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STUDENT AWARDEE PAPER

Females with Increased Costs Maintain Reproductive Output: A Field Experiment in a Common Songbird

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Synopsis Reproduction and self-maintenance are energetically costly activities involved in classic life history trade-offs. However, few studies have measured the responses of wild organisms to simultaneous changes in reproductive and self-maintenance costs, which may have interactive effects. In free-living female Barn Swallows (*Hirundo rustica*), we simultaneously manipulated reproductive costs (by adding or removing two nestlings) and self-maintenance costs (by attaching a ∼1 g weight in the form of a GPS tag to half of our study birds) and measured mass, immune status, blood glucose, feather growth, and reproductive output (likelihood of a second clutch, number of eggs, and time between clutches). GPS tags allowed us to analyze how movement range size affected response to brood size manipulation. Tagging altered females' immune function as evidenced by an elevated heterophil to lymphocyte (H:L) ratio, but all females were equally likely to lay more eggs. There was no evidence of interactive effects of the tagging and brood size treatment. Range size was highly variable, and birds with large ranges grew feathers more slowly, but analyzing the effect of brood size manipulation while accounting for variation in range size did not result in any physiological response. Our results support the theoretical prediction that short-lived vertebrates do face a trade-off between reproduction and self-maintenance and, when faced with increased costs, tend to preserve investment in reproduction at the expense of parental condition. This experiment also helps us to understand how movement patterns may be relevant to life history trade-offs in wild birds.

Introduction

Reproduction and self-maintenance are energetically costly activities involved in life history trade-offs: investment in reproduction should reduce energy available for parental maintenance, and vice versa (Stearns 1989). Variation in life history traits and associated behavioral and physiological traits occur along a fast-slow continuum (i.e., pace of life syndromes (POLS), Ricklefs and Wikelski 2002; Jablonszky et al. 2018; Mathot and Frankenhuis 2018), but empirical support for predicted trade-offs among life history traits remains mixed (Williams 2018). For example, a meta-analysis in birds found that increased investment in offspring does not necessarily reduce female survival (Santos and Nakagawa 2012). Individuals may vary in their ability to acquire and allocate resources, for example, by occupying more favorable environments (Zera and Harshman 2001; Laskowski et al. 2021). Furthermore, the costs of reproduction and parental care likely vary among individuals (Williams 2018). Variation among individuals may thus obscure expected trade-offs at the population level (van Noordwijk and de Jong 1986; Stearns 1989; Zera and Harshman 2001; Williams 2018). To better account for individual variation in life history trade-offs, we need empirical data on individual-level reproductive and somatic costs, while also taking into account relevant axes of individual variation, such as physiology, behavior, and resource access.

Costs of reproduction and parental care have been extensively studied in birds using brood size manipulations, where experimenters change the number of nestlings per nest (e.g., Velando and Alonso-Alvarez 2003; Reichert et al. 2014; Vitousek et al. 2017). Some parents, due to varying energetic costs associated with

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feeding nestlings, may respond to brood size manipulations behaviorally or physiologically more or less than expected (Laskowski et al. 2021). One potentially important, yet understudied, source of individual variation in energetic costs is movement range size. Animals foraging in poor-quality habitat or during times when prey are less abundant may have to expand their range to find resources (Henry et al. 2002; Bruun and Smith 2003; Pejchar et al. 2005; Santangeli et al. 2012). Animals with large ranges may be expending more energy to forage, provision offspring, and find mates than animals with small ranges. Maintaining a large range size may be particularly costly for aerial insectivores, who catch prey during energetically demanding powered flight (Tatner and Bryant 1993; Zhang et al. 2021). In most birds, and small birds in particular, we lack fine-scale movement data to understand range size during nestling feeding. Only recently, due to advances in the miniaturization of GPS tags, have we been able to gain any data about the fine-scale movements of small birds.

While many studies have manipulated reproductive costs (by changing the number of offspring, e.g., Zera and Harshman 2001; Ardia 2005) or self-maintenance costs (Wegmann et al. 2015a; Fowler and Williams 2017; by adding weights or wing-clipping, i.e., handicapping, Serota and Williams 2019), rarely have reproductive and self-maintenance costs been manipulated simultaneously (but see Wright and Cuthill 1990; Ratz and Smiseth 2018). We therefore lack empirical data on how these costs may jointly affect avian physiology. Physiological responses are particularly salient to analyze, as these measures provide insights into the real-time management of stress and changes in the allocation of energy induced by increased reproductive and self-maintenance costs (Ricklefs and Wikelski 2002).

Increased energetic costs, such as those imposed by brood enlargement or handicapping, may trigger physiological responses indicative of a worsening condition, such as mass loss, slowed growth, and the secretion of glucocorticoids (Blas 2015). In birds, the predominant glucocorticoid is corticosterone, which mobilizes the release of glucose into the blood stream and inhibits protein synthesis needed for repair of muscles and growth of feather tissue (Blas 2015). Blood glucose levels are correlated with life history traits at the interspecies level (Tomasek et al. 2019) and are increasingly used as a marker of the stress response in wild avian systems (Lill 2011; Malisch et al. 2018; McGraw et al. 2020; but see Taff et al. 2022). Corticosterone also alters immune function by increasing the circulation of heterophils (white blood cells involved in an emergency response to infection) while decreasing the circulation of lymphocytes (involved in adaptive immunity) (Harmon 1998; Davis et al. 2008). Corticosterone levels in adult birds peak and subside in individually repeatable ways (Jenkins et al. 2014; Vitousek et al. 2017). Unlike corticosterone, the ratio of heterophils to lymphocytes (H:L ratio) remains elevated over time after experiencing an environmental stressor, and is thus a useful measure of a longer-term response to perturbation in wild populations (Goessling et al. 2015; Davis and Maney 2018). For this study, we focused on a broad suite of metrics that are affected by increased somatic costs in different ways: mass, feather growth, blood glucose, and H:L ratio (Blas 2015; Goessling et al. 2015; Malisch et al. 2018). For simplicity, we refer to this group of metrics as physiological responses.

We simultaneously manipulated both reproductive costs (by adding or removing two nestlings in "enlarged" and "reduced" brood size treatments, respectively) and self-maintenance costs (by attaching a 1 g GPS tag or leaving females untagged) in wild female barn swallows (*Hirundo rustica*). Using new, miniaturized GPS technology, we further tracked female movements and tested whether female responses to brood size manipulation varied by movement range size, a potentially important axis of individual variation in energetic costs. Because of their variation in habitat use, the energetic demands of aerial foraging, and their propensity to breed multiple times in a season, barn swallows are an ideal study system to understand how reproductive costs, self-maintenance costs, and range size influence physiological and reproductive outcomes in a short-lived species.

Drawing from the POLS framework, we predicted that barn swallows might favor reproduction over self-maintenance. Specifically, we expected increased costs (tag and/or enlarged brood size) to result in increased metabolic demands (indicated by mass loss and/or slowed feather growth) and a stress response (increased H:L ratio and/or higher blood glucose) but not to affect the number of offspring produced in a breeding season (see Supplementary Material Fig. S1 for graphical predictions). Drawing from theoretical models of the costs and benefits of parental care (Ratz and Smiseth 2018), we predicted that the moderate increase in self-maintenance costs incurred by the 1 g GPS tag (∼5% of total body weight, typically 18–20 g in a breeding female) would not result in interactive effects between tagging and brood size treatments. Finally, we predicted that adjusting for individual variation in range size would result in a trade-off between current and future reproduction, specifically a negative effect of an enlarged first brood on reproductive investment in second clutches. Range size in barn swallows may be somewhat inflexible (Madden et al. 2022), perhaps due to the availability of foraging sites. If birds cannot adjust range size to allocate energy

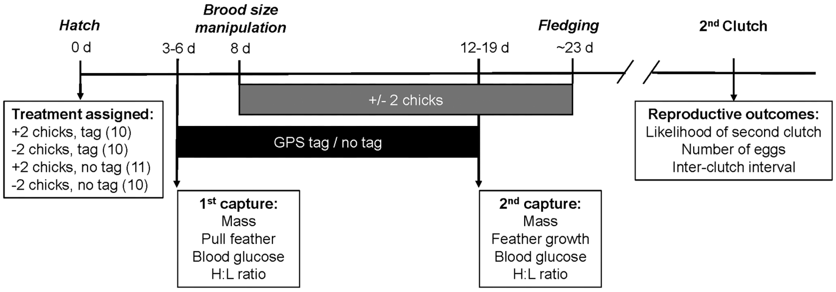

Fig. 1 Experimental design. At hatch, females were randomly assigned to receive a tag or not. Nests with the same hatch date were paired, with one nest randomly assigned to be reduced. Sample sizes at first capture are listed in parentheses for each treatment group. At day 3–6 post-hatch, we captured females, attached a tag (if she was in a tag treatment), and completed our first round of physiological measurements. At day 8 post-hatch, we completed the brood size manipulation. At day 12–19 post-hatch, we recaptured the female, removed her tag if she had one, and completed our second round of physiological measurements. After the fledging of the first brood, we continued monitoring females to document reproductive outcomes in the remainder of the breeding season.

toward tissue maintenance and/or reproduction, then we expect birds with large ranges to lose mass, grow feathers more slowly, and/or to invest less in second clutches.

Methods

Study system

Barn swallows are short-lived (average 2 years), small, migratory passerines who occupy both urban and rural habitats and nest on human-made structures (Brown and Brown 2020). They are aerial insectivores who forage on insects and are loosely colonial. In our study area in Boulder County, CO, United States, birds typically have 1–4 nest attempts of 3–5 eggs each and a maximum of 2 successful nests per breeding season. We monitored 15 colonies in 2019 and 2020 and paired nests by hatch date. We randomly assigned experimental nests to be enlarged or reduced within a pair, and randomly assigned each female to receive a GPS tag or not. See Supplementary Methods for more detail.

Adult captures and physiological measurements

Females were first captured using mist nets or by hand off the nest when nestlings were between 3 and 6 days old (see Fig. 1 for an overview of the experimental design). We collected blood to measure blood glucose and white blood cell counts using blood smears, and marked each bird with a USGS aluminum leg band and a unique combination of plastic color bands. We weighed each bird and collected one inner central tail feather, and attached a GPS tag if a female was to receive one. Handling time was similar between tagged and untagged birds, and the stress of capture was standardized by the waiting period before collecting blood glucose (see Supplementary Methods for more detail on physiological measurements and GPS tagging).

Females were recaptured using the same protocol described above when nestlings were between 12 and 19 days old (Fig. 1), except that we did not attempt capture by hand at the nest when nestlings were older than 14 days to minimize the risk of premature fledging. During the second capture, we again measured blood glucose, prepared blood smears, removed the GPS tag if the bird had received one, and measured body mass. Additionally, we checked the follicle of the inner rectrix previously collected to see if the feather had begun to regrow, and if so, we measured how long the feather had grown.

BSM manipulation

When nestlings in an experimental nest were 8 days old (Fig. 1), nestlings were transferred from the reduced to the enlarged nest in a cloth bag placed in a plastic container with no lid. Note that previous studies have found that foster parents do not discriminate between own and introduced nestlings (Hund et al. 2015; Vitousek et al. 2017). We chose the lightest and heaviest nestling in order to have an unbiased procedure that would work for nests with odd or even numbers of nestlings (Hund et al. 2015). The time nestlings were out of the nest was minimized as much as possible (range = 2–45 min, mean = 26 min).

Statistical analyses

We analyzed the data using R v. 4.1.2 (R Core Team 2021). We built a separate mixed model for each outcome (mass, feather growth, H:L ratio, blood glucose, likelihood of second clutch (yes or no), number of eggs, and inter-clutch interval). Using a multivariate model to simultaneously assess the effects of experimental manipulations on multiple outcomes was not possible as we had few birds for which all data were collected. We used a binomial distribution when the second clutch was the outcome, a Poisson distribution for the number of eggs, and a Gaussian distribution for all other models. In general, we followed the guidance for model fitting and checking outlined by Bolker et al. (2009) and Harrison et al. (2018). We checked model assumptions by inspecting residuals vs. fitted values for each model, each predictor within each model, and QQ-norm plots for all models, and random effect means. We also tested Poisson and binomial models for overdispersion. Outliers were identified using QQ-norm plots and Cook's distance. We set $\alpha = 0.05$ and effects were considered significant if $P < \alpha$. Goodness-of-fit was calculated using marginal (R^2GLMM_m) and conditional (R^2GLMM_c) pseudo-R^2 (Nakagawa and Schielzeth 2013). Across all models, continuous predictors were centered and scaled by their standard deviation to aid in model convergence and comparison of effect sizes, except time, which was already normalized to values between -1 and 1 following the linearization described below. We were not able to collect all measurements on all birds; therefore, sample sizes vary among the models and are reported with the results.

Reproduction vs. self-maintenance

To test whether physiological condition was affected by enlarged brood size and/or tags, we analyzed the combined effects of the tag and brood size treatments on each physiological response variable (within-individual change in mass, within-individual change in H:L ratio, blood glucose, and feather regrowth). We used tag (Y/N) and brood size treatment (enlarged/reduced) as categorical predictors, year (2019/2020) as a covariate, and breeding site as a random intercept. Within-individual change in mass and H:L ratio were calculated by subtracting the first measurement of each individual from the second measurement of that same individual. Prior work on blood glucose levels in birds has shown that body mass and time of day are potentially influential (Lill 2011; Tomasek et al. 2019; Taff et al. 2022), so we included these covariates in the model of blood glucose. Because time of day is a circular variable, we linearized it by taking the sine and cosine of 2π(time/24) and included both variables in the model (Cox 2006). It would not be appropriate to analyze within-individual change in blood glucose without taking into account the effects of body mass and time of day on each measurement, so we analyzed only the second blood glucose measurement rather than within-individual change in blood glucose.

To test whether reproductive investment was affected by enlarged brood size and/or tags, we built models for each reproductive outcome (whether or not females produce a second clutch, number of eggs in the second clutch, and inter-clutch interval) using the same set of predictors as above (tag, brood size treatment, year, and site). To model the likelihood of laying a second clutch, we also included the clutch initiation date of the first clutch as a covariate because birds that start breeding early may be more likely to re-lay regardless of the treatment they received.

Interactive effects of reproductive and self-maintenance costs

We further wished to understand the joint effects of tagging and brood size manipulation on physiological responses and reproductive outcomes. In all models, we tested for an interaction between the tag and brood size treatments using a likelihood ratio test; if the model including the interaction term was not significantly better ($P < 0.05$) than the model without, the interaction term was dropped. All other predictors were retained.

Influence of range size on response to reproductive costs

To test whether birds with large ranges would have worse condition, and whether brood size treatment influences female physiology or reproductive outcomes after adjusting for range size variation, we analyzed the influence of both range size and brood size treatment on each physiological response (mass, H:L ratio, blood glucose, and feather regrowth) and reproductive outcome (whether or not females produce a second brood, number of eggs in the second brood, and inter-clutch interval). First, we prepared the GPS data for analysis (see Supplementary Methods, GPS data). When analyzing range size, we considered the subset of tagged birds only because these are the only birds for which we were able to collect GPS locations. Predictors for this set of models included range size (km^2), brood size treatment (enlarged/reduced), and breeding site as a random intercept. Because this set of models had smaller sample sizes, we endeavored to reduce the number of predictors and therefore did not include year as a covariate in these models. Blood glucose was again modeled with mass and time of day as additional covariates, with time linearized as described above. To model the likelihood of laying a second clutch, we again included the clutch initiation date of the first clutch.

We tested for an interaction between range size and brood size treatment using a likelihood ratio test; if the model including the interaction term was not significantly better than the model without ($P < 0.05$), it was dropped. All other predictors were retained.

Results

Of the 41 birds for which we had collected initial physiological measurements, most were recaptured after 9–10 days (Supplementary Material Table S1), but 4 were never recaptured (3 in the no tag, reduced brood treatment and 1 in the no tag, brood enlarged treatment; Supplementary Material Table S1) despite the fact that they continued to feed nestlings until fledging and no experimental nests were abandoned. One (in the tag, brood-reduced group) was not recaptured until initiating a second clutch of eggs. These birds were excluded from analyses of physiological outcomes, but were included in analyses of reproductive outcomes if we were able to determine whether or not they laid a second clutch of eggs.

Reproduction vs. self-maintenance

Our models of each physiological and reproductive outcome included both tag and brood size treatment as predictors. Brood size treatment did not affect any physiological outcomes (Fig. 2, Supplementary Material Table S5, Supplementary Material Fig. S3), the likelihood of laying a second clutch, or the number of eggs in a second clutch. However, females with enlarged broods did have a significantly longer inter-clutch interval by 2.9 days (1 outlier excluded due to violations of normality and heteroscedasticity, $\beta = 2.89$ days, 95% CI 0.54–5.36, $P = 0.03$, and $n = 29$; Fig. 2, Supplementary Material Fig. S3, Supplementary Material Table S5) compared to females with reduced broods. Tagged birds had significantly elevated H:L ratios (1 outlier excluded due to violations of normality and heteroscedasticity, $\beta = 0.42$, 95% CI 0.08–0.77, and $P = 0.03$, and $n = 35$) but did not significantly differ from untagged birds in mass, feather growth, or blood glucose (Fig. 2, Supplementary Material Table S5, Supplementary Material Fig. S3). Tagged females did not differ from non-tagged females in the likelihood of laying a second clutch or the number of eggs in a second clutch, but had a significantly shorter inter-clutch interval by about 2.5 days (1 outlier excluded, $\beta = -2.55$ days, 95% CI -4.70—0.174, and $P = 0.03$, and $n = 29$; Fig. 2, Supplementary Material Table S5, Supplementary Material Fig. S3). Together with year, tag, and brood size treatments explained much of the variance in the inter-clutch interval ($R^2GLMM_m = 0.428$, Supplementary Material Table S5). We therefore see evidence that increased costs, particularly tags, negatively affect physiological condition but not the number of offspring produced.

Interactive effects of reproductive and self-maintenance costs

There was no evidence of interactive effects between the brood size and tagging treatments. For all physiological and reproductive outcomes, the model without an interaction term was either equivalent to or significantly better than a model with an interaction term (LRT, P values > 0.05).

Influence of range size on response to reproductive costs

Range size was highly variable (median $= 0.5\,km^2$, minimum $= 0.1\,km^2$, maximum $= 2.9\,km^2$, and $n = 18$, Fig. 3), with some females traveling nearly 30 times the area of other females and over larger ranges than had been previously assumed for barn swallows (Kusack et al. 2022). Females with larger ranges had significantly slower feather growth rates ($\beta = -0.14$ mm/day, 95% CI -0.24—0.04, $P = 0.04$, and $n = 10$; Fig. 4; Supplementary Material Table S7) and longer inter-clutch intervals ($\beta = 1.9$ days, 95% CI 0.72–3.23, $P = 0.04$, and $n = 11$; Fig. 4; Supplementary Material Table S7), but tended to lose less mass than females with small ranges across brood size treatments, although this trend was not significant ($\beta = 0.30$ g, 95% CI 0.01–0.60, $P = 0.07$, and $n = 18$; Fig. 4, Supplementary Material Table S7, Supplementary Material Fig. S4). After adjusting for range size, females with enlarged broods tended to grow their feathers more slowly than females with reduced broods, although this trend was not significant ($\beta = -0.25$ mm, 95% CI -0.47—0.03, $P = 0.08$, and $n = 10$, Fig. 4, Supplementary Material Table S7). Consistent with our analyses of all birds together, when analyzing the effect of brood size treatment and range size, tagged females with enlarged broods had longer inter-clutch intervals relative to females with reduced broods ($\beta = 3.03$ days, 95% CI 0.85–5.48, $P = 0.04$, and $n = 11$; Fig. 4, Supplementary Material Table S7, Supplementary Material Fig. S5). Range size and brood treatment did have interactive effects on inter-clutch interval ($\beta = -6.13$ days, 95% CI -10.23—2.02, $P = 0.02$, and $n = 11$; Fig. 4G, Supplementary Material Table S7), such that in females with reduced broods, range size and inter-clutch interval were positively correlated, but in females with enlarged broods, range size and inter-clutch interval were negatively correlated (Fig. 4G). Together, these variables explained a substantial amount of variation in the inter-clutch interval ($R^2GLMM_m = 0.646$). We therefore see evidence that range size is related to physiological condition (specifically feather growth). After adjusting for

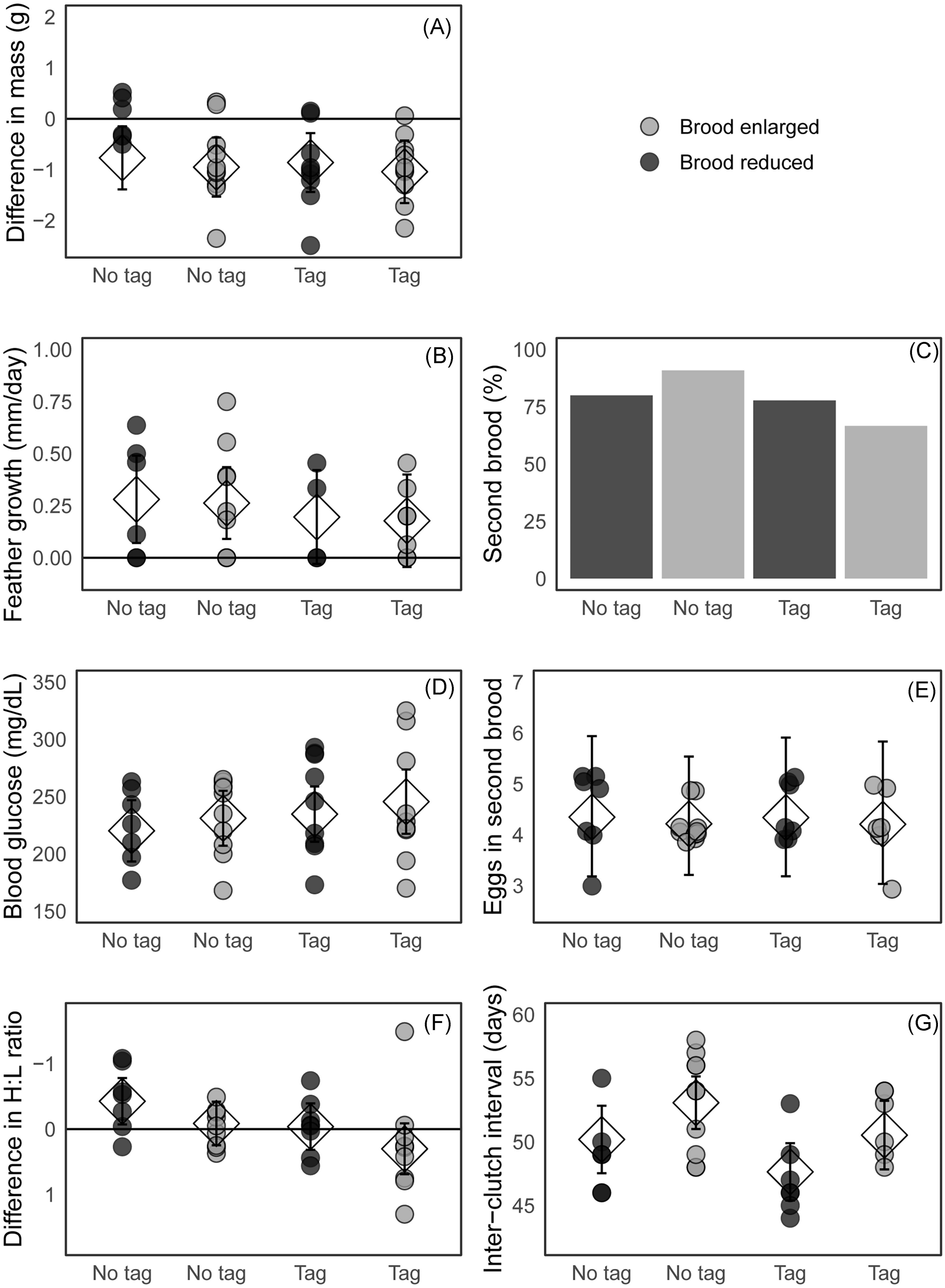

Fig. 2 The effect of tag and brood size treatments on physiological responses and reproductive outcomes. Each circle represents a single bird. Females with enlarged broods are shown in light gray, females with reduced broods are shown in dark gray. Unfilled diamonds represent the estimated marginal mean for each group as predicted by the linear models presented in Supplementary Table S5, and error bars are the 95% confidence interval around the estimated mean. Points in panel (E) are jittered to better show overlapping datapoints.

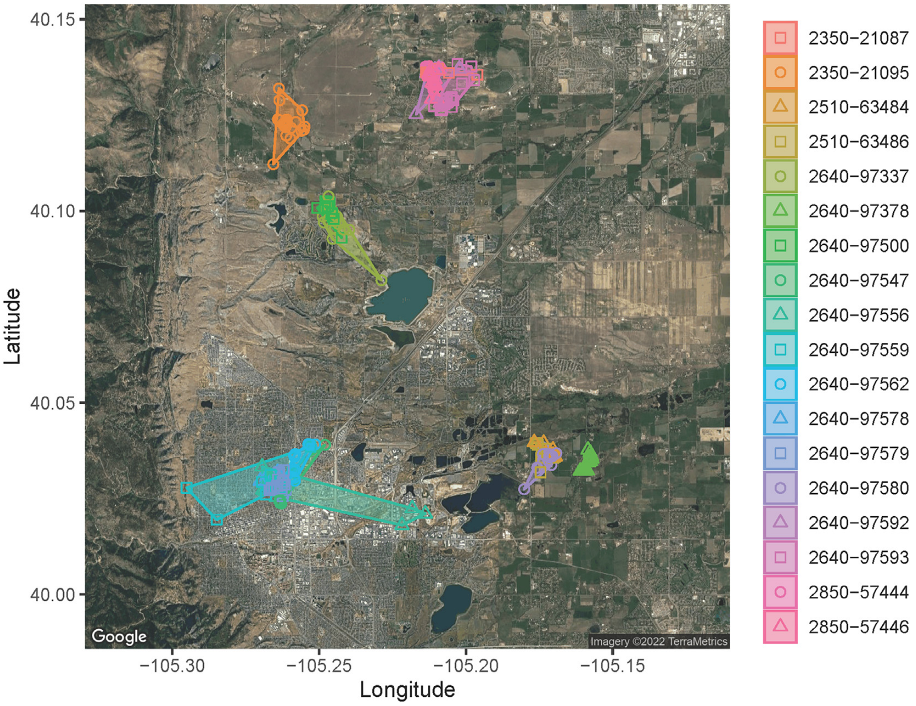

Fig. 3 Range sizes of tagged females used in the analysis of physiological outcomes ($n = 18$). Symbols show the GPS fixes collected for each female over nestling ages 9–10. Polygons show the minimum convex polygon fitted to the GPS fixes. Each color-symbol combination represents a different bird.

individual variation in range size, brood size treatment affected the inter-clutch interval but not other physiological or reproductive outcomes.

Discussion

We manipulated both reproductive costs (by adding or removing two nestlings from the nest) and self-maintenance costs (by attaching a 1g GPS tag or leaving females untagged) in wild female barn swallows (Fig. 1). Additionally, in tagged females, we tested the influence of range size on physiological and reproductive responses to brood size treatment. We found partial support for a trade-off between self-maintenance and reproduction favoring reproduction—there was no evidence that fitness (likelihood of a second clutch or the number of eggs) differed among treatment groups, but tagged females had elevated H:L ratios, providing evidence of a stress response (Fig. 2). There was no evidence of joint effects of tagging and brood size manipulation—in other words, the female response to brood size manipulation did not depend on whether she was tagged and vice versa. Range size seemed to be somewhat influential on female condition, in that females with large ranges grew feathers more slowly. However, when adjusting for range size, we found no evidence of a physiological response or change in the number of eggs produced after brood size manipulation (Supplementary Material Table S7). An important limitation of our study is that the ages of our subjects were unknown, and age is known to affect allocation among life history traits in general (Bradley and Safran 2014) and in barn swallows (Bradley et al. 2014). However, we did randomize our treatments, and therefore ages were randomly distributed among treatment groups.

Range size, a potentially important axis of individual variation in energetic costs, did not vary with brood size treatment. Range size was highly variable individually, with a 30-fold difference between the smallest and largest ranges we observed. Many of our study birds traveled over much larger distances than had previously been assumed (Kusack et al. 2022). Birds with large ranges grew feathers more slowly, but otherwise did not seem to be in worse condition. In fact, there was weak evidence that birds with large ranges lose less mass compared to birds with small ranges. Flight costs should be higher when birds have more mass and/or lower wing and tail area (Carrascal and Polo 2006), and thus birds with large ranges would benefit from compensating for range size by maintaining a lower body mass and putting more energy into feather growth (Senar et al. 2002), but we did not observe a pattern consistent with this type of compensation for flight costs. Without this compensation, maintaining a large range would likely be energetically demanding. In a related experiment on barn swallows within our population, range

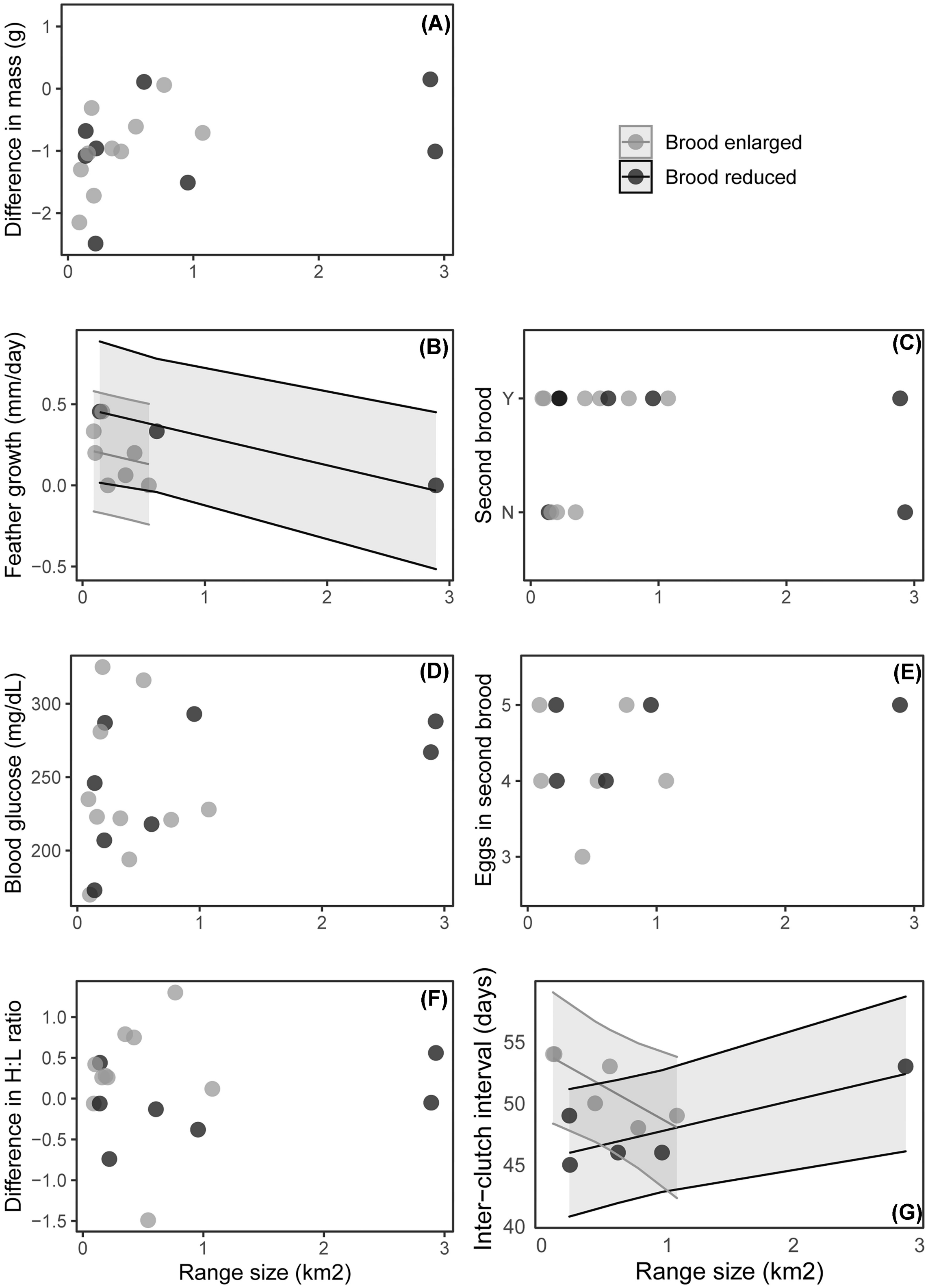

Fig. 4 The effect of range size and brood size treatment on physiological responses and reproductive outcomes. Each circle represents a single bird. Females with enlarged broods are shown in light gray, females with reduced broods are shown in dark gray. Regression lines and 95% prediction intervals shown for significant effect sizes only from the models in Supplementary Table S7.

size appeared to be somewhat inflexible—birds with more chicks visited their nests more often, but maintained their range size (Madden et al. 2022), raising the possibility that birds with large ranges have to work harder to provide the same level of parental care. It is possible that the additional resources provided in a larger range would offset the energetic costs of maintaining a large range size. The movement patterns of birds in our study suggest that they frequent profitable foraging areas such as ponds and agricultural fields (Fig. 3), and because these locations are at a fixed distance from their nest, it is not possible to find enough food without maintaining a certain range size.

We found that female barn swallows, when faced with increased reproductive and self-maintenance costs, still invest equally in reproduction but may trade this investment off with immune function. A related study in our population found that brood size manipulations influenced nestling outcomes, with parents of enlarged broods visiting more often but nestlings having a lower growth rate compared to reduced nests (Madden et al. 2022). Tagging influenced the visitation rates of males but not females and had no effect on nestling growth (Madden et al. 2022). In the present study, females with increased costs had elevated H:L ratios, which is an alteration of circulating white blood cells that is a downstream effect of corticosterone release (Harmon 1998; Davis et al. 2008). An elevated H:L ratio is indicative of an emergency life history strategy, where the circulation of phagocytotic cells increases to mount a broad response to possible infection, but the circulation of cells involved in adaptive immunity (i.e., the production of pathogen-specific antibodies) decreases (Goessling et al. 2015). This immunological profile may help females stave off emerging infections, such as bacterial infections caused by injury, but leaves them less able to produce antibodies that would target specific infectious agents and create future immunity. Our experiment took place over a single breeding season for each female, so we were not able to assess how our manipulations affected survival or reproduction in future breeding seasons. However, barn swallows in our population live on average 2 years, so one breeding season captures a substantial portion of lifetime reproductive potential. The responsiveness of H:L ratios we observed is consistent with results from other studies indicating that immune system traits may be important mediators of life history variation in birds (Norris 2000; Ardia 2005; Goessling et al. 2015; Wegmann et al. 2015b) and other vertebrates (Zera and Harshman 2001; Lee 2006; Davis and Maney 2018).

As predicted, and in agreement with the results from the few other empirical studies (Ratz and Smiseth 2018), we did not observe interactive effects of self-maintenance and reproductive costs on any of the outcomes we measured. This may be expected if self-maintenance costs are increased modestly (Ratz and Smiseth 2018), and in our study, the tags that female birds carried were ∼5% of their body weight (the generally recommended maximum for attaching tags to small birds), and carried for an average of 10 days. Both tagging and brood enlargement increase costs, but there may be important differences between these treatments. Tagging increases both reproductive and self-maintenance costs by increasing the weight a female must carry while foraging for herself and while provisioning her offspring. In contrast, brood enlargement only directly increases the costs of providing parental care (although there may be indirect effects on self-maintenance, for example, by reducing the amount of time available for self-feeding). In our experiment, there were also differences in the duration of these treatments. Because birds were tagged on average 3 days prior to the brood size manipulation, the second round of physiological measurements was conducted when females had been tagged for longer than their brood size had been manipulated. This might explain why tagging significantly elevated H:L ratios while the effect size of brood size manipulation was similar but not significant. The effect sizes of tagging and brood size manipulation were remarkably similar on all metrics of female physiology (Supplementary Material Fig. S3). In contrast, when we measured females' second brood attempts, they had at that point spent more time caring for additional (or fewer) nestlings than they had while being tagged. We might therefore expect a stronger effect of brood size manipulation compared to tagging on reproductive outcomes, but the effect sizes of the two treatments remained similar except for clutch timing.

Inter-clutch interval was the most flexible reproductive outcome, with females with enlarged broods taking longer to re-lay and tagged females taking less time to re-lay. Although we did not observe direct effects of tagging or brood size on our measures of fitness (likelihood of a second clutch or the number of eggs), it is possible that clutch timing could have indirect effects on fitness that we did not measure here, such as affecting food availability for offspring in second broods (Shipley et al. 2020). Flexibility in breeding timing may have allowed females to adjust recovery time in response to the energetic demands of increased brood size or tags. It appears that birds with enlarged broods or larger range sizes needed more time to recover, but once they did recover, they were able to produce as many offspring as birds who were not as energetically taxed. Under this explanation, however, we would assume that tagged birds would show a similar pattern. In contrast, tagged birds took less time to initiate a second clutch. It is unclear why the increased costs imposed by tagging and enlarged brood size would induce opposite responses in clutch timing, but perhaps the increased self-maintenance costs imposed by tagging induced a pattern of terminal investment, in which females assess their own survival probability to be low, and therefore increase their investment in reproduction (Ardia 2005; Bowers et al. 2015).

Here we present results from an experimental test of the simultaneous effects of tagging and brood size manipulation—to our knowledge, the first such study to measure physiological responses in a wild vertebrate. We made use of new, miniaturized tracking technology to study how range size, a potentially important source of individual variation in energy expenditure, may affect investment in reproduction. Overall, our results are consistent with the idea that short-lived vertebrates do face a trade-off between reproduction and self-maintenance and, when faced with increased costs, tend to preserve investment in reproduction at the expense of parental condition. Furthermore, we provide evidence to support the importance of immune system traits in life history studies (Norris 2000; Lee 2006). Range size was incredibly variable and was negatively related to feather growth, but controlling for this variation did not result in a predicted trade-off between current and future reproduction. Future research into other axes of individual variation, particularly those related to immune functioning, will be valuable.

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Supplementary data

Supplementary data available at *ICB* online.

Conflict of interest

The authors declare no conflict of interest.

Data availability

GPS data are available via MoveBank [DOI 10.5441/001/1.8c9b82qk]. Other data and R code underlying this manuscript are available via Figshare [DOI 10.6084/m9.figshare.21968714].

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