species, on the other hand, may be able to reduce their investment in offspring care (i.e. load-lightening) because large groups of alloparents at a nest may compensate for their decreased investment (Russell 2016; Guindre-Parker and Rubenstein 2017b). Multiple pre-breeding physiological traits paired with the buffering effects of caring for young in large groups may therefore shape parental and alloparental care in superb starlings. These results also suggest that there is not one single physiological trait that stands out as the primary driver of parental or alloparental care behavior in superb starlings, but rather different traits may be simultaneously and weakly related to investment in offspring care. Future work on the mechanism(s) underlying the cost of parental care should incorporate multiple physiological systems simultaneously, as many physiological mechanisms are likely to contribute to shaping parental care.

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TABLES

Table 3.1: Summary of previous findings, as well as ones presented within this study, supporting that multiple physiological mechanisms may act as constraints or costs of reproduction and have subsequent carry-over effects that influence fitness.

Trait	**Evidence from previous studies**			**Findings of this study**	
	Constraint	Cost	Carry-over	Measure used	Results
Glucocorticoid hormones	Pre-breeding corticosterone allowed for earlier breeding (Goutte et al. 2011; Dickens and Bentley 2014) or increased parental care (Ouyang et al. 2013); elevated corticosterone increased investment in second broods (Love et al. 2014)	Elevated parental care increased baseline corticosterone (Harding et al. 2009; Welcker et al. 2014; Madliger and Love 2016)	Elevated corticosterone during breeding lowered annual survival (Goutte et al. 2010) or delayed reproduction the following year (Crossin et al. 2013)	Baseline corticosterone	Constraint
Oxidative stress	Pre-breeding oxidative damage is correlated with a subsequent reduction in investment in litter size (Stier et al. 2012) or in parental care (Heiss and Schoech 2012)	Elevated parental care increased oxidative damage (Guindre-Parker et al. 2013; Sharick et al. 2015) or decreased antioxidants (Christe et al. 2012; Costantini et al. 2014)	Antioxidants increased annual survival (Saino et al. 2011); oxidative damage reduced annual survival and subsequent reproductive success (Costantini and Dell'Omo 2015)	Reactive oxygen metabolites; Antioxidant capacity	Constraint and Cost
Immune function	Pre-breeding exposure to a pathogen decreased investment in breeding (Griesser et al. 2017) and parental care (Råberg et al. 2000)	Elevated parental care decreased investment in immune function (Ardia 2005; Hanssen et al. 2005; Archie et al. 2014)	Post-breeding immune-suppression decreased annual survival (Nordling et al. 1998; Hegemann et al. 2013)	Bacterial killing ability	Carry-over effect
Mass/body condition	Pre-breeding body condition is correlated with earlier (Descamps et al. 2011) or increased investment in breeding (Rödel et al. 2016)	Elevated parental care led to decreased body mass (Bonnet et al. 1999; Velando and Alonso-Alvarez 2003; Canestrari et al. 2007)	Body mass (Haramis et al. 1986; Naef-Daenzer and Gruebler 2016) and condition (Bonnet et al. 1999) increased annual survival	Residuals of mass on tarsus; Within-individual change in mass	Constraint

Table 3.2: Results of LMMs testing whether incubation physiology constraints subsequent offspring care behavior during chick rearing: a) baseline corticosterone (*N* = 69), b) reactive oxygen metabolites (*N* = 66), c) antioxidant capacity (*N* = 69), d) bacteria killing ability (*N* = 33), e) male body condition (*N* = 24) and f) female body condition (*N* = 45).

	Estimate ± SE	***t-value***	***P-value***	**95% CI**
a) Baseline corticosterone (square root transformed)				
Intercept	1.82 ± 0.30	5.99	<0.001*	1.20 – 2.44
Nest guarding	0.03 ± 0.01	2.35	0.024*	0.004 – 0.04
Provisioning rate	-0.04 ± 0.23	-0.17	0.87	-0.52 – 0.44
Prey size	0.22 ± 0.30	0.74	0.46	-0.39 – 0.83
b) Reactive oxygen metabolites				
Intercept	0.81 ± 0.17	4.79	<0.001*	0.47 – 1.15
Nest guarding	-0.002 ± 0.003	-0.59	0.56	-0.008 – 0.005
Provisioning rate	-0.04 ± 0.07	-0.62	0.53	-0.19 – 0.10
Prey size	0.03 ± 0.08	0.40	0.69	-0.14 – 0.20
c) Antioxidant capacity				
Intercept	2.25 ± 0.16	14.1	<0.001*	1.93 – 2.58
Nest guarding	-0.006 ± 0.005	-1.05	0.30	-0.02 – 0.005
Provisioning rate	-0.11 ± 0.12	-0.90	0.37	-0.34 – 0.13
Prey size	0.39 ± 0.14	2.71	<0.001*	0.10 – 0.68
d) Bacteria killing ability (square root transformed)				
Intercept	2.96 ± 1.51	1.97	0.066	-0.21 – 6.14
Nest guarding	0.02 ± 0.04	0.56	0.58	-0.06 – 0.10
Provisioning rate	0.48 ± 0.96	0.50	0.63	-1.55 – 2.51
Prey size	0.48 ± 1.08	0.44	0.67	-1.81 – 2.76
e) Body condition in males				
Intercept	-2.68 ± 1.80	-1.49	0.17	-6.70 – 1.34
Nest guarding	-0.05 ± 0.11	-0.44	0.67	-0.30 – 0.20
Provisioning rate	-8.25 ± 5.48	-1.51	0.16	-20.5 – 3.96
Prey size	4.51 ± 2.34	1.93	0.08	-0.70 – 0.75
f) Body condition in females				
Intercept	0.50 ± 1.57	0.32	0.76	-2.81 – 3.80
Nest guarding	-0.05 ± 0.05	-0.89	0.38	-0.15 – 0.06
Provisioning rate	1.61 ± 1.07	1.50	0.15	-0.65 – 3.88
Prey size	-3.97 ± 1.55	-2.56	0.02*	-7.24 – -0.70

Table 3.3: Results of LMMs testing whether within-individual change in physiology from incubation to chick rearing is correlated to investment in offspring care behavior: a) baseline corticosterone (*N* = 23), b) reactive oxygen metabolites (*N* = 18), c) antioxidant capacity (*N* = 22), d) bacteria killing ability (*N* = 10), e) body mass (*N* = 23).

	Estimate ± SE	***t-value***	***P-value***	**95% CI**
a) Baseline corticosterone				
Intercept	1.79 ± 5.24	0.34	0.74	-10.1 – 13.7
Nest guarding	0.31 ± 0.17	1.84	0.10	-0.07 – 0.69
Provisioning rate	-3.88 ± 2.69	-1.44	0.18	-9.97 – 2.22
Prey size	1.86 ± 1.88	0.99	0.35	-2.39 – 6.11
b) Reactive oxygen metabolites				
Intercept	0.02 ± 0.12	0.20	0.85	-0.26 – -0.31
Nest guarding	0.006 ± 0.005	1.21	0.27	-0.006 – 0.02
Provisioning rate	0.20 ± 0.12	1.69	0.14	-0.09 – 0.49
Prey size	-0.35 ± 0.13	-2.62	0.04*	-0.67 – -0.02
c) Antioxidant capacity				
Intercept	-0.40 ± 0.24	-1.70	0.13	-0.95 – 0.15
Nest guarding	0.009 ± 0.009	1.08	0.31	-0.01 – 0.03
Provisioning rate	0.22 ± 0.15	1.48	0.18	-0.12 – 0.57
Prey size	-0.20 ± 0.24	-0.85	0.42	-0.76 – 0.35
d) Bacteria killing ability				
Intercept	34.2 ± 11.9	2.86	0.06	-3.81 – 72.1
Nest guarding	-0.48 ± 0.75	-0.65	0.58	-3.70 – 2.73
Provisioning rate	-4.31 ± 18.0	-0.24	0.83	-81.8 – 73.2
Prey size	-28.8 ± 19.6	-1.47	0.28	-113.1 – 55.5
e) Mass				
Intercept	-0.59 ± 1.90	-0.31	0.76	-4.90 – 3.71
Nest guarding	0.03 ± 0.08	0.33	0.75	-0.15 – 0.21
Provisioning rate	-0.21 ± 1.20	-0.18	0.86	-2.94 – 2.51
Prey size	-0.80 ± 1.05	-0.76	0.47	-3.19 – 1.58

Table 3.4: Results of LMMs testing whether chick rearing physiology was correlated to breeding status in subsequent short or long rainy seasons: a) baseline corticosterone (*N* = 36), b) reactive oxygen metabolites (*N* = 29), c) antioxidant capacity (*N* = 35), d) bacteria killing ability (*N* = 25), e) male body condition (*N* = 19) and f) female body condition (*N* = 17).

	Estimate ± SE	***F-value***	***P-value***	**95% CI**
a) Baseline corticosterone (square root transformed)				
Intercept	2.11 ± 0.40	35.2	<0.001*	1.29 – 2.93
Bred short rains	0.70 ± 0.52	1.17	0.29	-0.39 – 1.79
Bred long rains	-0.38 ± 0.48	0.63	0.43	-1.37 – 0.61
b) Reactive oxygen metabolites				
Intercept	0.74 ± 0.20	13.2	0.002*	0.33 – 1.16
Bred short rains	0.02 ± 0.17	0.19	0.67	-0.35 – 0.38
Bred long rains	-0.16 ± 0.15	1.07	0.32	-0.48 – 0.16
c) Antioxidant capacity				
Intercept	2.22 ± 0.16	212.3	<0.001*	1.88 – 2.55
Bred short rains	0.02 ± 0.19	0.05	0.82	-0.37 – 0.42
Bred long rains	-0.09 ± 0.18	0.26	0.62	-0.47 – 0.29
d) Bacteria killing ability (square root transformed)				
Intercept	4.39 ± 0.45	152.3	<0.001*	3.44 – 5.35
Bred short rains	2.69 ± 1.23	6.01	0.03*	0.08 – 5.31
Bred long rains	-0.18 ± 1.06	0.03	0.86	-2.43 – 2.06
e) Body condition in males				
Intercept	-1.24 ± 1.97	0.46	0.52	-5.80 – 3.31
Bred short rains	1.63 ± 3.64	0.18	0.69	-6.77 – 10.0
Bred long rains	-0.50 ± 3.10	0.03	0.88	-7.66 – 6.66
f) Body condition in females				
Intercept	-4.24 ± 1.87	10.6	0.02*	-9.04 – 0.56
Bred short rains	1.36 ± 4.41	0.07	0.80	-9.97 – 12.7
Bred long rains	-3.11 ± 4.41	0.50	0.51	-14.4 – 8.23

FIGURES

Figure 1: Multiple physiological traits during incubation constrained subsequent offspring care behaviors. Elevated A) baseline CORT during incubation increased nest guarding during chick provisioning, B) antioxidant capacity during incubation increased the size of prey items delivered to nestlings, and C) body condition in incubating females reduced the size of prey items delivered to nestlings. In the case of a significant effect of prey item size, we present results of models that included individuals that did not provision young (i.e. prey size of 0) in grey, and results that exclude these individuals in black. Only statistically significant regression lines are included (grey lines include individuals that did not provision young, black lines do not).

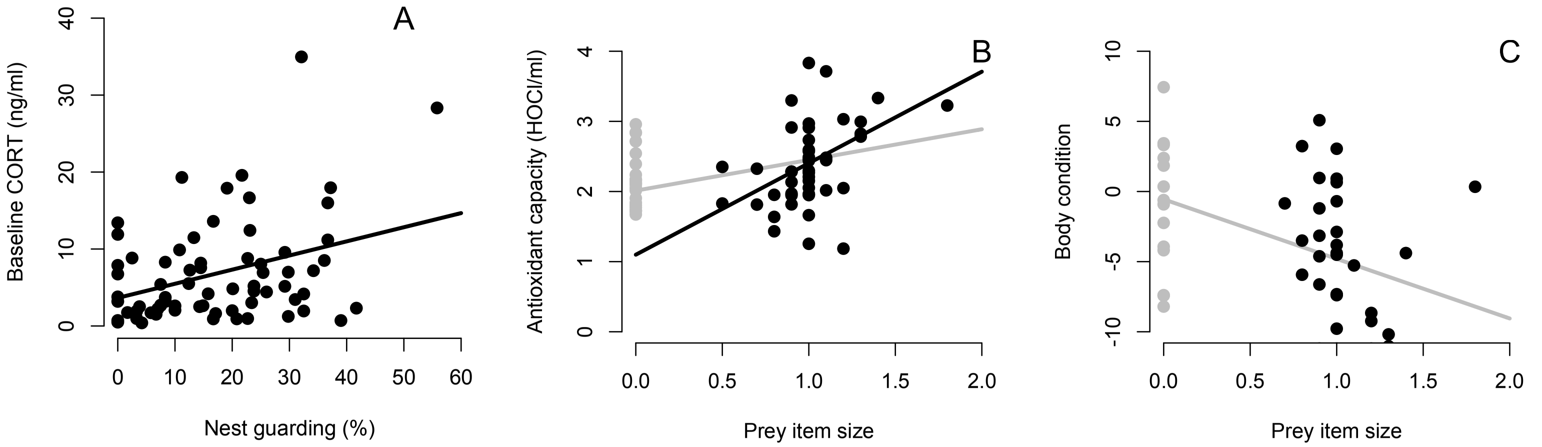
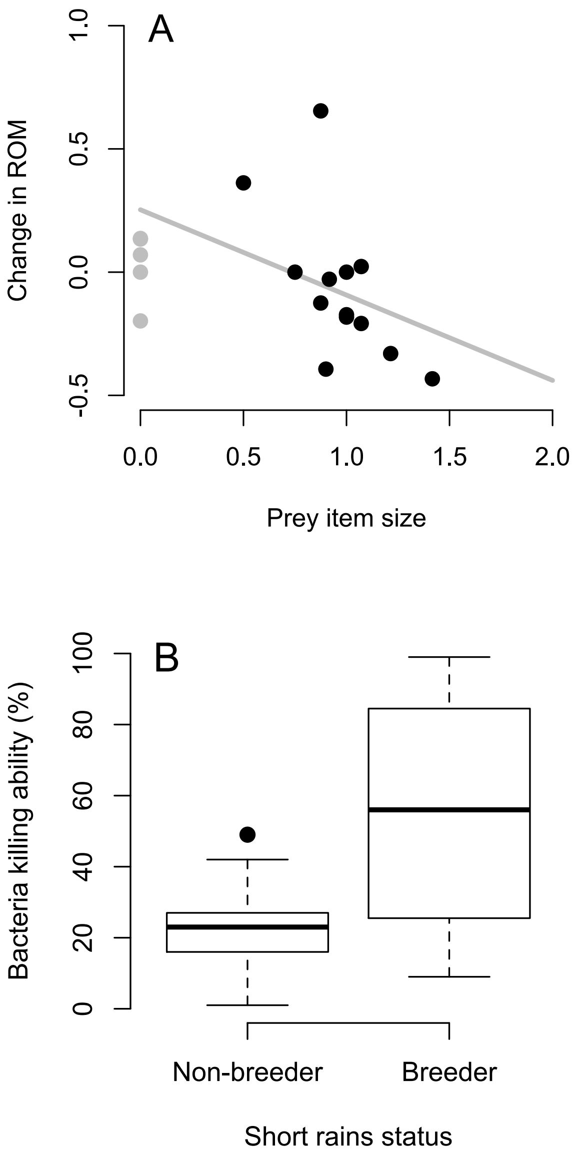


Figure 3.2: We only found evidence for one physiological trait which represented a cost of reproduction: A) within-individual declines in ROM from incubation to chick rearing occurred proportionally to the size of prey items delivered to nestlings (linear regression). We present results of models that included individuals that did not provision young (i.e. prey size of 0) in grey, and results that exclude these individuals in black. Only statistically significant regression lines are included (grey lines include individuals that did not provision young). Similarly, we found evidence of a single carry-over effect on the likelihood of breeding in future seasons: B) high chick rearing bacteria killing ability increased the likelihood of breeding in the following short rainy season (box and whiskers plot, where outliers are represented by closed circles).



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CHAPTER 4 — BREEDING COOPERATIVELY REDUCES THE OXIDATIVE COST OF REPRODUCTION

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ABSTRACT

The cost of reproduction is the trade-off between reproduction and survival, and has long been thought to favor the evolution of cooperative breeding because breeders can reduce parental care behavior when aided by alloparents. However, this idea has never been tested formally because we previously lacked a mechanistic understanding of the cost of reproduction. Oxidative stress—the imbalance between reactive oxygen species and neutralizing antioxidants—has emerged as a key mechanistic link between current reproduction and subsequent survival. To determine whether breeding cooperatively reduces the cost of reproduction, we examined whether a breeding attempt alters oxidative stress in synoptic cooperative and non-cooperative species of African starlings. We found that individuals of the non-cooperative species incurred an increase in reactive oxygen metabolites during breeding, but that breeders and alloparents of the cooperative species did not. While rearing young of both species requires a similar workload, the oxidative cost of reproduction was actually lower in the cooperative species because this breeding workload was divided among a larger group of individuals, resulting in lower per capita workloads. These findings demonstrate that cooperative breeders avoid the high physiological costs associated with parental care by sharing the workload. This study suggests that direct benefits in the form of reduced physiological costs of caring for young may promote sociality independently of kin selection.

INTRODUCTION

Since sexually reproducing organisms can invest valuable resources in current reproduction at the expense of their future survival they face a key life history trade-off, known as the ‘cost of reproduction’ (Williams 1966; Nur 1988). This trade-off is thought to shape the evolution of animal mating systems (Stearns 1992) because increasing the cost of offspring care favors a transition from uni- to bi-parental care (Webb et al. 2010). Similarly, when the cost of reproduction becomes prohibitively high, more than two individuals may be required to successfully rear young (i.e. cooperative breeding systems with alloparental care) (Brown 1978; Emlen 1982; Crick 1992; Heinsohn and Cockburn 1994; Langen 2000; Ligon and Burt 2004). The high cost of reproduction associated with parental care is thought to favor the evolution of cooperative breeding behavior because breeders can reduce their investment in offspring care when breeding in larger groups where alloparents aid in caring for young (i.e. load lightening) (Crick 1992; Hatchwell 1999; Heinsohn 2004; Johnstone 2011). The cost of reproduction may also favor delayed dispersal (Pyle et al. 1997; Hawn et al. 2007), which is thought to be an important precursor for the evolution of cooperative breeding (Emlen 1982; Brown 1987). The idea that the cost of reproduction favors the evolution of cooperative breeding was first proposed nearly 40 years ago (Brown 1978; Crick 1992), yet to our knowledge no study has demonstrated that costs of reproduction are reduced in cooperative relative to non-cooperative species. Testing this hypothesis has proven challenging because we have lacked a mechanistic understanding of the cost of reproduction (Harshman and Zera 2007). However, recent work suggests that oxidative stress (i.e. the imbalance between reactive oxygen species and neutralizing antioxidants) links current reproductive effort to future survival (Christe et al. 2012; Fletcher et al. 2013; Metcalfe and Monaghan 2013; Costantini and Dell’Omo 2015), making it possible to study how the cost of reproduction influences the evolution of cooperative breeding behavior. The heightened metabolic demand associated with caring for young can increase the production of reactive oxygen species, harmful chemicals that may be neutralized by antioxidant defenses. However, if reactive oxygen species overwhelm antioxidants, biomolecules begin to accumulate oxidative damage proportionally to investment in reproduction (Guindre-Parker et al. 2013; Cram et al. 2015; Sharick et al. 2015), which can lead to reduced survival (Saino et al. 2011).

Here we examined whether breeding cooperatively can lead to a reduced oxidative cost of reproduction by studying two co-occurring, closely related species of African starlings that differ in their social behavior: the obligate cooperatively breeding superb starling (*Lamprotornis superbus*), and the non-cooperative greater blue-eared glossy starling (*L. chalybaeus*). While greater blue-eared glossy starlings breed and care for young in pairs, superb starlings live in large social groups where both breeders and 1 to 14 alloparents care for young (Rubenstein 2016). Where we work in central Kenya, these two species are not only synoptic with overlapping territories, but they have similar life histories and breed simultaneously from March to June during the long rains. To determine if the cost of reproduction could influence the evolution of cooperative breeding behavior, we explored whether oxidative costs of reproduction were (i) greater in individuals of the non-cooperative species than in those of the cooperative species, (ii) greater in mothers than in fathers or alloparents in the cooperative species, and (iii) greater in individuals that perform more nest guarding, offspring provisioning, or have a higher per capita workload in either species.

To quantify oxidative stress in both species, we assessed reactive oxygen metabolites and total antioxidant capacity from plasma samples. We measured reactive oxygen metabolites (ROM) which are more stable derivatives of reactive oxygen species and represent a marker of early oxidative damage (Guindre-Parker et al. 2013; Costantini 2016). Although an indirect index of oxidative damage, ROM have been shown to correlate with increased rates of telomere loss (Geiger et al. 2011; Hau et al. 2015) and reduced survival in free-living birds (Geiger et al. 2011; Costantini and Dell'Omo 2015). We also quantified antioxidant defenses by measuring the general capacity of plasma antioxidants (OXY) to neutralize a strong of oxidant. We expected that individuals of the non-cooperative and cooperative breeding species would differ in their oxidative cost of reproduction. Specifically, we predicted that individuals of the non-cooperative species would experience either a greater increase in ROM or a greater decrease in OXY from incubation to chick rearing, leading to an imbalance in the ratio of ROM to OXY that would likely result in oxidative damage. We also expected that within the cooperative species, breeding roles would differ in the cost of reproduction they experienced because previous work in this system has shown that breeders and alloparents differ in how much they care for nestlings; on average, mothers perform the greatest proportion of offspring care, while fathers and alloparents perform significantly less (Rubenstein 2016). Therefore, we predicted that mothers of the cooperative species would experience a greater increase in ROM and/or a greater decrease in OXY from incubation to chick rearing relative to either fathers or alloparents. Additionally, since alloparents can provision young as well as guard the nest from predators, we further categorized alloparents as either provisioners or guards. We predicted that provisioning alloparents would have a greater increase in ROM or a greater decrease in OXY from incubation to chick rearing compared to guard alloparents because provisioning young is thought to be more costly than passively watching the nest (i.e. it requires finding and catching food (Marteinson et al. 2015)). Lastly, we expected that the cost of reproduction would be correlated with parental care behavior for individuals of both species. We predicted that individuals would have higher ROM and/or lower OXY during chick rearing when (i) guarding the nest a greater proportion of the time, (ii) feeding nestlings at a higher rate, or (iii) with a greater index of per capita workload. Since the number of individuals contributing to offspring care differs significantly in both species (greater blue-eared glossy: mean ± SD = 2 ± 0; superb starling: mean ± SD = 6.6 ± 4.1), we calculated per capita workload as the number of chicks in a nest multiplied by the number of days of feeding or guarding (chick*days, *sensu* Heiss and Schoech 2012) divided by the total number of birds caring for young at each nest.

METHODS

Study species

We continuously monitored populations of free-living superb and greater-blued eared glossy starlings from 2001 to 2015 at the Mpala Research Centre in central Kenya (0°17’N, 37°52’E). Non-cooperative greater blue-eared glossy starlings are socially monogamous pair-breeders, but they are seasonally gregarious and form larger flocks during the non-breeding season. In contrast, superb starlings are plural cooperative breeders that live in large social groups of up to 50 individuals with multiple breeding pairs per group (Rubenstein 2016) and between 1 and 14 alloparents at each nest (Rubenstein 2016). Individuals of both species have been marked with a unique combination of colored bands and a numbered metal band.

Both congeneric species of starlings not only occur in the same clade (Lovette and Rubenstein 2007), but they co-occur in parts of the their ranges across East Africa (Feare and Craig 1998). Non-cooperative greater blue-eared glossy starlings are larger than cooperative superb starlings (mean 85g versus 65g) (Feare and Craig 1998), but both species have similar clutch sizes (mean ± SD: greater blue-eared glossy = 3.0 ± 0.73; superb = 3.1 ± 0.87), and eggs of approximately the same size relative to adult body mass (7% of adult mass)(Feare and Craig 1998). Adults of both species are omnivorous, feeding on insects, berries, and seeds, but they provision their young exclusively with insects (Feare and Craig 1998). Greater blue-eared glossy starlings are cavity nesters and in our study area, pairs use natural cavities as well as human-made nest boxes (wooden boxes and beehives). Conversely, superb starlings build closed grassy-domed nests, primarily in acacia trees (Rubenstein 2016). Otherwise, both species have very similar life histories (Feare and Craig 1998) and often co-occur on the same territories.

Trapping and sampling birds

We trapped individuals of both species during the long-rains from 2013 to 2015 (March-June) using food-baited ground-traps or mist-nets around the focal nest, and then collected a small blood sample from the brachial vein within 3 min of capture. Both species were sampled during two breeding stages: (i) incubation, defined as post-clutch completion but before hatching to exclude potential costs of egg laying or nest building; and (ii) chick rearing, when nestlings were between 4 and 16 days of age. We re-sampled individuals across both stages whenever possible, but high nest predation rates made this difficult. Blood samples were centrifuged and plasma was stored frozen until analysis (up to 6 months) (Rubenstein et al. 2008).

Monitoring reproductive effort and breeding roles

We observed active nests with a spotting scope for 2 hours per observation period (mean ± SD observation periods per nest = 2 ± 1; n = 50 nests); during focal observations we monitored the identity of each bird that came within 20 m of the nest as well as those that delivered food to chicks (Rubenstein 2007a). We calculated two behaviors that reflect investment in offspring care: (i) *nest guarding*, defined as the proportion of time an individual spent within 20 m of the nest—but not inside—relative to the length of the observation period; and (ii) *offspring provisioning*, defined as the number of trips per hour where an individual delivered food into the nest. We also calculated per capita breeding workload—the number of chicks in a nest multiplied by the age of the chicks on the day of each adult's capture divided by group size—which accounts for additional aspects of offspring care that are not captured by guarding or provisioning behavior alone. For both starling species, the mother was identified as the bird with a brood patch, while the social father was identified as the male closely following the incubating female (Rubenstein 2007b). For superb starlings, where the large nest contingent occasionally made identifying the parents more difficult, we also confirmed parentage genetically using microsatellite markers (Rubenstein 2007c).

Physiological analyses

We measured ROM using a commercially available kit (dROM test, Diacron International, Italy) according to standard protocols (Costantini et al. 2007; Costantini 2016) with slight modifications (Costantini et al. 2008; Baldo et al. 2015). Briefly, we diluted 10 μL of plasma with 400 μL of a 1:100 mixture of the alkyl-amine solution and acetate buffer. Similarly to previous studies (Costantini et al. 2011; Guindre-Parker et al. 2013), we found that a precipitate (i.e. lipids) formed at the bottom of the wells—therefore, instead of running the assay in a 96-well plate, we ran the assay in 1.5mL eppendorf tubes before pipetting the liquid layer into a 96-well plate. We incubated reagents and plasma in eppendorf tubes for 75 mins at 37°C and centrifuged the tubes at 10,000 rpm for 30 sec to isolate the precipitate at the bottom of the tube. We then pipetted 190 μL of the liquid into duplicate wells of a flat-bottomed 96-well plate (Costantini et al. 2011; Guindre-Parker et al. 2013). On each plate, we ran a standard curve of H_2O_2 (a strong oxidant; range = 0.16-5.12 mg H_2O_2/dL). Absorbance was read at a wavelength of 490 nm and concentrations of reactive oxygen metabolites are given in mg H_2O_2/dL.

We measured OXY using a commercial kit (OXY test, Diacron International, Italy). This assay therefore measures the general capacity of plasma antioxidants to neutralize a strong oxidant *in vitro* (Guindre-Parker et al. 2013). Briefly, we diluted plasma samples to 1:100 with deionized water and added 5 μL of diluted samples in triplicate to wells of a flat-bottomed 96-well plate with 200 μL of the HOCl solution (Costantini et al. 2008, 2011; Guindre-Parker et al. 2013). The plate was shaken at 450 rpm for ten seconds and incubated at 37°C for 10 min. On each plate, we included a standard curve of HOCl (range = 0.425-6.8 μmol HOCl/mL). We then added 2μL of the color-changing chromogen solution to each well (N,N-diethylparaphenilendiamine) and read the plate at 490 nm after shaking for 30 sec. Concentrations of antioxidants are expressed in μmol of HOCl/mL.

Both assays were performed within 6 months of sampling, and multiple samples from a single individual were run on the same plate, but positions within a plate were randomized. For the dROM assay, the mean intra-assay coefficient of variation was 5.9% and the inter-assay coefficient of variation was 6.8% while for the OXY assay the mean intra-assay coefficient of variation was 5.4% and the mean inter-assay coefficient of variation was 7.9%.

Statistical analysis

ROM was square-root transformed to meet the assumptions of normality, while OXY did not require a transformation. We originally ran models with and without year included as a predictor but ended up removing year from all models because this did not alter the results and doing so improved model fit (ΔAIC>3). We used mixed effect ANOVAs to determine whether ROM or OXY differed across breeding stage, species, breeding role, or the interactions between breeding stage and species or role. We included individual ID as a random effect because some individuals were sampled during both breeding stages (non-cooperative = 11; cooperative = 17), whereas others were only sampled at one stage (incubation: non-cooperative = 26, cooperative = 52; chick rearing: non-cooperative = 5, cooperative = 12). Next, we investigated whether chick rearing ROM or OXY reflected investment in offspring care using linear mixed effect models including nest guarding, provisioning rates, and their interactions with species as independent variables. In a separate set of models, we examined whether chick rearing ROM or OXY were related to per capita breeding workload and the interaction between per capita workload and species. Because we sampled multiple individuals at each nest, these behavior and workload analyses included nest ID as a random effect (conversely, each individual was included only once in these analyses so we did not include a random effect of individual ID). All analyses were performed in R version 3.2.4(R Core Team 2016), using ‘nlme’ and restricted maximum likelihood. Finally, a subset of individuals was captured during both incubation and chick rearing, so we repeated our analyses examining within-individual change in ROM or OXY from incubation to chick rearing as the dependent variable. The results of within-individual analyses are qualitatively identical to those of the other analyses and are presented in Appendix 4.1.

RESULTS

First, we examined whether individuals of the non-cooperative greater blue-eared glossy starling experienced a greater cost of reproduction relative to that in the cooperative superb starling by measuring how a single breeding attempt shaped changes in ROM or OXY. Although we found that individuals of both species had similar ROM during incubation, breeders of the non-cooperative greater-blue eared glossy starling increased significantly in ROM from incubation to chick rearing, whereas individuals of the cooperative superb starling did not (interaction of breeding stage by species: $F_{1,43} = 7.1$, $P = 0.01$; Figure 4.1A). In contrast, individuals of both species had similar OXY during incubation and chick rearing (species: $F_{1,110} = 0.3$, $P = 0.57$), and neither species exhibited a change in OXY over the course of a breeding attempt (breeding stage: $F_{1,49} = 1.1$, $P = 0.30$; interaction between breeding stage and species: $F_{1,49} = 2.5$, $P = 0.12$; Figure 4.1C). These findings are consistent with the idea that species that breed cooperatively are likely to experience reduced costs of reproduction relative to those that do not.

Next, we examined whether individuals of different breeding roles in the cooperatively breeding superb starling experienced differential costs of reproduction. We found that breeders (both mothers and fathers) and alloparents (both guards and provisioners) had similar ROM in both breeding stages (breeding role: $F_{4,43} = 1.1$, $P = 0.37$; interaction between breeding role and breeding stage; $F_{4,43} = 1.9$, $P = 0.12$; Figure 4.1B). Similarly, breeders and alloparents had similar OXY during incubation and chick rearing (breeding role: $F_{4,49} = 0.3$, $P = 0.82$), and no individuals exhibited a change in OXY over the course of a breeding attempt (breeding stage: $F_{1,49} = 1.1$, $P = 0.30$; interactions between breeding stage and breeding role: $F_{4,49} = 0.46$, $P = 0.77$; Figure 4.1D). These results suggest that irrespective of breeding role, superb starlings do not appear to experience an oxidative cost of reproduction.

To address our third hypothesis that an individual's oxidative cost of reproduction is proportional to its investment in offspring care, we examined whether ROM or OXY measured during chick rearing reflected offspring care workload in both species of starlings. We found that chick rearing ROM was unrelated to nest guarding (*t* = -0.19, *DF* = 16, *P* = 0.84; interaction between species and guarding: *t* = 1.1, *DF* = 16, *P* = 0.30) or provisioning rate in both species (*t* = 0.63, *DF* = 16, *P* = 0.53; interaction between species and provisioning rate: *t* = -0.11, *DF* = 16, *P* = 0.91). Similarly, OXY was unrelated to nest guarding (*t* = 0.05, *DF* = 17, *P* = 0.96; interaction between species and guarding: *t* = 0.27, *DF* = 17, *P* = 0.79) and provisioning rate in both species (provisioning rate: *t* = 0.57, *DF* = 17, *P* = 0.57; interaction between species and provisioning rate: *t* = 1.2, *DF* =17, *P* = 0.26). However, we found that chick rearing ROM increased with per capita workload (*t* = 2.5, *DF* = 20, *P* = 0.02), and did so similarly for both species (interaction between species and per capita workload interaction: *t* = -1.2, *DF* = 20, *P* = 0.24; Figure 4.2A). Additionally, chick rearing OXY was unrelated to per capita workload in both species (*t* = -0.1, *DF* = 23, *P* = 0.94; interaction between species and workload; *t* = 1.0, *DF* = 23 *P* = 0.31; Figure 4.2B). Finally, although we found that an individual's offspring care workload increased ROM production similarly in both species, the per capita workload was significantly lower in cooperative superb starlings than in non-cooperative greater blue-eared glossy starlings (*t* = 2.68, *DF* = 19, *P* = 0.015; Figure 4.2C), resulting in the absence of an oxidative cost of breeding in the cooperatively breeding species.

DISCUSSION

Together our results suggest that both breeders and alloparents in an obligate cooperatively breeding species forgo an oxidative cost of reproduction by sharing the breeding workload, while individuals of a non-cooperative closely related species face an oxidative cost of reproduction because they are unable to divide the parental care duties beyond the parents. Although this is the first study contrasting the cost of reproduction in co-occurring species with very different social systems but similar life histories, our results are consistent with an intraspecific study in the facultative cooperatively breeding white-browed sparrow weaver (*Plocepasser mahali*) showing that the oxidative cost of reproduction was reduced in individuals belonging to larger social groups (Cram et al. 2015). Thus, the oxidative cost of reproduction may favor the evolution of obligate cooperative breeding, as well as larger social groups with more alloparents in facultatively cooperative species. Unlike white-browed sparrow weavers (Cram et al. 2015), however, cooperatively breeding superb starlings showed no evidence for an oxidative cost of reproduction regardless of group size. We attribute this difference in our findings to the reduced per capita workload of individual superb starlings relative to those in white-browed sparrow weavers. For example, superb starling individuals perform up to a maximum of 5 feeds per hour, whereas white-browed sparrow weaver individual can provision up to 15 times per hour (Cram et al. 2015).

Although the oxidative differences we observed between superb and greater blue-eared glossy starlings are consistent with differences in their social behavior, it is difficult to rule out other factors in a comparison of two species. For example, it is also possible that the cost of reproduction may be tissue specific, and damage may accumulate in different tissues or organs rather than circulating plasma markers of oxidative stress (Schmidt et al. 2014). However, there is no reason to believe that greater blue-eared glossy starlings would incur damage that can be measured in plasma, whereas a close relative living in the same environment would accumulate damage exclusively in different tissues. It is also possible that an individual's oxidative state prior to breeding may affect subsequent breeding decisions in cooperatively breeding starlings. For example, only individuals with low pre-breeding oxidative stress invested in reproduction in cooperatively breeding Seychelles warblers (*Acrocephalus sechellensis*) (van de Crommenacker et al. 2011) and Florida scrub-jays (*Aphelocoma coerulescens*) (Heiss and Schoech 2012). While superb starling breeders and alloparents had similar ROM and OXY during the incubation stage, oxidative status in the dry pre-breeding season could influence breeding decisions such as the adoption of different roles, which would represent an oxidative constraint on reproduction that we did not explore in this study. Since previous work in superb starlings has demonstrated that pre-breeding physiology (i.e. glucocorticoid stress hormones) is central to shaping breeding roles (Rubenstein 2007d), it will be interesting to examine carryover effects of oxidative stress across life history stages in this and other species.

Our results demonstrate that ROM increases with per capita workload, but oxidative stress was unrelated to nest guarding or provisioning rates for both species. The cost of reproduction in African starlings thus reflects total breeding effort, a complex set of behaviors (Guindre-Parker et al. 2013; Baldo et al. 2015) that may be poorly captured by a single behavioral measure such as nest guarding or nestling provisioning rate. Since dividing the breeding workload among more individuals effectively reduces the workload that each individual must perform in cooperatively breeding superb starlings, it remains unclear why greater blue-eared glossy starlings do not avoid potential physiological costs of reproduction by also breeding cooperatively. One key difference between these two species is that superb starlings experienced much higher rates of nest failure due to nest depredation over the course of this study relative to greater blue-eared glossy starlings; approximately 1 in 4 nests escaped predation in the greater blue-eared glossy starling, but only 1 in 10 did so in the superb starling (Guindre-Parker and Rubenstein, unpublished data). As a result, superb starlings typically re-nested within a breeding season at a higher rate than greater blue-eared glossy starlings (Rubenstein 2016)—this is a pattern observed more generally across the tropics, as cooperative breeders typically re-nest more frequently within a season than their non-cooperative counterparts (van den Heuvel and Ridley 2012). Since the cost of reproduction likely accumulates with each nesting attempt, selection for reducing oxidative costs of reproduction via cooperative breeding may have been stronger in the superb starling than in the greater blue-eared glossy starling. Thus, the evolution of cooperative breeding may not just be associated with the reproductive benefits of multi-brooding (Rubenstein and Lovette 2007; Rubenstein 2011), but also the reduced physiological costs associated with re-nesting. We were only able to include two species in this study, but future tests of the hypothesis that cooperative breeding reduces the oxidative cost of reproduction will need to make use of a comparative approach across a larger number of species.

Ultimately, our study suggests that the oxidative costs of reproduction should be considered as one of the many potential factors that can influence the evolution of cooperative breeding behavior. Indeed, direct benefits in the form of reduced physiological costs of caring for young may not only promote sociality, but do so independently of kin selection. Although superb starlings live in kin-based family groups, relatedness values are surprisingly low and a large proportion of the male breeders—the primary philopatric sex—are immigrants to the group (Pollack and Rubenstein 2015; Rubenstein 2016). This suggests that direct benefits like the one we uncovered here are likely to be important to the evolution or maintenance of alloparental care, and that kinship may play a limited role in favoring cooperative breeding in superb starlings. Thus, in social species where the costs of rearing young are high, the benefits of increased nest defense and reduced physiological costs of re-nesting are likely to have played a much stronger role in favoring alloparental care than has previously been considered, even in those species where kin structure within social groups exists.

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FIGURES

Figure 4.1: (A) Reactive oxygen metabolites (ROM) increased significantly from incubation to chick rearing in non-cooperative greater blue-eared glossy starling fathers (filled circle) and mothers (filled diamond). In contrast, (B) ROM did not change over the course of reproduction for cooperatively breeding superb starling fathers (filled circle), mothers (filled diamond), provisioners (open circle) or guards (open square). Total antioxidant capacity (OXY) did not differ significantly from incubation to chick rearing for (C) non-cooperative or (D) cooperative starlings. Symbols represent the mean ± standard error and lower case letters represent groups that differ from each other according to Tukey post-hoc comparisons. *NS* represents cases when no significant differences were detected.

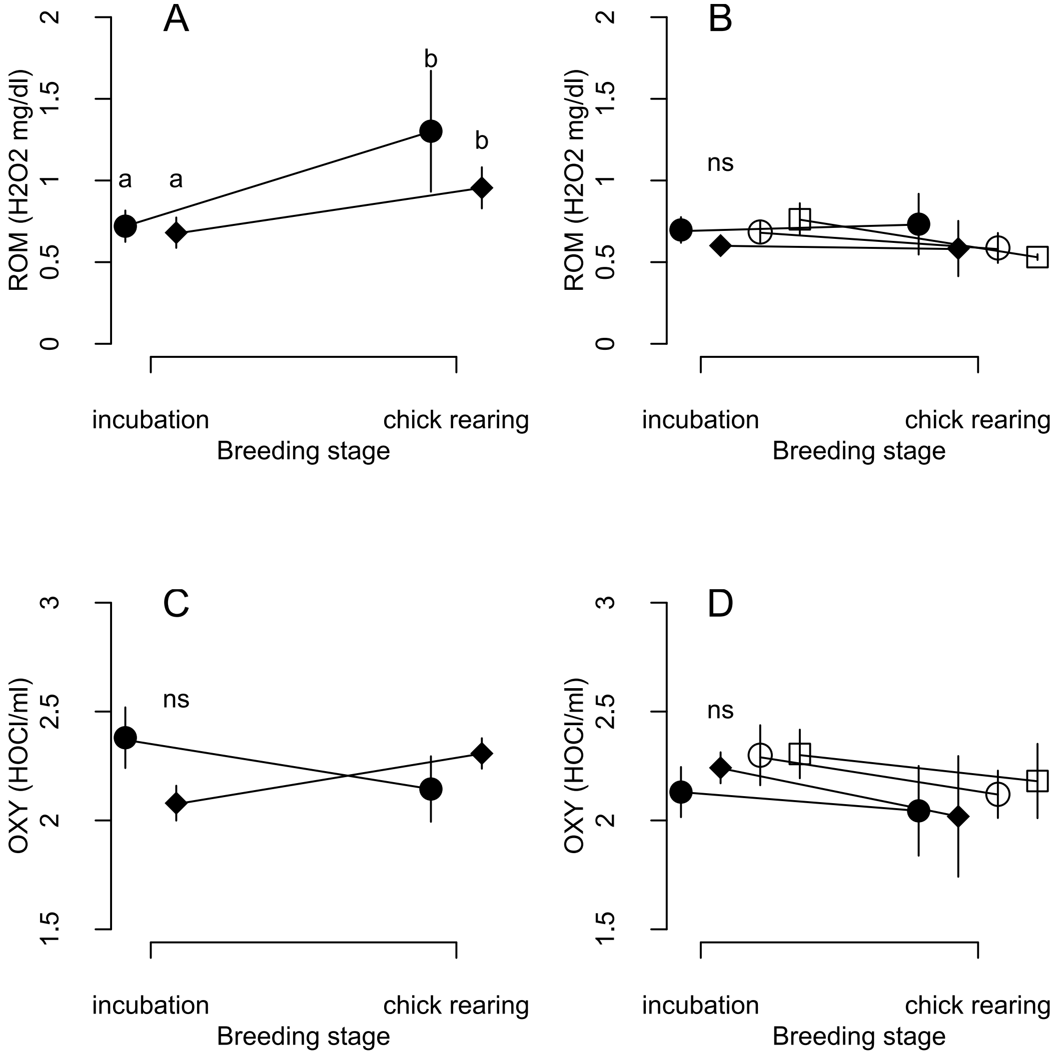
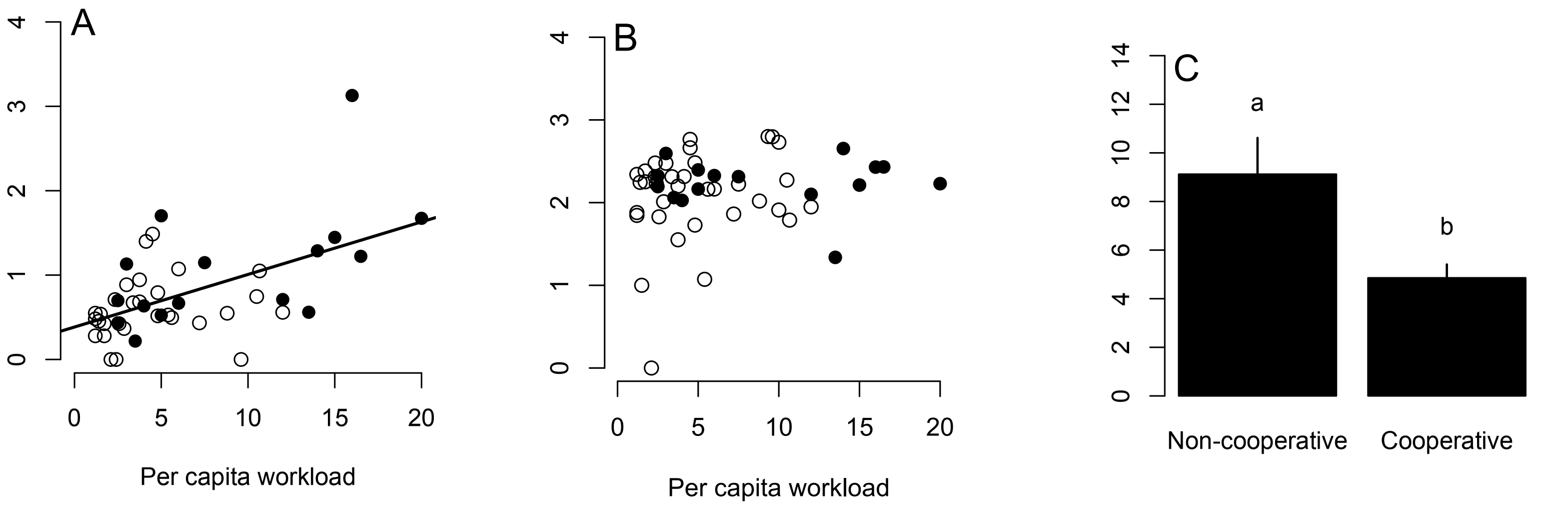


Figure 4.2: Breeding workload, which accounts for the number of birds that shared offspring care at each nest (i.e. per capita workload, calculated as chick*days/group size), was (A) positively correlated to reactive oxygen metabolites (ROM) in glossy starlings (black circles) and superb starlings (open circles) but (B) unrelated to total antioxidant capacity (OXY) in both species. (C) Individuals of the non-cooperative species have a breeding workload twice as high as individuals of the cooperative species. Bars represent the species mean ± standard error and lower case letters indicate that the means differed in a two-tailed *t*-test.



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APPENDICES

CHAPTER 1 APPENDICES

Appendix 1.1: Ageing superb starlings

In our study population, 49% of the individuals were banded at the nest and are therefore of known age. However, the other half of individuals in our nine banded social groups were aged according to their iris color, a morphological trait which can vary with age and has been used to age individuals in other avian species (Bond 1919; Scholten 1999; Nogueira and Alves 2008), including other starlings (Sweijd and Craig 1991; Feare and Craig 1998). In the African pied starling (*Lamprotornis bicolor*), iris color transitions from dark brown to creamy white with age due to changes in pigmentation of the anterior border layer of the iris (Sweijd and Craig 1991). A similar transition occurs in the superb starling (Feare and Craig 1998), a close relative of the African pied starling (Lovette and Rubenstein 2007). Iris color has been validated to correspond to age in the African pied starling as follows: (i) juveniles have a completely brown iris; (ii) sub-adults have irises where the outer ring is becoming white, while the inner iris remains brown around the pupil; and (iii) adults 2 years or older have completely white irises.

We assembled a collection of photographs displaying the iris color of banded superb starlings to determine whether, like in pied starlings, iris color in superb starlings changes from dark to light with age (Table S1.1). Our results show that individuals with a completely brown iris are recently fledged young—these juvenile individuals can further be identified by their visible yellow gape flange at the base of the beak and their plumage (i.e. non-iridescent black plumage on their head, face and throat, as well as lacking the white breast band) (Feare and Craig 1998). We found no evidence that juvenile birds maintain a completely brown iris outside of the breeding season during which they fledged, so juvenile birds are likely 4 months or less (i.e. the typical length of a breeding season). Conversely, individuals with mixed brown and white irises are several months of age (e.g. 5 months and 7 months, see Table S1.1). Finally, adults with entirely white irises appear to be 2 years of age or older—although we did not examine the timing of the color transition or the extent of among-individual variation in iris color, our results support that iris color changes with age in superb starlings as in African pied starlings.

Thus, for any bird captured outside of the nest, we assigned age according to the iris color: juvenile birds with a brown iris were classified as being a maximum of 4 months of age, sub-adults with a mixed brown and white iris were classified as being a maximum of 1 year of age, and adults with a pure white iris were classified as being a minimum of 2 years of age.

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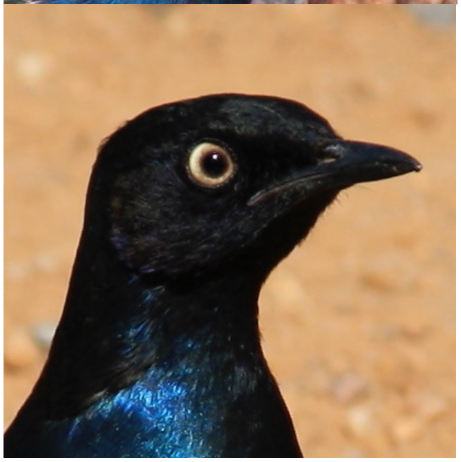
Table S1.1: Collection of photographed superb starlings of known ages born in our study population with visible variation in iris color. Birds were identified individually in the photographs via their unique color band combination and/or their metal band number (when captured and handled). S. Guindre-Parker took all photographs within our study population.

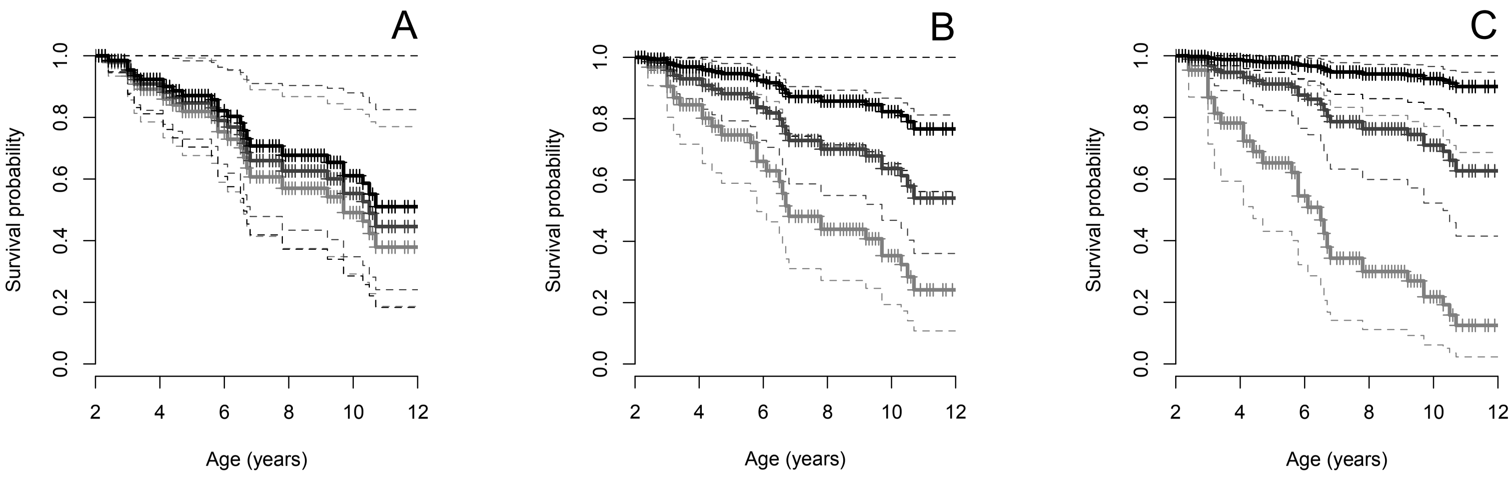
	Original Photo	Iris close-up	Bird details
Taken July 13th 2015			BB-12748 (Color bands added at a later date) Banded in nest June 3rd 2015 **Age in Photo:** **1 month**
Taken May 4th 2016			BB-17270 (Pink-Red-Yellow) Banded in nest November 25th 2015 **Age in Photo:** **5 months**
Taken May 18th 2013			BB-11901 (Black-Yellow-Brown) Banded in nest October 16th 2012 **Age in Photo:** **7 months**

	Original Photo	Iris close-up	Bird details
Taken May 16th 2015			BB-11960 (Brown-Orange-Pink) Banded in nest May 12th 2013 **Age in Photo: 2 years**
Taken March 28th 2014			BB-9489 Pink-Blue-Green) Banded in nest June 2nd 2011 **Age in Photo: 2 years and 10 months**
Taken May 13th 2016			BB-9462 (Light blue-Black-Orange) Banded in nest October 16th 2010 **Age in Photo: 5 years and 7 months**

Appendix 1.2: Grass cover shapes male survival

In males, two of our four models found an interactive effect of grass cover and group size on male survival. We present the results as estimated by our original model, which suggest that males on average or above average territories (measured by grass cover) gain fitness benefits from being in larger groups significantly more than males in smaller groups (Figure S1.1); under these favourable environmental conditions males in larger groups have higher survival relative to smaller groups. Conversely, males on territories with below average territories do not benefit from being in larger groups, as their survival is equivalent regardless of group size. These results should be interpreted with caution, however, as only 2 of 4 models in our sensitivity analyses supported this finding.

Figure S1.1: Superb starling male survival was sometimes correlated with an interaction between the group size and grass cover. When territory grass cover was (A) below average (i.e. 1 standard deviation below average) group size did not to increase male survival. However when grass cover was (B) average or (C) above average (i.e. 1 standard deviation above average), group size significantly increased male survival. The thick survival curves represent survival model predictions with other parameters set to their mean values while the small vertical hatches indicate a death event. Line colors represent group size – (i) above average (i.e. 1 standard deviation above average; black), (ii) average (dark grey) or (iii) below average (i.e. 1 standard deviation below average; pale grey). Thin dotted lines represent 95% confidence intervals.



Appendix 1.3: Group size and average age of group members

Our results suggest that group size is positively correlated to increased survival rates in both male and female superb starlings. This study is correlative in nature since we were not able to manipulate social group size; as a result, it remains unclear whether the lifespan of group members increases social group size (i.e. group size increases because group members remain in the group over longer periods), or whether living in larger groups confers benefits that increase the survival of group members.

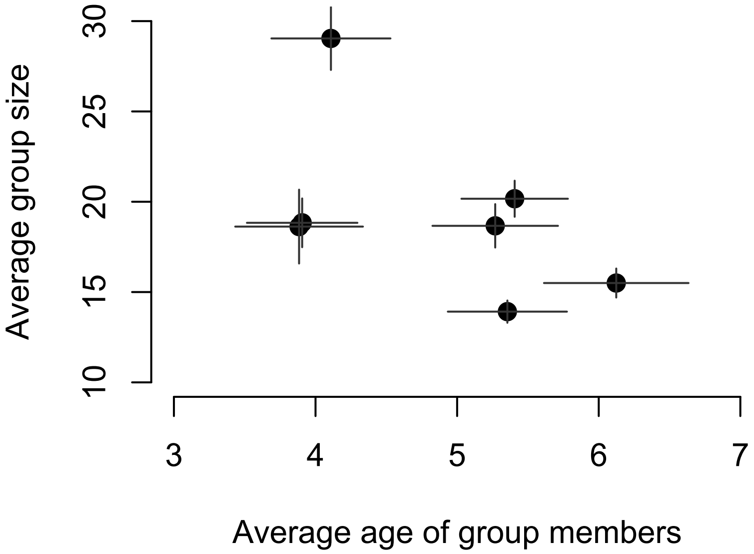
To examine the possibility that group member lifespan increases the size of social groups, we calculated the average age of all living group members in each social group for each breeding season. We used a generalized linear mixed model (GLMM) to test whether there was a positive correlation between group size and the average age of all group members. Group size and average age were computed for each breeding seasons, so we included social group ID as a random effect to account for repeated measurements within each group. All analyses were performed in R version 3.2.4 (R Core Team 2016), using the 'lme4' package.

We found that social group size was negatively correlated to the average age of group members (Table S1.3), suggesting that larger groups are unlikely to be shaped by the longevity of members within the group. Instead, these results suggest that larger groups are more likely to recruit or accept younger individuals, which would decrease the average age of group members while simultaneously increasing social group size (Figure S1.3). Larger group sizes therefore likely confer benefits that increase adult survival, rather than the other way around. We recognize, however, that in order to test the causality of this relationship, manipulating group size or the average age of members within a social group will be necessary.

Table S1.2: Parameter estimates and 95% Wald confidence intervals for a GLMM examining how total group size is correlated to the average age of all living group members. The model assumes a poisson error distribution and a random effect of group ID because we estimated group size repeatedly across seasons within each group. Asterisks highlight significant variables.

Fixed Effects	**Estimate ± SE**	***Z***	***P***	**95% Wald CI**	
Intercept	3.12 ± 0.09	36.4	<0.001*	2.93	3.30
Group size	-0.04 ± 0.01	-4.55	<0.001*	-0.05	-0.02

Figure S1.2: The group members of the largest social groups are younger on average, relative to the group members of the smaller social groups. Symbols indicate means whereas lines indicate the standard error of the mean (vertical for group size; horizontal for group member age).



CHAPTER 2 APPENDICES

Appendix 2.1: Alloparents at a nest are independent of group size

The number of alloparents providing offspring care to a brood of young can be correlated to, or constrained by, group size in many cooperatively breeding systems. However, superb starlings do not follow this pattern because non-breeding members of these plural breeding social groups can forgo alloparental care. In other words, non-breeding individuals can make the decision to provide alloparental care or to forgo providing any alloparental care (termed non-breeder/non-alloparents). Therefore, the common correlation observed between group size and the number of alloparents partaking in offspring is not seen in superb starlings (Table S2.1). Future work will be necessary to address why and when individuals choose to serve as alloparents rather than become non-breeder/non-alloparents.

Table S2.1: Parameter estimates and 95% Wald confidence intervals for a GLMM examining whether the number of alloparents counted at a nest is correlated to the size of the social group. The model assumes a negative binomial error distribution and nest ID as a random effect to account for re-nesting attempts. Asterisks highlight significant variables.

	Estimate ± SE	***Z***	***P***	**95% Wald CI**	
A) Dependent variable: Number of alloparents					
Intercept	0.62 ± 0.12	5.34	<0.001*	0.39	0.85
Group size	0.09 ± 0.01	6.65	<0.001*	0.06	0.11

Appendix 2.2: Alloparents correcting for observation length

In our study, nest predation events prevented further observation periods from being performed. Nests that are observed for a longer period of time typically represent nests that survive to a later stage of nestling development (i.e. with older nestlings). Nestling age and observation length are therefore likely to confound the number of alloparents observed at a nest; nests with older chicks typically have more numerous alloparents present, and longer observation periods are more likely to capture alloparental care by infrequent alloparents. Nest age and observation length are correlated, so to correct for these potential confounding factors we examined whether the number of alloparents observed at a nest increased with the cumulative observation time or the age of nestlings during the last observation performed at a nest. We built two GLMMs to predict the number of alloparents at a nest using a negative binomial error distribution and a random effect of nest ID, and selected the best model using an information theoretic approach. Briefly, the number of alloparents increased with the length of cumulative focal observations performed at a nest (Figure S2.1) as well as the age of nestlings (Figure S2.2). However, the model accounting for the length of focal observations was a better fit for our dataset ($AIC = 936.1$) relative to the model accounting for age of nestlings ($AIC = 950.2$) or the null model ($AIC = 968$). Therefore, we chose to calculate the residuals of the number of alloparents on cumulative observation length (Table S2.2) to get an index of the residual number of alloparents at a nest. A greater positive value indicates that the nest had a larger contingent of alloparents then nests observed for a comparable length of time, and vice versa. We checked whether nestling age could still account for significant variation in this index of residual alloparents using a GLMM with Gaussian error distribution, but found no significant correlation (estimate ± SE = 0.01 ± 0.007, $t = 1.54$, $N = 127$, $P = 0.12$). Similarly, the residual number of alloparents on observation length was strongly correlated with the residual number of alloparents on nest age (estimate ± SE = 0.85 ± 0.05, $t = 17.6$, $N = 127$, $P < 0.001$). These results suggest that correcting the number of alloparents observed by taking the residuals on observation length also satisfactorily accounts for variation in nest age.

Table S2.2: Parameter estimates and 95% Wald confidence intervals for a GLMM examining how total cumulative observation time at a nest shapes the number of alloparents observed at that nest. The model assumes a negative binomial error distribution and nest ID as a random effect to account for re-nesting attempts. Asterisks highlight significant variables.

	Estimate ± SE	***Z***	***P***	**95% Wald CI**	
A) Dependent variable: Number of alloparents					
Intercept	1.03 ± 0.29	3.55	<0.001*	0.46	1.60
Observation time	0.004 ± 0.01	0.33	0.73	-0.02	0.03

Figure S2.1: The number of alloparents counted at a nest increased with the length of cumulative observation time at that nest.

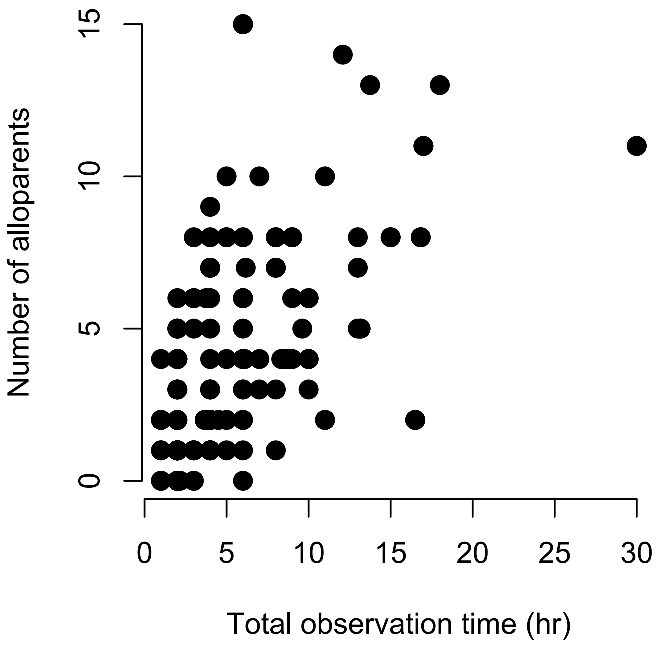
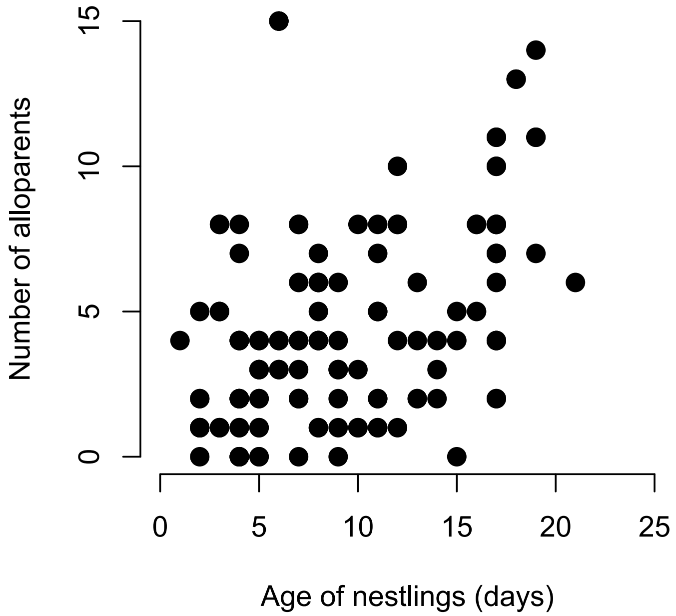


Figure S2.2: The number of alloparents observed at a nest increased with the age of nestlings during the last observation performed before either fledging or nestling death.



CHAPTER 4 APPENDICES

Appendix 4.1:Within-individual analyses of oxidative costs

Within-individual analyses revealed that the change in ROM was significantly related to species ($F_{1,\ 1.9}$ = 7.7, *P* = 0.01; Figure S4.1) but not role ($F_{3,\ 1.5}$ = 1.5, *P* = 0.13) or the interaction between species and role ($F_{1,\ 1.0}$ = 4.0 P = 0.06). Conversely, within-individual change in OXY from incubation to chick rearing was unrelated to species ($F_{1,\ 1.1}$ = 1.1, *P* = 0.07), role ($F_{3,\ 0.03}$ = 0.03, *P* = 0.99), or the interaction between the two ($F_{1,\ 0.3}$ = 1.1, *P* = 0.31; Figure S4.1). Within-individual change in ROM was similarly unrelated to nest guarding (*t* = 2.6, *P* = 0.06) or provisioning rate (*t* = 2.0, *P* = 0.12), or their interaction with species (*t* < 1.4, *P* > 0.22). Nest guarding (*t* = 1.6, *P* = 0.19) and provisioning (*t* = 2.0, *P* = 0.12) were similarly unrelated to within-individual change in OXY from incubation to chick rearing. Additionally, interactions between species and guarding (*t* = 1.4, *P* = 0.22) or provisioning (*t* = -0.15, *P* = 0.88) were also not significant. Finally, within-individual change in ROM was significant related to per capita workload (*t* = 2.9, *P* = 0.03; Figure S4.2). However, the change in OXY within individuals was unrelated to per capita workload (*t* = 0.3, *P* = 0.78; Figure S4.2).

Figure S4.1: Within-individual (A) reactive oxygen metabolites (ROM) increased in non-cooperative greater blue-eared but not in cooperative superb starlings. Conversely, (B) the species did not differ in the change in total antioxidant capacity (OXY) between incubation and chick rearing. Bars represent the mean ± standard error and lower case letters indicate that the means differed between the two species in a two-tailed *t*-test. *NS* represents cases when no significant differences were detected.

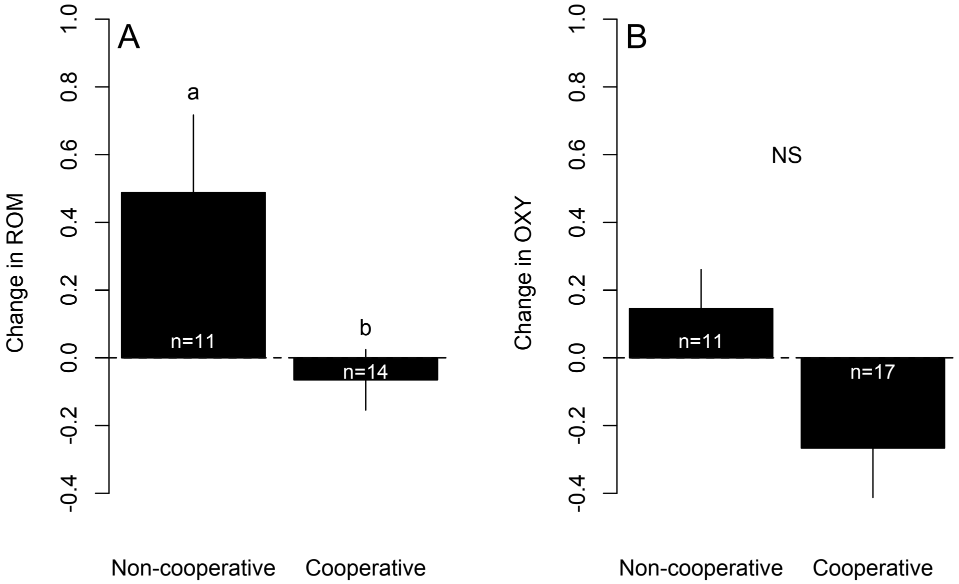


Figure S4.2: When accounting for the number of birds that are sharing breeding workload at each nest, (A) within-individual ROM increased with per capita workload similarly in non-cooperative glossy starlings (black circles) and cooperative superb starlings (open circles), but (B) OXY did not change with per capita workload in either species.