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RESEARCH ARTICLE

No short-term physiological costs of offspring care in a cooperatively breeding bird

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ABSTRACT

The cost of reproduction results in a life-history trade-off where investment in current reproduction via costly parental care decreases subsequent fitness. Although this trade-off is thought to occur ubiquitously across animals, there is equivocal evidence that parental care behaviours 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. Here, we compare four traits believed to mediate the cost of parental care by examining whether glucocorticoids, oxidative stress, immune function or body condition represent a cost of performing offspring care and shape subsequent fitness. We use a 4 year dataset collected in free-living cooperatively breeding superb starlings (*Lamprotornis superbus*), a species in which parental and alloparental care effort varies widely among individuals and across years. Our results showed that within-individual change in physiology was unrelated to investment in offspring care, and physiological state during chick rearing did not predict the likelihood that an individual would breed in subsequent seasons. Instead, individuals that had elevated baseline corticosterone during incubation performed more nest guarding, suggesting that this hormone may play a preparatory role for investing in offspring care. Together, our results indicate that superb starlings modify their investment in offspring care according to their physiological state during incubation, despite there being no evidence of a short-term physiological cost of parental or alloparental care. Thus, breeding cooperatively appears to provide individuals with the flexibility to adjust their investment in offspring care and overcome any potential costs of reproduction.

KEY WORDS: Cost of reproduction, Cooperative breeder, Glucocorticoid, Oxidative stress, Antioxidant, Immune function, Body condition

INTRODUCTION

Sexually reproducing organisms are faced with a fundamental decision – how to allocate valuable resources optimally when investment in current reproduction can come at the expense of future fitness. This life-history trade-off is called the 'cost of reproduction' (Stearns, 1992; Williams, 1966), and occurs across taxa including humans (Lycett et al., 2000), other mammals (Speakman, 2008), birds (Descamps et al., 2009), fish (van den Berghe, 1992), snakes (Chamberlain and Gifford, 2016), insects (Kotiaho and Simmons, 2003; Salmon et al., 2001) and plants (Obeso, 2002). Although theory suggests that individuals investing more heavily in their current breeding attempt should incur the largest cost of reproduction via reduced subsequent survival or reproductive success, this has not always been supported empirically. For example, a number of studies have found that current and future fitness are actually positively rather than negatively correlated (Cam et al., 2002; Sanz-aguilar et al., 2008; Weladji et al., 2008), or that breeders have higher future fitness than non-breeding conspecifics (Beauplet et al., 2006; Lescroël et al., 2009). Furthermore, while meta-analyses generally suggest that experimentally manipulating investment in current reproduction results in a cost of reproduction (Elliott et al., 2014; Santos and Nakagawa, 2012), a number of studies altering investment in current reproduction have failed to detect any effect on future fitness (Hare and Murie, 1992; Madliger and Love, 2016; Wheelwright et al., 1991). As a result, it remains unclear why the cost of reproduction is detected in some systems but not in others (Harshman and Zera, 2007; Williams and Fowler, 2015), as well as what factors shape the likelihood that such a cost will be detected.

One of the primary challenges with reconciling results from studies on the cost of reproduction is that we lack a general understanding of the mechanisms by which investment in current reproduction can inhibit future fitness (Harshman and Zera, 2007; Speakman, 2008; Williams and Fowler, 2015). In general, the cost of reproduction is thought to occur when individuals have a limited pool of resources; once allocated to the current breeding attempt, these resources are no longer available for self-maintenance, which can lead to reduced survival or future reproductive success (van Noordwijk and de Jong, 1986). However, the nature of these resources – or the currency of the cost of reproduction – remains unclear. Multiple physiological traits have been proposed to link metabolism with fitness in vertebrates, and could thus underlie the cost of reproduction; these include glucocorticoid hormones, oxidative stress, immune function and body condition or fat reserves (Alonso-Alvarez and Velando, 2012; Harshman and Zera, 2007).

Briefly, we summarize why each of these four physiological traits has been suggested to play a mediating role in driving the cost of reproduction. Glucocorticoid hormones such as corticosterone (hereafter CORT) circulating at baseline concentrations has been proposed to shape the cost of reproduction because of this hormone's role in mediating homeostasis (Love et al., 2014; McEwen and Wingfield, 2003). Life-history stages requiring elevated energy expenditure are expected to result in increased baseline CORT (McEwen and Wingfield, 2010), where CORT generally increases with offspring care workload (Bonier et al., 2009a, 2011; Crossin et al., 2013), and chronically elevated levels can decrease survival (Breuner et al., 2008; Suorsa et al., 2003).

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Oxidative stress, the imbalance between harmful reactive oxygen metabolites (ROM) and neutralizing antioxidants, has been suggested to be 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). Individuals providing greater offspring care may face elevated metabolic activity, which results in an increased production of reactive oxygen species (Cram et al., 2015; Guindre-Parker et al., 2013). If reactive oxygen species are not neutralized by antioxidant defences, these molecules can damage DNA or tissues and reduce survivorship (Cadenas and Davies, 2000; Saino et al., 2011). As producing an immune response to a pathogen is energetically demanding (Lochmiller and Deerenberg, 2000; Smith et al., 2017), immune responses can trade-off with investment in current reproduction and individuals may become immunosuppressed during breeding if energetic resources are preferentially allocated to offspring care (Cox et al., 2010; Graham et al., 2017; Hanssen et al., 2004, 2005). However, immune defences represent a vital barrier to pathogens, making individuals that become immunocompromised more susceptible to pathogens and their sublethal or lethal effects (Knowles et al., 2009; Zylberberg et al., 2012). Lastly, body mass represents an integrated trait resulting from the trade-off between the benefit of storing energy and the cost of locomotion (Rogers, 2015). An individual's mass or body condition reflects a combination of the availability of energy in the environment (i.e. availability or predictability of food) (Witter et al., 1995), their metabolic requirements (Cuthill et al., 2000) and their investment in protein or fat reserves (Labocha and Hayes, 2012). As investment in parental care results in increased energy expenditure (Welcker et al., 2015) and can lead to decreased investment in body mass (Canestrari et al., 2007; Velando and Alonso-Alvarez, 2003), declines in body condition have been suggested to represent a potential cost of parental care (Bryant, 1988). In turn, body condition can link investment in current reproduction to future fitness because lower body condition can reduce survival (Cox and Cresswell, 2014; Haramis et al., 1986; Naef-Daenzer and Gruebler, 2016) as well as future reproductive success (Salton et al., 2015).

For a physiological trait to mediate the cost of reproduction, the trait in question must reflect investment in current reproduction, and in turn shape future survival or reproductive success. Glucocorticoid hormones, oxidative stress, immune function and body mass are linked to an animal's metabolism (Bourgeon et al., 2010; Schull et al., 2016; Zheng et al., 2014), while also having the potential to impact fitness (Bonier et al., 2009b; Milenkaya et al., 2015; Saino et al., 2011; Tieleman, 2018). As a result, each trait has been shown to reflect investment in current breeding, where individuals investing more in current reproduction generally decline in physiological condition (Crossin et al., 2013; Fowler and Williams, 2017; Hanssen et al., 2005; Velando and Alonso-Alvarez, 2003). Similarly, each trait has been shown to shape future fitness, where individuals in poor condition often have reduced future fitness (Blums et al., 2005; Bonier et al., 2009b; Møller and Saino, 2004; Saino et al., 2011; van de Crommenacker et al., 2017). Despite a variety of empirical studies examining whether each of these traits can mediate the cost of reproduction, the relative importance of each trait in contributing to a potential cost of reproduction remains unknown because (i) studies have found conflicting support about whether each trait reflects investment in offspring care and shapes subsequent fitness (Harshman and Zera, 2007; Speakman, 2008; Williams and Fowler, 2015), and (ii) few studies have simultaneously quantified multiple traits to determine whether multiple physiological systems act in concert to shape the cost of reproduction (but see Fowler and Williams, 2017). Importantly, life-history trade-offs are likely to be mediated by multiple physiological systems that together shape individual state rather than any one trait alone (Williams, 2012).

Here, we aimed to compare four physiological traits (baseline glucocorticoid hormones, oxidative stress, immune function and body condition) suggested to mediate the cost of reproduction using a study system where investment in offspring care varies considerably and naturally among individuals: the plural cooperatively breeding superb starling, *Lamprotornis superbus*, a species in which up to 14 individuals aid in offspring care at a single nest (Rubenstein, 2006). Although it is widely assumed that cooperative breeding behaviour in vertebrates results in part from costly parental care (Emlen, 1982), the physiological basis of the cost of reproduction in social species has only been explored in a few studies (Cram et al., 2015; Guindre-Parker and Rubenstein, 2018a; Heiss and Schoech, 2012; Schoepf et al., 2017). We chose to perform our study using a cooperatively breeding species for two reasons. First, the population that we worked with is free-living and resources crucial for reproduction – such as insects – vary unpredictably with the amount of rainfall during breeding (Rubenstein, 2006, 2016). It is under these types of harsh environments (compared with a laboratory environment where food is provided *ad libitum*) that the cost of reproduction is most likely to be elevated and detectable (Erikstad et al., 1998; Speakman and Garratt, 2014). Second, cooperatively breeding species are ideal systems to explore how natural variation in care shapes the cost of rearing offspring (Costantini, 2016a; Speakman and Garratt, 2014) because care involves a combination of parental and alloparental forms. In superb starlings, investment in offspring care is highly variable from one individual to the next (Guindre-Parker and Rubenstein, 2018b), making it possible to explore whether individuals that invest most in caring for offspring pay a greater cost of reproduction. For example, mothers of this species tend to invest the most in rearing their young relative to fathers or alloparents (Guindre-Parker and Rubenstein, 2018b; Rubenstein, 2006), although offspring care behaviour is also highly variable from one individual to the next within each breeding role (Guindre-Parker and Rubenstein, 2018b).

We explored the relationship between parental or alloparental care and physiological state by monitoring the post-hatching offspring care behaviour of individuals. Specifically, we examined within-individual changes in glucocorticoids (i.e. CORT), oxidative stress, immune function and body mass over the course of a single breeding attempt. We also examined whether an individual's physiological state immediately prior to offspring care (i.e. during incubation) influenced its investment in care in order to test whether individuals adjust care according to their intrinsic state immediately preceding offspring care. Individuals can adjust their investment in reproduction according to their pre-breeding condition (Descamps et al., 2011; Ouyang et al., 2013; Stier et al., 2012), which could mask our ability to detect a cost of reproduction unless differences in physiological state prior to parental care are accounted for. Additionally, we determined whether within-individual declines in physiological state reflect investment in offspring care in order to test whether individuals pay a physiological cost of caring for offspring. Importantly, we examined declines in the four physiological traits concurrently to determine whether multiple traits might contribute to shaping a cost of offspring care in superb starlings. Lastly, we monitored individuals over the subsequent year to determine whether physiological state in one breeding season shaped reproductive opportunities in subsequent seasons. As superb starlings can live for up to 14 years (Rubenstein, 2016), we were unable to examine the lifetime fitness consequences of offspring care and physiological state in the current study.

Our research addressed three related questions, which together contribute to our understanding of which physiological trait(s) represent a cost of reproduction. (i) Does an individual's physiological state during incubation predict subsequent investment in offspring care behaviour post-hatching? (ii) Do individuals that invest more heavily in offspring care behaviour face a greater decline in physiological state over the course of a single breeding attempt? (iii) Does an individual's physiological state during offspring care in one season shape the likelihood that it will breed in the following breeding seasons? First, we predicted that controlling for breeding role and sex, individuals in a superior physiological state during incubation may be more likely to perform increased offspring care upon hatching at the focal nest. Specifically, we expected that individuals with lower baseline CORT, lower oxidative damage, higher antioxidants, higher immune responsiveness or higher body condition during incubation would guard the nest a greater proportion of the time, would be more likely to provision the young and, if they provisioned, would do so at a higher rate. Second, we predicted that individuals providing greater offspring care would face a greater physiological cost of reproduction. That is, individuals that guarded or provisioned the young at a higher rate would face a greater increase in baseline CORT, increase in oxidative stress, decrease in antioxidants, decrease in immune responsiveness or decrease in body condition from incubation to chick rearing relative to individuals that invested less in offspring care. Third, we predicted that individuals in poor physiological state during chick rearing (i.e. higher baseline CORT, higher oxidative stress, lower antioxidants, lower immune responsiveness or lower body condition) would be less likely to breed in the subsequent short or long rains breeding season. Lastly, we predicted that multiple physiological traits (rather than a single trait) would co-vary with investment in offspring care and the likelihood of breeding in future seasons. This prediction arises from previous work in this system demonstrating that pair-breeding greater blue-eared glossy starlings (*Lamprotornis chalybaeus*), which live sympatrically with superb starlings, faced elevated damage from oxidative stress during breeding whereas this physiological change was absent in superb starlings (Guindre-Parker and Rubenstein, 2018a). It remains unclear whether superb starlings face alternative physiological costs of rearing young, so our current study expands upon this work by simultaneously investigating multiple physiological traits which could together shape a cost of reproduction. Thus, these traits are not mutually exclusive because they can co-vary and each can simultaneously reflect an individual's energetic state and shape fitness.

MATERIALS AND METHODS

Study species

A population of superb starlings, *Lamprotornis superbus* (Rüppell 1845), 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 ring and a combination of four coloured leg bands. Individuals of this species live in large social groups of up to 50 individuals and defend year-round territories (Rubenstein, 2016). Breeding occurs twice per year, during the short (October–November) and long rainy seasons (March–June) (Rubenstein, 2011). Superb starlings are plural cooperative breeders, in which up to 7 pairs in each social group will attempt to breed in a given breeding season (mean±s.e.m. breeding pairs for the short rains: 2.0±0.11; long rains: 2.6±0.13). Non-breeding individuals in the group either act as alloparents at one of the active nests by guarding and/or provisioning the young, or forgo participating in breeding and alloparental care completely (termed 'non-breeder/non-alloparents') (Guindre-Parker and Rubenstein, 2018b). Individuals of both sexes are thought to compete for breeding opportunities, which appear to be shaped by dominance hierarchies (Rubenstein, 2016). Specifically, breeders are dominant to all non-breeding individuals, while alloparents are subordinate to non-breeder/non-alloparents (Pikus et al., 2018; Rubenstein, 2007).

Sampling birds

We trapped birds during the long rains from 2013 to 2016 using baited ground traps or mist nets around focal nests. For both trapping methods, we collected a small blood sample from the brachial vein within 3 min of capture (up to a maximum of 450 µl of plasma) which was used to measure baseline CORT, oxidative stress and immune function. Blood samples were centrifuged in the field and plasma was stored frozen until analysis (less than 8 weeks for immune assays, and up to 6 months for CORT and oxidative stress assays). Before releasing individuals, birds were weighed with a Pesola scale and their tarsus was measured with callipers.

Individuals were captured during two breeding stages: (i) incubation, before hatching but post-clutch completion (to exclude potential costs of egg laying or nest building); and (ii) chick rearing, when nestlings were 4–16 days post-hatching. We re-sampled individuals across both stages when possible, but high nest predation rates made it difficult to recapture every individual during chick rearing. Over the 4 years of the study, we were able to sample 103 individuals during incubation, although many of the nests at which these individuals were providing offspring care were depredated before hatching or before we could perform focal observations. As a result, only 30 of 103 birds were observed performing subsequent care. Similarly, we were able to capture 52 individuals during chick rearing, of which only 23 had been previously sampled during the incubation period.

Measuring offspring care behaviour

Superb starling mothers typically perform the majority of incubation, which lasts 2 weeks (Feare and Craig, 1998), while both breeders and alloparents contribute to offspring care upon hatching via provisioning and guarding the young (Guindre-Parker and Rubenstein, 2018b). Hatchlings typically remain in the nest for up to 23 days before fledging (Feare and Craig, 1998), although they can leave the nest after 2 weeks and still survive (S.G.-P. and D.R.R., unpublished data). We observed active nests with a spotting scope for a minimum of 2 h (mean±s.e.m. duration of focal observations per nest: 4.4±0.4 h), typically when nestlings were 4 or 5 days old (mean±s.e.m. 4.6±0.5 days). We observed nests on multiple days when possible, although high nest predation rates meant that we were not always able to perform multiple focal observations at each nest (>90% of nests were depredated). Excluding nests with shorter focal observation periods (i.e. depredated early on) would bias our dataset towards groups that invested more in offspring care, as cumulative care at a nest increases the likelihood of fledging more young (Guindre-Parker and Rubenstein, 2018b). During focal observations, we monitored the identity of each bird that came within 20 m of the nest as well as whether individuals delivered food into the nest in order to quantify several behaviours that reflect investment in offspring care (Guindre-Parker and Rubenstein, 2018b). First, we measured nest guarding, defined as the time an individual spent within 20 m of the nest – but not inside it – relative to the total length of the observation period. This behaviour constitutes guarding because individuals perched near the nest produce alarm calls and attack predators that approach the nest (Guindre-Parker and Rubenstein, 2018b; Rubenstein, 2006). Second, because not all individuals provision the young, we assessed the likelihood of provisioning by classifying individuals as non-provisioners or provisioners (i.e. a binary classification where provisioners delivered food into the nest at least once while non-provisioners did not). Lastly, for those individuals that did provision the young, we calculated provisioning rate as the number of trips per hour where an individual delivered food into the nest. In this system, there exists little variation in the size of the prey items delivered to young (relative to adult bill length) – the mean±s.e.m. prey size delivered by mothers is 1.1±0.06, by fathers it is 1.2±0.09, and by alloparents it is 1.2±0.09 – making provisioning rate a stronger indicator of offspring care (Guindre-Parker and Rubenstein, 2018b). For this reason, prey size was not included in our analyses. Our study focused on investment in current reproduction during offspring care (i.e. guarding and provisioning nestlings) because these behaviours increase current breeding success in superb starlings (Guindre-Parker, 2017; Guindre-Parker and Rubenstein, 2018b; Rubenstein, 2006) and are costly to perform in many other species of birds (e.g. Cram et al., 2015; Markman et al., 1995; van de Crommenacker et al., 2011; van den Berghe, 1992). Both guarding and provisioning rates are repeatable (R=0.137±0.037 and R=0.13±0.056, respectively), suggesting that even at nests observed for a single observation period, we were capturing some degree of consistency in among-individual variation in offspring care.

In addition to characterizing each individual's investment in current offspring care, we also monitored the likelihood that individuals would breed in the following year as an index of future fitness. The primary cause of breeding failure in superb starlings is predation, so there is a certain degree of stochasticity to reproductive success (Rubenstein, 2016). Nevertheless, as the number of breeding attempts an individual undertakes over their lifetime is the primary predictor of lifetime reproductive success (Apakupakul and Rubenstein, 2015), we chose to assess future fitness as the likelihood that individuals would acquire a breeding position in the future. We monitored whether individuals sampled in one season attempted to breed in the following short or long rains breeding season (i.e. the very next breeding season starting in November or a year from the current season starting in March, respectively). We note that over the course of this long-term study (2001–2016), there were significantly fewer breeding pairs in the short rains breeding season compared with that in the long rains season (t=4.3, d.f.=237, P<0.001), so the intensity of competition for breeding positions is expected to differ between these two breeding seasons. To determine breeding status in subsequent seasons, we surveyed territories as part of routine long-term data collection and performed focal observations to identify the breeding pair at active nests (Rubenstein, 2006). We were not able to determine whether physiological state in one season shaped subsequent annual mortality as short-term re-sighting probabilities are not very meaningful in this long-lived bird because it is not uncommon for an individual to go unobserved for a breeding season in our study population, only to return at a later time (Guindre-Parker and Rubenstein, 2018b; Pollack and Rubenstein, 2015).

Physiological analyses

Baseline CORT

We measured baseline CORT using a commercially available enzyme immunoassay (EIA) kit according to the manufacturer's directions (Enzo Life Sciences corticosterone kit). We thawed plasma samples before adding 1% steroid displacement buffer for 5 min and diluting samples 1:10 in assay buffer. This protocol was validated for superb starlings, where 5 dilutions (1:6, 1:8, 1:11, 1:15 and 1:20) of the same plasma sample yielded similar hormone concentrations (mean 6.9% difference in CORT between dilutions, $F_{4,10}$=1.7 and P=0.29). We also included a standard curve (32–20,000 pg ml^{-1}) and two blank controls on each plate. We ran samples in duplicate 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; absorbance was analysed at 405 nm with a Bio-Rad model 680 XR plate reader, and circulating CORT concentrations were calculated from the absorbance relative to that of the standard curve and expressed in ng ml^{-1}. The intra- and inter-assay coefficients of variation were 2.5% and 12.7%, respectively.

Oxidative stress

To quantify oxidative stress, we measured ROM and total antioxidant capacity (hereafter OXY) from plasma samples. ROM – a more stable derivative of reactive oxygen species – represents a marker of early oxidative damage (Costantini, 2016b). We used a commercially available kit (dROM test, Diacron International, Grosseto, Italy) according to standard protocols (Baldo et al., 2015; Costantini et al., 2008) that have been validated for this species (Guindre-Parker and Rubenstein, 2018a). We diluted 10 µl of plasma with 400 µl of a 1:100 mixture of alkyl-amine solution and acetate buffer. As we found that a lipid precipitate formed at the bottom of the wells, we ran the assay in 1.5 ml Eppendorf tubes before pipetting the liquid layer into a 96-well plate (Costantini et al., 2011; Guindre-Parker et al., 2013) – we incubated the reagents and plasma mixture in Eppendorf tubes for 75 min at 37°C, centrifuged the tubes at 10,000 rpm for 30 s, and pipetted 190 µl of the liquid into duplicate wells of a flat-bottomed 96-well plate. We included a standard curve of H_2O_2 on each plate (0.16–5.12 mg H_2O_2 dl^{-1}) and absorbance was read at a wavelength of 490 nm. The ROM concentration is given in mg H_2O_2 dl^{-1}. Each year, multiple samples from a single individual were run on the same plate, but positions within a plate were randomized. The mean intra- and inter-assay coefficients of variation were 5.9% and 10.4%, respectively.

We quantified antioxidant defences by measuring the general capacity of plasma antioxidants (OXY) to neutralize a strong oxidant (hypochlorous acid, HOCl). We measured OXY using a commercial kit (OXY test, Diacron International) that has been validated for this species (Guindre-Parker and Rubenstein, 2018a). We diluted plasma samples 1:100 with deionized water and added 5 µl of diluted samples in triplicate to wells of a flat-bottomed 96-well plate. We then added 200 µl of HOCl solution to each well, and the plate was shaken at 450 rpm for 10 s and incubated at 37°C for 10 min. Following this incubation period, we added 2 µl of colour-changing chromogen solution to each well (*N*,*N*-diethylparaphenilendiamine) and shook the plate for 30 s before reading the absorbance of each well at 490 nm. On each plate, we included a standard curve of HOCl (0.43–6.8 µmol HOCl ml^{-1}) and concentrations of antioxidants are expressed in µmol HOCl ml^{-1}. Multiple samples from the same individual in the same year were run on the same plate, but positions within a plate were randomized. The mean intra-assay coefficient of variance was 5.4% whereas the mean inter-assay coefficient of variance was 11.9%.

Immune function: bacteria-killing ability

We measured the ability of plasma to kill a standardized bacterial strain to assess constitutive innate immune function (Matson et al., 2006; Tieleman et al., 2005). This test represents a broad and integrative measure of immunity, including antibodies, acute phase proteins and complement activity (Tieleman et al., 2005). The assay involves exposing plasma to a known quantity of quality-controlled colonies of *Escherichia coli* (Lyfo disk derived from control strain ATCC 8739) and determining how many colonies are killed by the plasma sample *in vitro*. Preliminary analyses revealed that bacteria-killing ability (hereafter BKA) is not repeatable in plasma samples frozen for more than a few months or thawed multiple times (S.G.-P., unpublished data), so we only performed BKA assays in the field from 2014 to 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±s.e.m. 86±5.5 units) and incubated each sample on agar for 12 h in triplicate (Rubenstein et al., 2008). We counted the number of bacteria colonies present on each plate and averaged counts across triplicates of the same sample. BKA was defined as the proportion of colonies killed by plasma, which we calculated by subtracting the number of colonies that grew despite exposure to plasma from the number of colonies on the positive control (bacteria only on agar, i.e. 100% colony growth) and dividing the resulting value by the number of colonies on the positive control. We always included a negative control to ensure that agar plates were not contaminated with other bacteria colonies that could influence the results of this assay. The mean intra-assay coefficient of variation was 13.4%.

Body condition

We measured body condition (hereafter BC) by taking the residuals of a linear regression of mass on tarsus length (Rubenstein, 2007), 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 and would not change over a few weeks (Potti and Merino, 1994).

Statistical analysis

We first examined whether an individual's investment in offspring care was shaped by physiological state during incubation using three generalized linearized mixed models (GLMMs). Rather than modelling provisioning rate or the percentage of time spent guarding the nest – which do not conform to common error distributions – we modelled the number of provisioning trips or the total time spent guarding the nest along with an offset controlling for the total length of focal observations performed at a nest. This approach accounts for the fact that data are provided as the raw number of feeding visits or minutes spent guarding, whereas we are ultimately interested in the total number of feeding visits or minutes spent guarding per hour of observation (http://ms.mcmaster.ca/~bolker/misc/nceas_nonlinear/owls/owls.pdf). The first model included the number of minutes an individual spent guarding the nest as the dependent variable. As it is common for some superb starlings to forgo performing nestling provisioning in a breeding season, provisioning rates were zero-inflated (Guindre-Parker and Rubenstein, 2018b). As a result, we created two GLMMs to examine whether physiological state during incubation predicted subsequent provisioning behaviour. The first of these models included the likelihood that an individual provisioned the young (yes/no) as the dependent variable, whereas the second of these models included the number of trips where individuals delivered food into the nest as the dependent variable (excluding individuals that did not perform any provisioning). The three GLMMs mentioned above included baseline CORT, ROM, OXY, BKA, BC, breeding role (breeder or non-breeder) and sex as predictor variables. We chose to include only breeding role in our models because alloparent age, sex and relatedness to breeders/offspring account for few differences in alloparental care behaviour (Rubenstein, 2006). Similarly, these models included an offset controlling for the total length of focal observations performed at the nest as mentioned previously. The GLMMs predicting guarding and provisioning rates assumed a negative binomial distribution, while the GLMM predicting the likelihood that an individual would perform provisioning (yes/no) assumed a binomial error distribution.

Next, we examined whether within-individual changes in physiological state from incubation to chick rearing (i.e. chick rearing minus incubation physiological trait) were correlated to an individual's investment in offspring care behaviour using general linear mixed models (LMMs). We built one model for each physiological trait (i.e. baseline CORT, ROM, OXY, BKA, BC), which included within-individual change in physiology or body mass as the dependent variable. Each model included nest guarding, provisioning rate (including individuals that did not provision the young, i.e. a rate of 0), the number of alloparents at the nest, breeding role and sex as predictor variables.

Finally, we examined whether physiological state during chick rearing shaped the likelihood of breeding during subsequent short or long rains breeding seasons using two GLMMs with an individual's breeding status (yes/no) in the short or long rainy season as the dependent variable. While an alternative possibility would have been to test whether within-individual change in physiology from incubation to chick rearing was correlated with future breeding opportunities, we chose to use chick rearing physiology as a dependent variable because it is the final state in which individuals are left after performing parental or alloparental care behaviours that should shape subsequent fitness and doing so increased our sample sizes (N=30 compared with N=23 for within-individual analyses). Both models included baseline CORT, ROM, OXY, BKA, BC, breeding role and sex as predictor variables and assumed a binomial error distribution.

All models included a random effect of individual ID nested within year to control for individuals that were resampled across seasons. Similarly, all continuous predictor variables were standardized (with a mean of 0 and a standard deviation of 1) to improve model convergence. We checked that predictor variables were not linearly related to one another [all variance inflation factors <2]. Furthermore, baseline CORT, ROM, OXY, BKA and BC did not co-vary (P>0.08; with one exception where ROM and OXY during chick rearing were positively correlated, P=0.041). GLMMs were run in the package *glmmADMB* version 0.8.3.3 and LMMs were run in the package *nlme* version 3.1-131 in R version 3.4.1 (http://www.R-project.org/).

Performing model selection using Akaike's information criterion (AIC) does not alter the results of this study, so we have chosen to include the results of the full models outlined above (i.e. the variables identified as statistically significant predictors from full

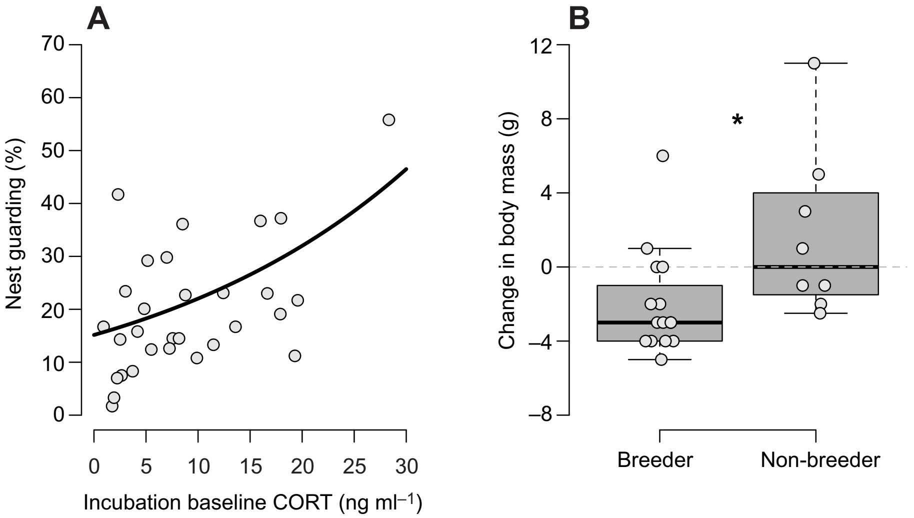

Fig. 1. Physiology and offspring care. (A) While few physiological traits measured during incubation predicted the degree of offspring care that birds would later perform, individuals with higher circulating baseline corticosterone (CORT) levels during incubation guarded the nest a greater proportion of the time post-hatching. (B) Although we generally failed to find evidence that individuals decline in physiological condition if they perform greater offspring care, the average breeder experienced a greater declined in body mass during a breeding attempt relative to non-breeding alloparent individuals. *$P<0.05$.

models are the only predictors consistently included in the set of top models using model selection by AIC). As environmental conditions may also play a role in shaping physiology and mediating life-history trade-offs (Erikstad et al., 1998), we originally included rainfall measured during the breeding season as a predictor variable in our models. However, rainfall was not a statistically significant predictor and did not significantly improve the fit of GLMMs or LMMs (ΔAIC>3), so we excluded rainfall from further analyses to prevent overfitting our models. We were not able to control for age in our models because breeding role is correlated with age and breeders are more likely to be older.

RESULTS

First, we investigated whether an individual's physiological state during incubation predicted their subsequent investment in offspring care behaviours. We found that individuals with higher baseline CORT prior to hatching performed greater nest guarding during the nestling stage (Fig. 1A), whereas ROM, OXY, BKA and BC measured during incubation were unrelated to subsequent nest-guarding behaviour (Table 1). While breeding role was not a significant predictor of investment in nest guarding, males guarded the nest less than females (Table 1). Similarly, we found that sex, but not breeding role, shaped the likelihood that an individual would

Table 1. Parameter estimates for three GLMMs examining how an individual's physiological state during incubation shaped their subsequent investment in nest guarding, provisioning behaviour (yes/no), and if they did provision, in their provisioning rates

	Estimate±s.e.m.	*Z*-value	*P*-value	95% CI	
Time spent guarding the nest					
Intercept	**−1.38±0.13**	**−10.34**	**<0.001**	**−1.64**	**−1.12**
Baseline CORT	**0.20±0.10**	**2.08**	**0.04**	**0.01**	**0.39**
ROM	−0.03±0.10	−0.31	0.76	−0.23	0.17
OXY	0.03±0.08	0.44	0.66	−0.12	0.18
BKA	−0.06±0.11	−0.55	0.58	−0.29	0.16
BC	−0.06±0.09	−0.60	0.55	−0.24	0.13
Role (non-breeder)	0.02±0.20	0.10	0.91	−0.38	0.42
Sex (male)	**−0.65±0.20**	**−3.31**	**<0.001**	**−1.04**	**−0.27**
Random effects (variance, s.d.)	Year 0.0000001, 0.0003			Bird ID:year 0.18, 0.43	
Likelihood of provisioning					
Intercept	0.86±0.93	0.93	0.35	−0.96	2.67
Baseline CORT	−0.80±0.64	−1.24	0.21	−2.05	0.46
ROM	0.11±0.57	0.20	0.84	−1.01	1.24
OXY	0.16±0.57	0.28	0.78	−0.96	1.27
BKA	0.27±0.63	0.42	0.67	−0.97	1.51
BC	−1.00±0.66	−1.51	0.13	−2.30	0.30
Role (non-breeder)	−1.36±1.32	−1.03	0.30	−3.94	1.23
Sex (male)	**−2.87±1.33**	**−2.15**	**0.03**	**−5.48**	**−0.26**
Random effects (variance, s.d.)	Year 0.00003, 0.006			Bird ID:year 0.0000005, 0.0007	
Number of provisioning trips					
Intercept	−0.23±0.17	−1.40	0.16	−0.56	0.09
Baseline CORT	0.18±0.15	1.17	0.24	−0.12	0.48
ROM	0.20±0.20	1.00	0.32	−0.19	0.58
OXY	−0.11±0.17	−0.62	0.54	−0.44	0.23
BKA	−0.22±0.27	− 0.81	0.42	−0.76	0.32
BC	0.02±0.18	0.12	0.90	−0.33	0.37
Role (non-breeder)	−0.03±0.50	−0.06	0.96	−1.00	0.95
Sex (male)	**−1.05±0.52**	**−2.03**	**0.04**	**−2.06**	**−0.04**
Random effects (variance, s.d.)	Year 0.0000001, 0.0003			Bird ID:year 0.0000001, 0.0003	

GLMMs, generalized linearized mixed models; CORT, corticosterone; ROM, reactive oxygen metabolites; OXY, antioxidant capacity; BKA, bacteria-killing ability; BC, body condition. Bold indicates significant effects.

provision young, as well as the provisioning rate that they would perform. As was the case with nest guarding, males were less likely to feed nestlings and, when they did, males provisioned at a lower rate than females. Individual state during incubation was not correlated with the likelihood of provisioning the young, or the provisioning rate performed for those individuals that did deliver food to the nest (Table 1).

Next, we examined whether parental or alloparental care effort shaped within-individual changes in physiology from incubation to chick rearing, which could represent a physiological cost of caring for young. We found that the within-individual change in baseline CORT was unrelated to an individual's breeding role, sex, the number of alloparents contributing to offspring care at the nest or the individual's offspring care behaviour (i.e. nest guarding or provisioning rates; Table 2). Similarly, within-individual changes in ROM, OXY or BKA were unrelated to investment in offspring care, as well as role, sex and the size of the alloparent contingent at the nest (Table 2). Within-individual change in mass was correlated with breeding role, such that breeders were more likely to decline in body mass over the course of a breeding attempt compared with non-breeding alloparent individuals (Fig. 1B). Change in mass was not related to sex, offspring care behaviours (nest guarding or provisioning rates) or the number of alloparents at the nest (Table 2).

Finally, we determined whether future breeding opportunities were shaped by an individual's physiological state during chick rearing. We found that breeding role, sex and an individual's physiological state (i.e. baseline CORT, ROM, OXY, BKA or BC) during chick rearing were unrelated to the likelihood that they would breed in the subsequent short rainy season (Table 3). Similarly, the likelihood of breeding in the following long rainy season (when a greater number of breeding pairs typically attempt to nest) was unrelated to sex or individual state (Fig. 2). However, we found that breeders in one long rainy season were more likely to breed in the following long rainy season compared with non-breeding alloparents (Fig. 2).

DISCUSSION

We examined four physiological traits that have been suggested to mediate the cost of parental care and shape subsequent fitness in a variety of avian species: baseline CORT concentrations, oxidative stress, immune function and body condition. We found that none of the traits we examined in this study were correlated to investment in

Table 2. Parameter estimates for five LMMs examining how an individual's investment in offspring care shaped within-individual changes in baseline CORT, ROM, OXY, BKA or body mass over the course of one breeding attempt

	Estimate±s.e.m.	*t*-value	*P*-value	95% CI	
Change in baseline CORT					
Intercept	7.76±4.29	1.80	0.09	−1.44	17.0
Nest guarding	3.43±1.89	1.81	0.09	−0.61	7.48
Provisioning rate	−4.55±2.60	−1.75	0.10	−10.1	1.02
Contingent size	−2.91±2.04	−1.43	0.17	−7.29	1.46
Role (non-breeder)	−7.56±4.41	−1.71	0.11	−17.0	1.90
Sex (male)	−0.13±4.27	−0.03	0.97	−9.28	9.02
Random effects (variance, s.d.)	Year 42.6, 6.53			Bird ID:year 56.3, 7.50	
Change in ROM					
Intercept	−0.09±0.11	−0.86	0.41	−0.34	0.15
Nest guarding	0.10±0.07	1.33	0.22	−0.07	0.27
Provisioning rate	0.09±0.08	1.03	0.33	−0.10	0.27
Contingent size	−0.10±0.07	−1.38	0.20	−0.27	0.07
Role (non-breeder)	0.12±0.15	0.80	0.45	−0.22	0.26
Sex (male)	0.02±0.14	0.12	0.90	−0.31	0.35
Random effects (variance, s.d.)	Year 0.00000000005, 0.000007			Bird ID:year 0.08, 0.27	
Change in OXY					
Intercept	−0.23±0.18	−1.26	0.23	−0.62	0.16
Nest guarding	0.10±0.11	0.88	0.40	−0.14	0.34
Provisioning rate	0.14±0.13	1.02	0.33	−0.15	0.43
Contingent size	0.10±0.11	0.87	0.40	−0.14	0.34
Role (non-breeder)	0.04±0.25	0.17	0.87	−0.50	0.59
Sex (male)	−0.11±0.24	−0.47	0.65	−0.64	0.41
Random effects (variance, s.d.)	Year 0.03, 0.16			Bird ID:year 0.18, 0.43	
Change in BKA					
Intercept	8.10±13.4	0.60	0.59	−34.7	50.9
Nest guarding	−0.06±15.6	−0.003	0.99	−49.8	49.7
Provisioning rate	−7.83±13.1	−0.60	0.59	−49.4	33.7
Contingent size	−13.0±12.0	−1.09	0.36	−51.1	25.0
Role (non-breeder)	3.08±18.8	0.16	0.88	−56.7	62.8
Sex (male)	−8.56±19.9	−0.43	0.70	−71.8	54.7
Random effects (variance, s.d.)	Year 0.000001, 0.001			Bird ID:year 0.05, 21.7	
Change in mass					
Intercept	−2.16±1.20	−1.81	0.09	−4.73	0.41
Nest guarding	0.74±0.86	0.86	0.41	−1.11	2.59
Provisioning rate	0.32±0.98	0.32	0.75	−1.78	2.42
Contingent size	−0.12±0.83	−0.14	0.89	−1.89	1.65
Role (non-breeder)	**4.46±1.90**	**2.34**	**0.03**	**0.38**	**8.54**
Sex (male)	−0.35±1.87	−0.19	0.85	−4.36	3.67
Random effects (variance, s.d.)	Year 0.0000002, 0.0004			Bird ID:year 14.1, 3.75	

LMMs, linear mixed models; Bold indicates significant effects.

Table 3. Parameter estimates for two GLMMs examining how an individual's physiological state during chick rearing in one season shaped their likelihood of breeding in the following long or short rainy season

	Estimate±s.e.m.	*Z*-value	*P*-value	95% CI	
Likelihood of breeding in the following long rainy season					
Intercept	**11.6±5.57**	**2.08**	**0.04**	**0.67**	**22.5**
Baseline corticosterone	−28.1±28.4	−0.99	0.32	−83.7	27.6
ROM	−0.13±8.00	−0.02	0.99	−15.8	15.6
OXY	3.98±5.07	0.79	0.43	−5.95	13.9
BKA	−0.06±4.88	−0.01	0.99	−9.61	9.50
BC	2.12±5.38	0.39	0.69	−8.42	12.7
Role (non-breeder)	**−32.9±11.3**	**−2.92**	**0.004**	**−55.0**	**−10.8**
Sex (male)	−3.53±6.78	−0.52	0.60	−16.8	9.75
Random effects (variance, s.d.)	Year=0.00001, 0.003			Bird ID:year 0.003, 54.7	
Likelihood of breeding in the following short rainy season					
Intercept	0.44±5.02	0.09	0.93	−9.39	10.3
Baseline corticosterone	−7.34±16.8	−0.44	0.66	−40.3	25.6
ROM	13.8±28.2	0.49	0.63	−41.5	69.1
OXY	5.70±10.0	0.57	0.57	−14.0	25.4
BKA	12.2±22.0	0.56	0.58	−30.9	55.4
BC	41.9±80.4	0.52	0.60	−115.7	199.5
Role (non-breeder)	−57.8±108.1	−0.53	0.59	−269.7	154.1
Sex (male)	−11.4±24.7	−0.46	0.64	−59.9	37.1
Random effects (variance, s.d.)	Year 0.0000000000000006, 0.00000002			Bird ID:year 0.47, 0.69	

Bold indicates significant effects.

offspring care behaviour or shaped the likelihood that birds would attain a breeding position in subsequent short or long rains breeding seasons. In light of these results, it is difficult to conclude whether (i) a cost of reproduction occurs in superb starlings and/or whether (ii) these traits mediate a cost of reproduction in superb starlings. This is in contrast to recent evidence that a suite of physiological traits are associated with the cost of reproduction in European starlings (*Sturnus vulgaris*), including oxidative stress, immune function, oxygen-carrying capacity and triglyceride levels (Fowler and Williams, 2017). It is possible that our study failed to detect a physiological cost of reproduction because unlike Fowler and Williams (2017), we did not manipulate breeding effort (i.e. by manipulating brood size or handicapping parents and alloparents). Therefore, individuals in our study were left unmanipulated and were allowed to select their own investment in current reproduction (i.e. either parental or alloparental care effort). In unmanipulated systems such as our own, individuals may thus invest in current reproduction optimally, making it challenging to detect a cost of reproduction (Metcalfe and Monaghan, 2013). As a result, it is not possible to identify which physiological mechanism may best mediate a cost of reproduction in this species. Nevertheless, our study compared investment in offspring care by breeders and alloparents across their natural range of variation, which has been suggested to represent a powerful comparison to explore the physiological underpinnings of the cost of reproduction (Costantini, 2016a; Speakman and Garratt, 2014). Regardless of whether manipulating investment in offspring care would have unveiled a cost of reproduction in superb starlings, our study suggests that in free-living cooperative breeders where individuals can select their contribution to parental or alloparental care, individuals did not incur a short-term cost of offspring care as measured across diverse physiological systems.

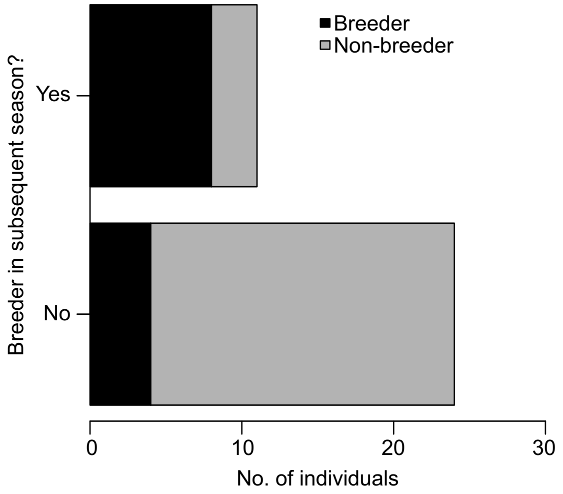

Fig. 2. Only breeding role predicted future breeding opportunities. The likelihood of breeding the following year (in the long rainy season) was unrelated to an individual's physiological state in the previous season, but individuals that were breeders in one season were more likely than non-breeder alloparents to acquire a breeding position in the following long rainy season.

An alternative explanation for why we did not find evidence for a decline in physiological state in our study is that the time scale for measuring such a cost of offspring care may extend across multiple breeding attempts (Love et al., 2014) or even multiple breeding seasons (Bonnet et al., 1999) rather than over a single breeding attempt. Superb starlings are multi-brooded within a breeding season and re-nest up to 6 times in one breeding event (Rubenstein, 2016). Similarly, individuals of this long-lived species may initiate reproduction in up to 16 seasons over their lifetime (S.G.-P. and D.R.R., unpublished data). It is possible then that the physiological cost of reproduction may only manifest once enough 'wear and tear' has accumulated, and a single breeding attempt may represent too short a time frame to measure a decline in physiological state. Unfortunately, we were not able to re-sample individuals across multiple breeding attempts or seasons to test this possibility. Ideally, a cost of reproduction would be studied over the entire lifespan of individuals, as this is the time scale at which natural selection will act. Despite these potential limitations, previous work in a non-cooperative syntopic species – the greater blue-eared glossy starling – did detect an oxidative cost of reproduction in male and female breeders using an identical field sampling protocol, while the same cost was absent in the cooperatively breeding superb starling (Guindre-Parker and Rubenstein, 2018a). This suggests that we are able to detect an oxidative cost of reproduction using our study design, and that such a cost may simply be low or absent in superb starlings. Our current study builds on this previous work by simultaneously assessing four traits known to mediate the cost of parental care in other species (Alonso-Alvarez and Velando, 2012; Harshman and Zera, 2007), each of which provides independent and repeated support that superb starlings face little to no realized cost of parental or alloparental care. This is particularly true as our measures of CORT, oxidative stress, immune function and body condition did not co-vary. Our present study therefore supports similar results from comparative work in the same system (Guindre-Parker and Rubenstein, 2018a) suggesting that breeding cooperatively can result in a low or absent realized physiological cost of caring for young. Although our study did not find that alloparent contingent size influenced declines in the body mass of breeders over a breeding attempt, superb starlings only experience incomplete load-lightening in offspring care behaviours when the number of alloparents present at a nest increases (Guindre-Parker and Rubenstein, 2018b). Therefore, the number of alloparents caring for young at a nest can only account for limited among-individual variance in offspring care behaviours.

We found that breeders were more likely to decline in body mass over the course of a breeding attempt, which could be interpreted as evidence for a cost of reproduction because breeders typically provide greater offspring care relative to alloparents (Guindre-Parker and Rubenstein, 2018b). Body mass has been shown to decline for individuals performing increased offspring care in other cooperatively breeding species of birds, including white-browed sparrow weavers (*Plocepasser mahali*) (Cram et al., 2015) and carrion crows (*Corvus corone corone*) (Canestrari et al., 2007). However, within-individual changes in body mass in superb starlings were not related to nest guarding or provisioning behaviour, suggesting that this loss in body mass did not stem from differences in offspring care. Instead, a breeding role-specific decline in body mass may reflect a cost of maintaining social status, even once a breeding attempt is underway. For example, it is possible that breeders and non-breeding alloparents face differences in the cost of maintaining their social status or enforcing alloparental contributions to offspring care, although it remains unknown whether alloparental care is coerced in this species. Within-group social conflict is thought to be high in this species (Rubenstein and Shen, 2009), and individuals may experience social conflict differently depending on their breeding status (Pikus et al., 2018), such that greater mass loss in breeders could represent a cost of elevated social conflict during reproduction.

We also found that an individual's physiological state during incubation shaped subsequent offspring care behaviour during chick rearing. Specifically, incubation baseline CORT was positively correlated with nest guarding during chick rearing, where birds that had elevated hormone levels prior to hatching invested in offspring care to a greater extent. These results add to a growing body of evidence supporting that elevated baseline CORT prior to reproduction may prepare individuals for the energetic demands associated with parental care (Bowers et al., 2016; Dupoué et al., 2016; Hennin et al., 2014; Love et al., 2014; Ouyang et al., 2013). As elevated baseline CORT did not decrease the likelihood that superb starlings would breed in subsequent seasons, our results suggest that a pre-emptive elevation in baseline CORT during incubation may actually be beneficial and does not come at a cost to subsequent reproductive attempts. While we did not find differences in baseline CORT measured during incubation across sex or breeding roles, elevated baseline CORT during the dry pre-breeding period has been shown to influence the adoption of different breeding roles (Rubenstein, 2007; Rubenstein and Shen, 2009). Together, these results suggest that baseline CORT may play a mechanistic role in influencing breeding decisions in superb starlings during several key periods.

Our study adds to previous work in superb starlings indicating that breeders and alloparents have flexible offspring care decision rules, which vary with environmental and social context (Guindre-Parker and Rubenstein, 2018b). For example, previous work demonstrated that alloparents provision young at higher rates following pre-breeding seasons with greater rainfall (i.e. benign environments), suggesting that they invest in offspring care preferentially under favourable environmental conditions (Guindre-Parker and Rubenstein, 2018b). Similarly, mothers and fathers experienced load lightening such that they provided lower offspring care when there was a larger contingent of alloparents assisting at their nest (Guindre-Parker and Rubenstein, 2018b). Findings from the current study suggest that in addition to their extrinsic environment (e.g. rainfall or social context), superb starlings may also adjust their investment in offspring care according to their intrinsic condition during incubation (i.e. circulating glucocorticoid hormones). In light of this flexibility, it is perhaps not surprising that superb starlings are able to adjust their offspring care decisions to minimize the realized cost of providing parental or alloparental care in any one breeding attempt. One important benefit of breeding cooperatively may be to provide individuals with the flexibility to adjust their investment in offspring care (Guindre-Parker, 2017; Valencia et al., 2003). In contrast, species with uniparental or biparental care may have less flexibility to modify their investment in offspring care according to their physiological state or the environmental conditions they currently face. For example, one parent may not be able to completely compensate for reduced investment in offspring care by its partner (Schwagmeyer et al., 2002). In contrast, individuals of cooperatively breeding species may be able to reduce their investment in offspring care because large groups of alloparents at a nest can compensate for their decline in care (i.e. load lightening) (Guindre-Parker and Rubenstein, 2018b; Russell, 2016).

Conclusions

The results from this study suggest that cooperatively breeding superb starlings did not face a physiological cost of parental or alloparental care over the course of a single breeding attempt. Our null results make it difficult to conclude which of the physiological traits we investigated – baseline CORT, oxidative stress, immune function or body condition – most strongly mediates a cost of reproduction (as there does not appear to be a cost of reproduction in this species). Nevertheless, our study highlights the importance of simultaneously investigating a suite of physiological traits that could mediate life-history trade-offs because had we investigated a single physiological trait, it would be tempting to conclude that our sampling design simply missed a cost of reproduction. Instead, our study found similar results from four separate physiological systems, which supports our conclusion that superb starlings do not experience a physiological cost of caring for young over the course of one breeding attempt (Guindre-Parker and Rubenstein, 2018b). Our finding that physiology during incubation shapes the offspring care behaviour of superb starlings supports the alternative conclusion that breeding cooperatively provides individuals with the flexibility to adjust their investment in offspring care. Individuals of this species can alter their investment in parental or alloparental care according to intrinsic and extrinsic conditions, including baseline CORT during incubation as we showed here, as well as pre-breeding rainfall and the number of alloparents contributing to offspring care at a nest (Guindre-Parker and Rubenstein, 2018b). In species that live in unpredictable environments (Jetz and Rubenstein, 2011; Lukas and Clutton-Brock, 2017), where harsh breeding conditions fluctuate unexpectedly across years – which would potentially otherwise lead to elevated costs of reproduction (Erikstad et al., 1998) – behavioural flexibility may be particularly important in order to minimize the realized or experience cost of reproduction. Future studies should aim to experimentally manipulate the cost of parental and alloparental care across harsh and benign conditions in order to test this hypothesis.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.G.-P., D.R.R.; Methodology: S.G.-P.; Formal analysis: S.G.-P.; Writing - original draft: S.G.-P., D.R.R.; Supervision: D.R.R.; Funding acquisition: S.G.-P., D.R.R.

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Data availability

Raw data are available from the corresponding author upon request.

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