

Success despite the stress: violet-green swallows increase glucocorticoids and maintain reproductive output despite experimental increases in flight costs

James W. Rivers^{*,1}, Gretchen N. Newberry^{†,2}, Carl J. Schwarz³ and Daniel R. Ardia⁴

¹Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA; ²Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA; ³Department of Statistics and Actuarial Science, Simon Fraser University, Burnaby, BC V5A 1S6, Canada; and ⁴Department of Biology, Franklin and Marshall College, Lancaster, PA 17604, USA

Summary

1. Glucocorticoid steroid hormones play a central role in regulating the metabolic state of animals, especially when they cope with unanticipated stressors in their environment. The cort-adaptation hypothesis predicts that baseline concentrations of glucocorticoids are adjusted upward to match energetic needs and promote fitness when individuals are faced with physiological challenges, including those associated with reproduction.

2. We tested the cort-adaptation hypothesis in the violet-green swallow (*Tachycineta thalassina*) by experimentally increasing flight costs during the offspring rearing period. Individual females were assigned to one of three treatments: light feather clipping (removal of four wing feathers), heavy feather clipping (removal of eight wing feathers) or a sham-clip control.

3. We measured baseline corticosterone and body mass immediately prior to implementing treatments during the offspring rearing period and then 10 days after initial manipulations took place. We also quantified risk-taking behaviour, offspring feeding rate and the number of offspring fledged. Finally, we examined how treatments influenced offspring phenotype via measurements of nestling body mass and baseline corticosterone, as both measures have been associated with post-fledging survival.

4. We found that handicapped females significantly increased baseline corticosterone between the two sampling periods, with the magnitude of change in the light clipping and heavy clipping treatments 2.5× and 6.1× greater than controls, respectively. All individuals lost mass between the two sampling periods, but the degree of loss was greater for females in both clipping treatments relative to unmanipulated controls.

5. In contrast, we found no evidence of treatment differences in female risk-taking, offspring provisioning or in the number of offspring fledged. Offspring raised by females in both handicapped treatments did have significantly elevated baseline corticosterone relative to those in control broods, but we detected no treatment differences in offspring body mass.

6. Our study found that handicapped females increased circulating glucocorticoids and were able to maintain critical parental care behaviours and raise a similar number of offspring as unmanipulated controls. Thus, increases in baseline cort of handicapped females appeared to have allowed them to maintain fitness despite increased physiological challenges, providing support for the cort-adaptation hypothesis.

Key-words: cort-adaptation hypothesis, corticosterone, feather clipping, glucocorticoids, *Tachycineta thalassina*, violet-green swallow

Introduction

Understanding how organisms respond to environmental change remains an important topic in the fields of

*Correspondence author. E-mail: jim.rivers@oregonstate.edu

[†]Present address. Department of Biology, University of South Dakota, Vermillion, South Dakota 57069, USA.

physiological and behavioural ecology (Wingfield *et al.* 1998, 2011; Nemeth, Bonier & MacDougall-Shackleton 2013). Glucocorticoid steroid hormones (i.e. corticosterone and cortisol; hereafter 'cort') play a key role in regulating metabolism and energy balance (Sapolsky, Romero & Munck 2000) and are therefore essential for understanding how organisms respond to acute and chronic stressors. Cort fluctuates adaptively on circadian and seasonal scales in a manner that facilitates undertaking activities that require additional energetic demands, such as long-distance migration or offspring rearing (McEwen & Wingfield 2003; Landys, Ramenofsky & Wingfield 2006; Romero, Dickens & Cyr 2009). Additionally, cort serves as a modulator of physiological and behavioural processes that occur when individuals encounter acute, unexpected stressors in the environment and face an emergency life-history stage (*sensu* Wingfield *et al.* 1998). For example, cort concentrations increase in response to short-term, unexpected stressors (e.g. storms, predator attacks) to promote survival during the period of interaction with the stressor(s) before returning to typical levels after the threat has subsided (Romero, Reed & Wingfield 2000; Romero 2004). Thus, cort serves to regulate energetic responses of organisms during regular activities, as well as during periods when individuals respond to unpredictable environmental stressors (Sapolsky, Romero & Munck 2000; Romero 2004; Landys, Ramenofsky & Wingfield 2006).

Many investigations examining organism health have focused on the relationship between cort and fitness and invoked the traditional assumption that elevated baseline cort concentrations are indicative of individuals in poor condition and of reduced fitness (i.e. the cort-fitness hypothesis; McEwen & Wingfield 2003; Walker, Boersma & Wingfield 2005; Bonier *et al.* 2009). Although many of these investigations have found support for the cort-fitness hypothesis, there remains a sizable number of studies whose results do not fit the expected relationship for this hypothesis (Bonier *et al.* 2009). In turn, this has led to a recognition that the link between baseline cort and fitness is more complex than initially thought. The cort-adaptation hypothesis has been put forth as a reformulation of the cort-fitness hypothesis and posits that increased energetic costs that arise from environmental challenges can lead to increases in baseline cort that ultimately enhance fitness (Bonier *et al.* 2009; Bonier, Moore & Robertson 2011). Testing the cort-adaptation hypothesis is essential for understanding how organisms adjust cort concentrations to cope with the increased energetic demands during stressful periods, including during reproduction, yet nearly all studies undertaken on this topic are observational in nature (Ebensperger *et al.* 2011, 2013; Ouyang *et al.* 2011a; Escribano-Avila *et al.* 2013). A notable exception was undertaken by Bonier, Moore & Robertson (2011), who altered brood sizes in the tree swallow (*Tachycineta bicolor*) during the offspring rearing period to manipulate the energetic costs of reproduction. Relative to female swallows with unmanipulated broods, female swallows

subjected to brood enlargement significantly increased baseline cort concentrations after their broods were altered and those increases in baseline cort were positively linked to increased feeding rates and reproductive success, providing support for the cort-adaptation hypothesis (Bonier, Moore & Robertson 2011). However, it is unclear whether organisms generally respond to increased energetic costs by increasing baseline cort and whether such increases promote the maintenance of fitness. Thus, additional studies are needed that test the generality of this hypothesis, including those that use experimental approaches to alter parental condition during the period of reproduction.

In this study, we tested a key prediction of the cort-adaptation hypothesis: whether breeding individuals respond to experimentally increased energetic costs by elevating baseline cort concentration; in turn, we also tested whether increased cort concentrations resulted in changes in reproductive output relative to unmanipulated individuals (Bonier *et al.* 2009; Bonier, Moore & Robertson 2011). We tested this prediction in the violet-green swallow (*T. thalassina*), a close relative to the tree swallow, during the offspring rearing period. However, to more directly affect workload and minimize the effect of brood size alternations on offspring phenotype, we experimentally removed primary flight feathers at two different intensities (i.e. 4-feather removal, 8-feather removal) to increase flight costs to females. Removal of flight feathers is a widely used technique that increases wing loading (Pennycuik 1989) and, in turn, elevates the energetic costs of flight (Slagsvold & Dale 1996; Love & Williams 2008a; Harding *et al.* 2009; Leclaire *et al.* 2011; Cantarero *et al.* 2014). In *Tachycineta* swallows, feather removal has been shown to reduce adult body condition, offspring feeding rates and reproductive output (Winkler & Allen 1995; Noor, Dunn & Whittingham 2005; Ardia & Clotfelter 2007), making it an ideal technique to invoke changes in baseline cort during breeding. Moreover, feather removal provides an especially strong handicap for species that are restricted to flight as their only locomotory behaviour and obtain all of their food from the air column via powered flight (Winkler & Allen 1995), such as the violet-green swallow.

The cort-adaptation hypothesis posits that individuals increase baseline cort in response to energetic challenges in a way that promotes fitness (Bonier *et al.* 2009; Bonier, Moore & Robertson 2011). Thus, our experimental design allowed us to assess the effects of feather-clipping treatments on physiological and behavioural measures during the period of offspring rearing. We therefore predicted that females subjected to feather-clipping treatments would have elevated mean baseline cort relative to unmanipulated females and that individuals subjected to the heavy clipping treatment (eight primary feathers) would have higher mean baseline cort than those subjected to the light clipping treatment (four primary feathers). In addition, we predicted that handicapped females would experience a post-treatment decrease in body mass such that mass loss in females would be lowest in the control group,

intermediate in the light clipping treatment and highest in the heavy clipping treatment, following previous studies (Winkler & Allen 1995; Nooker, Dunn & Whittingham 2005; Ardia & Clotfelter 2007). Despite these expected changes in cort and body mass, however, we predicted that increases in baseline cort of handicapped females would mobilize energy in a way that allowed females to maintain reproductive output and produce a similar number of offspring as unmanipulated females.

The cort-adaptation hypothesis assumes that breeding individuals make behavioural trade-offs to maintain short-term reproductive output. Thus, females faced with increased energetic expenditure from handicapping treatments may be expected to reduce other energetically expensive behaviours such as risk-taking (Mathot *et al.* 2015), which can be assayed by the propensity to attend offspring in the presence of an object of unknown potential harm (e.g. Mettke-Hofmann, Winkler & Leisler 2002; Martin & Reale 2008; Lendvai, Bokony & Chastel 2011; Ensminger & Westneat 2012). Thus, we predicted that the propensity of females to enter nest boxes and feed offspring in the presence of a novel object would be inversely linked to the degree of handicapping (Love & Williams 2008a; Patterson, Winkler & Breuner 2011). Finally, if the cort-adaptation hypothesis is supported, handicapped females that experience increased energetic challenges are expected to maintain per capita offspring provisioning rates and produce young that are phenotypically similar to those of unmanipulated females. Therefore, we assessed the downstream consequences of female handicapping on offspring phenotype by quantifying body mass and baseline cort. Both of these measures serve as indices of offspring quality, as post-fledging survival has been shown to be positively associated with body mass (Magrath 1991; Verboven & Visser 1998; Naef-Daenzer, Widmer & Nuber 2001) and baseline cort (Rivers *et al.* 2012). Given that females were expected to increase baseline cort to maintain reproductive output, we predicted that there would be no difference in per capita provisioning rates between females in the three treatments,

nor differences in offspring body mass or offspring baseline cort. Taken in its entirety, our study provides a critical test of the cort-adaptation hypothesis while assessing additional fitness-related measures that may be impacted by experimental-induced increases in flight costs.

Materials and methods

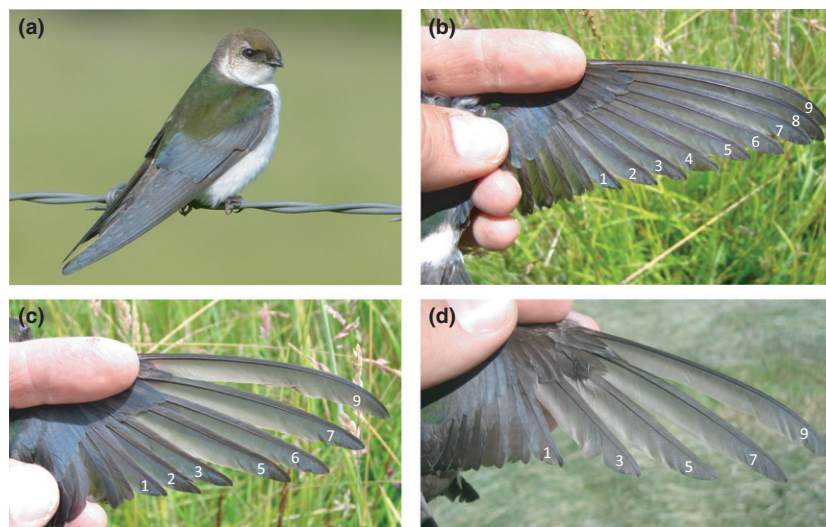
STUDY AREA AND SPECIES

We conducted our study during the 2010–2012 breeding seasons in a box-nesting population of the violet-green swallow (hereafter swallow; Fig. 1a) in the vicinity of Corvallis, Oregon (44°35'N, 123°15'W). Our study area comprised 250–320 nest boxes that were located at five sites within ~13 km of each other and functioned as a single population, as a small proportion of individuals switched between study sites in successive years (J.W., Rivers, unpublished data). Swallows typically arrived on study sites in early spring but did not initiate nesting until the latter half of May, with the first egg laid 23–26 May in each year of this study. We regularly checked boxes every 2–3 days early in the breeding season to establish the date of the first egg laid within each box (i.e. nest initiation date) and to determine final clutch size. After incubation commenced, we reduced the frequency of nest visits until the expected time of hatching to determine hatching date; we also regularly visited nests around the expected time of fledging to quantify the timing and number of offspring that fledged from each nest. All experimental procedures described in this study were approved by appropriate institutional, state and federal authorities.

EXPERIMENTAL FEATHER-CLIPPING TREATMENTS

At each nest on the day of initial capture (i.e. nestling day 3, where day 0 is the day of hatching), we randomly assigned the female to one of three feather-clipping treatments. Females in the control group were handled similarly to those in the clipping treatments except they were sham-clipped such that no feathers were removed (Fig. 1b). Females in the light clipping treatment had a total of four flight feathers removed (primary feathers #4 and 8 on each wing; Fig. 1c) whereas females in the heavy clipping treatment had a total of eight flight feathers removed (i.e. primary feathers #2, 4, 6 and 8 on each wing; Fig. 1d). We used a sharp pair of dissecting scissors to trim feathers at their base, resulting in complete removal of targeted feathers.

Fig. 1. This study investigated the degree to which glucocorticoid steroid hormones mediated responses to handicapping treatments in (a) female violet-green swallows that were subjected to experimentally increased flight costs. This species has nine primary feathers on each wing, and each female was assigned to one of three treatments: (b) control treatment, where no primary flight feathers were removed, (c) light feather-clipping treatment, where two flight feathers were removed from each wing (primaries #4 and #8), or (d) heavy feather-clipping treatment, where four flight feathers were removed from each wing (primaries #2, #4, #6 and #8). Numbers in white within panels (b–d) indicate primary feathers that were retained after treatments were applied.



We attempted to balance sample sizes across treatments and nest initiation date by using a blocking approach. Initially, we randomly assigned one female to each treatment for each group of three females captured in succession. However, nest abandonment occurred at a greater rate in the heavy clipping treatment so we modified our approach by assigning a lower proportion of nests to the heavy treatment. Our initial sample size was 57 individuals for all treatments and years combined (control, $N = 18$; 4-feather clip, $N = 26$; 8-feather clip, $N = 13$), and some females within each treatment group abandoned their nest shortly after release on the day of initial capture. Our goal was to assess how cort changed in response to clipping treatments so we excluded data from all females that abandoned, which reduced our sample size to a total of 28 nests (control, $N = 13$; 4-feather clip, $N = 12$; 8-feather clip, $N = 3$). Given the potential link between nest abandonment and individual condition (Love *et al.* 2004; Ouyang, Quetting & Hau 2011b), we explore elsewhere how cort and other physiological measures taken at the time of capture may have influenced the propensity for brood desertion (J.W. Rivers & D.R. Ardia, unpublished data).

PHYSIOLOGICAL MEASUREMENTS TAKEN DURING CAPTURE

A primary goal of our study was to evaluate how feather-clipping treatments influenced individual physiology and behaviour, which required us to measure females at two distinct points in time: immediately prior to the implementation of experimental clipping treatments (nestling day 3) and 10 days after treatments were implemented (nestling day 13). We trapped individual females by either placing a hand over the nest box entrance, using a trap door that was placed inside the nest box or using a permanent on-trap nest (<http://golondrinas.cornell.edu/>). We detected no significant differences in mean baseline cort among the three trapping approaches ($F_{2,35} = 2.4$, $P = 0.106$), so we did not consider trapping method further in our analysis. The timing of capture and bleeding occurred between 0650 and 1420 local time and varied non-systematically among treatments, so any variation in cort caused by circadian rhythms would have introduced random noise across all treatments rather than systematic bias. Nevertheless, we found no evidence for an association between the time of day when bleeding occurred and baseline cort for either pre-treatment ($F_{1,15} = 0.2$, $P = 0.700$) or post-treatment measures ($F_{1,19} = 1.4$, $P = 0.250$). Within 3 min of capture, which represents pre-stress cort concentrations (Romero & Reed 2005), we took a small blood sample (~75 μ L) by piercing the brachial vein on the wing with a sterile needle and collecting blood into a heparinized microcapillary tube. This sample was then transferred to a centrifuge tube and immediately placed on ice. We took additional measures for a separate study that evaluated how physiological measurements were associated with brood desertion (J.W. Rivers & D.R. Ardia, unpublished data), but we note that all females in all treatments in this study were subjected to the same measurements. We then measured body mass with an electronic scale (± 0.01 g) and then banded the bird with a uniquely numbered USGS aluminium leg band. Finally, we colour-marked each female with a small amount of non-toxic paint in a unique combination on the leading edge of each wing to facilitate identification during offspring feeding visits. Individual handling time averaged 43.5 min (95% CI: 41.5–45.5), and we found no significant difference in mean handling time among females in the three treatments ($F_{2,43} = 2.6$, $P = 0.086$).

MEASURES OF RISK-TAKING, PARENTAL CARE, OFFSPRING PHENOTYPE AND REPRODUCTIVE OUTPUT

To assess how risk-taking behaviour varied among females that were subjected to handicapping treatments, we conducted a novel

object test during the morning (0700–1200 local time) of nestling day 6. In our population, female swallows reduced offspring brooding markedly by this stage of the nesting cycle and typically entered nest boxes with food as soon as they returned to the nest site (Garlick, Newberry & Rivers 2014); thus, we assumed that females returning to their nest box during novel object trials were returning to feed their offspring and were similarly motivated to enter the nest box. We initiated novel object tests by remotely monitoring nest boxes from >25 m until the colour-marked female left the nest on her own accord. Once the female left the vicinity of the nest box, we placed a yellow toy rubber duck on top of the nest box before returning to our original hiding location; the duck was centred over the entrance hole and sat approximately 8 cm from the front edge of the roof of the box. Because females were marked with a unique colour combination of non-toxic paints, we were able to determine when each female returned to her nest box area, which we defined as a 4-m sphere centred on the nest box. Once the focal female entered the nest box area, we recorded the amount of time that elapsed before she completely entered the nest box to feed young. We ended the trial when the female entered the box or 30 min after the female entered the nest box area, whichever came first. For analysis, we evaluated the proportion of females that entered the nest boxes to feed within the 30-min limit.

We also assessed how per capita feeding by females varied relative to feather-clipping treatments by recording feeding visits with digital camcorders. Swallows typically have a bolus of food held within the mouth when they enter nest boxes (Garlick, Newberry & Rivers 2014), making it impossible to estimate food load size through visual observations. Therefore, we used the number of feeding visits divided by the number of young in the nest as an estimate of per capita feeding rate. Detailed study of adult provisioning in the closely related tree swallow concluded that the number of visits to nests served as an overall measure of food delivery (McCarty 2002), so we assumed that this was the case in our study. To collect feeding data, we recorded adults provisioning offspring for 1.5–2.0 h between 0700 and 1030 on the morning of nestling day 8 (with one exception, where filming starting at 1315). We assessed both female and male feeding rates, as males may have compensated for reductions in feeding by their mates and such responses have the potential to mask the effects of the female handicapping treatments on offspring phenotype. Females could be readily distinguished from males on videos because of their distinct plumage and because they were individually marked with acrylic paints; indeed, we were able to identify the sex of adults entering nest boxes on >99.6% of >3500 feeding visits.

To assess the consequences of feather-clipping treatments on offspring phenotype, we took two developmental measures of nestlings that are linked to post-fledging survival in songbirds (Magrath 1991; Verboven & Visser 1998; Naef-Daenzer, Widmer & Nuber 2001; Rivers *et al.* 2012). First, we measured offspring baseline cort on nestling day 12 as described above by arbitrarily sampling as many nestlings as possible in each brood (range: 1–3 nestlings/nest) within 3 min of initial contact to obtain pre-stress cort concentrations (Romero & Reed 2005). At the same time, we also quantified offspring body mass. Finally, we assessed the number of young that fledged from each nest as a measure of offspring production.

CORTICOSTERONE ASSAYS

Blood samples were kept on ice for up to 8 h before being centrifuged to separate plasma. Plasma samples were then frozen at -80°C until cort assays were conducted. To assay corticosterone, we used two commercially available enzyme immunoassay (EIA) kits: Cayman Chemical Company, Ann Arbor, MI, USA, item #500655, $n = 10$ kits; and Arbor Assays, Ann Arbor, MI, USA, item K014-H5, $n = 3$ kits. Samples from different treatments were

randomized across plates so any variation in cort measures caused by variation in plates would have introduced random noise across all treatments rather than systematic bias. Nevertheless, we detected no difference in mean or coefficient of variation (CV) values for cort among different kit types (D.R. Ardia & J.W. Rivers, unpublished data).

Initially, we extracted samples in methylene chloride followed by drying under a gentle stream of nitrogen and then reconstituted samples in assay buffer. We assayed samples in duplicate on a plate that included both positive and negative controls, as well as a standard curve using the manufacturers' recommendations. The assay detection limit for the Cayman kit was 30 pg mL⁻¹ and for Arbor Assays kit was 16.9 pg mL⁻¹; no sample concentration fell below these limits. We randomly allocated samples among plates and average intra- and inter-plate variation was 6.7% and 2.3%, respectively; we used assay kit concentration standards as our standards, which ranged from 19.5 pg mL⁻¹ to 10 000 pg mL⁻¹.

STATISTICAL ANALYSIS

We used a mixed linear modelling approach in the SAS (v9.4) (SAS Institute Inc., Cary, NC, USA) statistical environment. To assess cort in females, we modelled handicapping treatment (three levels: control, light clipping, heavy clipping), capture period [two levels: pre-treatment (nestling day 3), post-treatment (nestling day 13)] and a treatment × capture period interaction as fixed effects, with nest initiation date and **pre-treatment body mass as covariates**. We did not include a year effect in our model because of modest sample sizes; in addition, we did not include female age as a covariate because female swallows cannot be aged via physical characteristics (Brown, Knott & Damrose 2011). We used a similar approach when testing how treatments influenced per capita feeding rate, offspring baseline cort, offspring body mass and the number of young produced. For offspring baseline cort and body mass, however, we first calculated a brood-level mean because of the non-independent nature of young that are raised together within a nest. Additionally, we also used brood size as a covariate for these measures because offspring baseline cort and body mass may be influenced by brood size.

We used a chi-square test to evaluate the proportion of females in each treatment that entered nest boxes with a novel object present; we did not model time to enter because there were so few females that entered nest boxes during novel object trials (see Results). We used the Kenward–Rogers method to calculate degrees of freedom for contrasts and estimates, and we used a Tukey–Kramer adjustment for all multiple treatment comparisons. Because of the unbalanced nature of our data, we report least-squares marginal means (lsmeans) for effect sizes and their 95% confidence intervals (CIs) which take into account the mean of each covariate within the model; significance levels for all tests were set at $P < 0.05$. Additionally, we report effect sizes (β) and their 95% CIs from the full model (i.e. adjusted for covariates) for (i) the estimated change in mean values between pre- and post-treatment capture measurements for female baseline cort and female body mass, and (ii) the estimated change in mean values between treatments for female provisioning rate, offspring body mass, offspring baseline cort and the number of young produced.

Results

We detected no significant differences among females in the three treatment groups with respect to mean body mass ($F_{2,20} = 2.83$, $P = 0.083$), mean baseline cort ($F_{2,14} = 1.81$, $P = 0.199$), mean clutch size ($F_{2,21} = 0.61$, $P = 0.555$) or mean brood size ($F_{2,21} = 2.54$, $P = 0.103$) when they were measured prior to feather clipping.

PHYSIOLOGICAL RESPONSE TO HANDICAPPING TREATMENTS

Overall, females subjected to feather-clipping treatments were found to have increased baseline cort compared to controls after treatments were implemented (Fig. 2a). We detected a significant main effect of handicapping treatment ($F_{2,25} = 13.3$, $P = 0.001$), capture period ($F_{1,25} = 129.0$, $P < 0.001$) and a treatment × capture period interaction ($F_{2,25} = 22.8$, $P < 0.001$). Pre-treatment mean baseline cort concentrations of control females [2.67 ng mL⁻¹ (95% CI: 1.55, 3.80)] were not statistically different from those of females in the light clipping [2.69 ng mL⁻¹ (95% CI: 1.65, 3.73); $t_{1,25} = 0.0$, $P = 1.000$] and heavy clipping treatments [1.97 ng mL⁻¹ (95% CI: -0.16, 4.09); $t_{1,25} = 0.6$, $P = 0.990$]. In contrast, post-treatment baseline cort concentrations in control females [4.65 ng mL⁻¹ (95% CI: 3.46, 5.84)] were significantly lower than females in both the light clipped [7.54 ng mL⁻¹ (95% CI: 6.45, 8.64); $t_{1,25} = 3.7$, $P = 0.012$] and heavy clipped treatments [14.07 ng mL⁻¹ (95% CI: 12.24, 15.90);

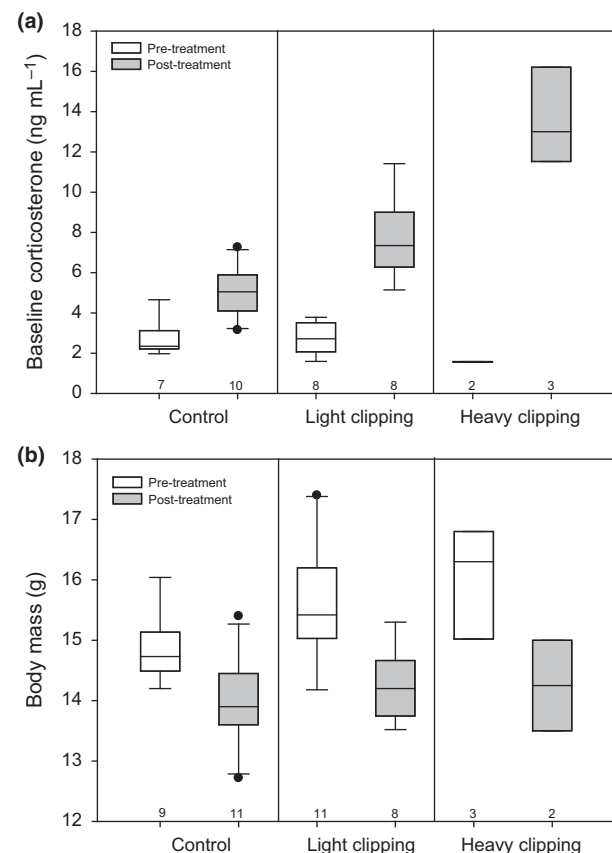


Fig. 2. Box plots for (a) baseline corticosterone and (b) body mass of female violet-green swallows during pre-treatment (white shading) and post-treatment measurements (grey shading). The boundary of the box closest to zero indicates the 25th, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 10th and 90th percentiles. Outliers are shown as individual data points, and sample sizes are reported below each box plot.

$t_{1,25} = 8.7$, $P < 0.001$]. Within each treatment, mean baseline cort increased from the pre-treatment capture period to the post-treatment capture period: control: $\beta = 1.97$ (95% CI: -0.44 , 4.39), $t_{1,25} = 2.5$, $P = 0.155$; light clipping: $\beta = 4.85$ (95% CI: 2.62 , 7.09), $t_{1,25} = 6.7$, $P < 0.001$; and heavy clipping: $\beta = 12.10$ (95% CI: 8.16 , 16.05), $t_{1,25} = 9.5$, $P < 0.001$. Relative to females in the control group, the magnitude of increase in mean baseline cort between the two capture periods was $2.5\times$ (95% CI: 1.1 , 5.7) and $6.1\times$ (95% CI: 2.7 , 13.9) greater in females subjected to the light clipping and heavy clipping treatments, respectively (Fig. 2a).

We found that female body mass significantly decreased between the two capture periods ($F_{1,14.7} = 48.9$, $P < 0.001$; Fig. 2b), but we did not detect a significant effect of either treatment ($F_{2,23} = 1.9$, $P = 0.173$) or a treatment \times capture period interaction ($F_{2,15} = 2.5$, $P = 0.118$). The mean decrease in body mass between capture periods was significant for females in the control treatment [$\beta = -0.95$ (95% CI: -1.75 , -0.14), $t_{1,16} = 3.8$, $P = 0.017$] as well as both handicapping treatments [light clipping: $\beta = -1.07$ (95% CI: -1.88 , -0.26), $t_{1,15.3} = 4.3$, $P = 0.007$; heavy clipping: $\beta = -2.12$ (95% CI: -3.67 , -0.58), $t_{1,14.3} = 4.5$, $P = 0.005$; Fig. 2b]. However, the magnitude of decrease in mean body mass of females in the light and heavy clipping treatments was $1.2\times$ (95% CI: 0.6 , 2.3) and $2.2\times$ (95% CI: 1.2 , 4.5) greater, respectively, that of females in the control treatment.

BEHAVIOURAL RESPONSE TO HANDICAPPING TREATMENTS

Females provided the majority of feedings to nestlings in all treatments, and the proportion of the total number of feeding visits by females was similar among treatments [control: 55% (95% CI: 51–58%), light clipping: 65% (95% CI: 58–71%), heavy clipping: 54% (95% CI: 50–57%)]. In contrast to changes in baseline cort, we found no effect of handicapping treatment on per capita feeding rate by female swallows ($F_{2,15} = 0.2$, $P = 0.846$), with no difference in female visitation rates detected between control and light clipping treatments [$b = 0.25$ (95% CI: -1.32 , 1.81), $t_{1,15} = 0.4$, $P = 0.912$] or between control and heavy clipping treatments [$b = 0.47$ (95% CI: -1.82 , 2.76), $t_{1,15} = 0.5$, $P = 0.854$; Fig. 3a]. We did not detect significant variation in male response to the handicapping of their social partner, as males exhibited similar per capita feeding rates in all treatments ($F_{2,15} = 1.8$, $P = 0.197$). Similarly, we found no significant effect of the handicapping treatments on overall (i.e. male + female) per capita feeding rate ($F_{2,15} = 1.0$, $P = 0.390$), although rates were slightly higher at control nests [6.8 feeding visits h^{-1} (95% CI: 5.4, 8.2)] relative to nests in the handicapping treatments [light clipping: 5.6 visits h^{-1} (95% CI: 4.1, 7.1), heavy clipping: 5.4 visits h^{-1} (95% CI: 2.9, 7.9)]. Finally, we found no support for an effect of the handicapping

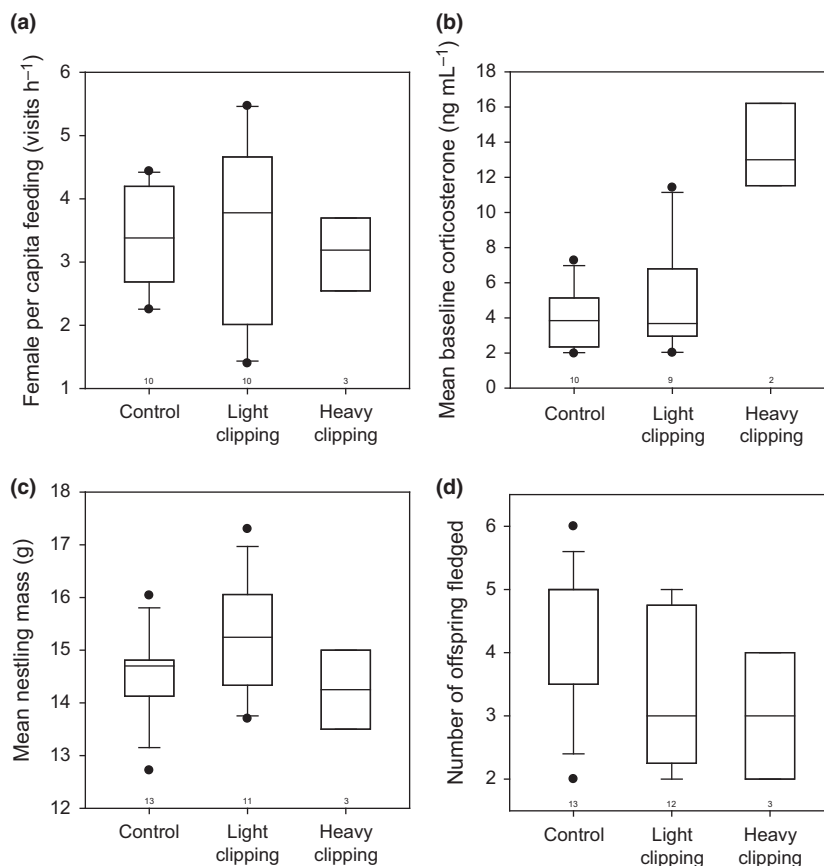


Fig. 3. Box plots for (a) female per capita feeding rate, (b) mean offspring baseline corticosterone, (c) mean offspring body mass and (d) number of offspring fledged for the three treatment groups. The boundary of the box closest to zero indicates the 25th, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 10th and 90th percentiles. Outliers are shown as individual data points, and sample sizes are reported below each box plot.

treatments on risk-taking ($X^2 = 1.24$, $P = 0.536$), as only four females entered nest boxes with a novel object present (control: 2 of 12 females, light clipping: 1 of 12 females, heavy clipping: 1 of 3 females).

MEASURES OF OFFSPRING PHENOTYPE AND REPRODUCTIVE OUTPUT

We found a significant effect of handicapping on mean offspring baseline cort ($F_{2,15} = 11.0$, $P = 0.001$), with concentrations increasing with the degree of handicapping (Fig. 3b). We detected significant pairwise differences between the control and light clipping treatments [$\beta = 3.19$ (95% CI: 1.04, 5.33), $t_{1,15} = 3.9$, $P = 0.004$] and between the control and heavy clipping treatments [$\beta = 4.65$ (95% CI: 1.42, 7.89), $t_{1,15} = 3.7$, $P = 0.005$], but not between the light and heavy clipping treatments [$\beta = 1.47$ (95% CI: -1.79 , 4.74), $t_{1,15} = 1.2$, $P = 0.490$]. In contrast, we did not detect a significant treatment effect on offspring body mass on ($F_{2,19} = 0.6$, $P = 0.540$; Fig. 3c), with no treatment-level differences detected when comparing control and light clipping treatments [$\beta = 1.07$ (95% CI: -1.36 , 3.50), $t_{1,19} = 1.1$, $P = 0.276$] or when comparing control and heavy clipping treatments [$\beta = 0.53$ (95% CI: -3.29 , 4.35), $t_{1,19} = 0.4$, $P = 0.933$]. Similarly, we did not detect a significant treatment effect on the mean number offspring fledged ($F_{2,20} = 1.0$, $P = 0.394$; Fig. 3d), with no statistically significant differences when comparing control and light clipping treatments [$\beta = 0.11$ (95% CI: -0.48 , 0.70), $t_{1,20} = 0.5$, $P = 0.891$] and control and heavy clipping treatments [$\beta = 0.52$ (95% CI: -0.43 , 1.47), $t_{1,20} = 1.4$, $P = 0.363$].

Discussion

Our study found strong support for the cort-adaptation hypothesis: female swallows that underwent experimental feather clipping were found to have elevated baseline cort concentrations, and the magnitude of the increase in cort was positively associated with the degree of handicapping. That we detected significant treatment differences in baseline cort between control females and females in the heavy clipping treatment despite modest sample sizes in the latter treatment is a testament to the strong effect that handicapping treatments had on breeding swallows. Despite undergoing physiological stress, however, females in handicapping treatments appeared to (i) maintain offspring feeding rates, (ii) raise nestlings of similar size and (iii) fledge a similar number of offspring as female in control treatments. Because cort is a metabolic hormone (Sapolsky, Romero & Munck 2000), we suspect that the greater increases in baseline cort experienced by handicapped females led them to work harder which, in turn, allowed them to achieve reproductive success to the same degree as control females as predicted by the cort-adaptation hypothesis (Bonier *et al.* 2009; Bonier, Moore & Robertson 2011).

Although increases in cort did appear to benefit females, the increased stress from handicapping treatments was associated with differential decreases in body mass that were, on average, 1.2 \times greater for females in the light clipping treatment and 2.2 \times greater for females in the heavy clipping treatment relative to those in the control treatment. Although mass loss in aerial insectivores during the offspring rearing period can be adaptive (e.g. Boyle, Winkler & Guglielmo 2012), we found that (i) the degree of mass loss, although not statistically significant, was still greater for handicapped females than for control females, and (ii) increased mass loss was coupled with increased baseline cort. Together, this suggests that handicapped females were under greater physiological stress than unmanipulated females. Whether these responses led to fitness reductions is unclear, although we found a similar proportion of females subjected to control (38%, 5 of 13) and light clipping treatments (33%, 4 of 12) returned to breed at our study area in at least one subsequent year after treatments were applied; in contrast, none of the three female subjected to heavy clipping treatments were detected on our study site (J.W. Rivers, unpublished data). It was beyond the scope of this study to assess the long-term consequences of feather clipping on survival, but it is clear that handicapped females increased baseline cort concentrations and produced a similar number of young in the season during which they were handicapped, a result that supports the cort-adaptation hypothesis.

In addition to treatment effects on female physiology, we also found that young in both feather-clipping treatments had significantly higher baseline cort concentrations relative to control young. Given results from previous handicapping studies (e.g. Love & Williams 2008b), it was surprising that we did not also find treatment effects on female per capita provisioning or offspring body mass. One explanation for this finding is that because effect size estimates had large variation associated with them for these two measures, it may have been difficult to detect differences between control and handicapping treatments. Alternatively, there may have indeed been no treatment effects on female provisioning or offspring body mass, and the downstream consequences of handicapping swallows were instead limited to differences in offspring cort. Regardless of the role of provisioning and body mass may have played, that offspring in the different treatment groups varied in their baseline cort levels is notable because baseline cort concentrations have been linked to post-fledging survival (Rivers *et al.* 2012). The mechanism behind treatment differences in offspring cort is unknown, but one possibility is that handicapped females took longer to resume parental care duties than control females, with longer recovery times leading to greater developmental stress of young. The hypothalamic–pituitary–adrenal (HPA) axis of nestlings is highly sensitive to developmental stress (Pravosudov & Kitaysky 2006; Monaghan 2008; Schoech, Rensel & Heiss 2011), so additional recovery

time by handicapped females is a reasonable hypothesis for increased baseline cort in offspring. Such an effect may have been enhanced in this study because (i) offspring brooding is restricted to female parents (Brown, Knott & Damrose 2011) and (ii) handicapping treatments were implemented early in nestling development when young are unable to thermoregulate and when feeding by male swallows is limited. Although we did not record the time it took females to return to the nest to brood and/or feed young after release during initial capture, a previous study of female tree swallows that used the same light clipping treatment (Ardia & Clotfelter 2007) found that handicapped females took more than twice as long to return to the brood as unmanipulated females (mean return time for females in control treatment: 70 min, mean return time to females in the light clipping treatment: 155 min; D.R. Ardia, unpublished data). If this is a general response by swallows to handicapping, females in the heaving clipping treatment might have taken even longer to return than those in the light clipping treatment. Although female swallows did not differ in per capita feeding rate in our study on nestling day 8, it is possible there were differences in feeding or other forms of parental care (e.g. brooding) in the days between the implementation of handicapping treatment and when we measured parental provisioning. Whether offspring baseline cort levels were influenced by an acute period of offspring neglect or a longer reduction in the amount of parental care is unknown, and a more detailed examination of these alternatives may prove fruitful to understand better how offspring phenotype is altered in response parental handicapping.

Our findings regarding how baseline cort changes with increased flight costs are consistent with the only other investigation to experimentally test the cort-adaptation hypothesis (Bonier, Moore & Robertson 2011). In that study, female tree swallows that were confronted with an experimentally enlarged brood also had increased baseline cort, and those elevated baseline cort concentrations appeared to enhance feeding rates and result in greater reproductive output (Bonier, Moore & Robertson 2011). That study did not report per capita feeding rates, however, making it difficult to compare feeding behaviour of female tree swallows directly to the violet-green swallows in this study. Regardless, when our overall results are compared to those of Bonier, Moore & Robertson (2011), they indicate that female *Tachycineta* swallows elevate baseline cort when faced with experimental increases in parental effort during the brood rearing period, and this increase in baseline cort appears to allow them to maintain feeding rates and reproductive output. Both studies found similar results despite using notably different approaches to increase the cost of rearing young [i.e. feather clipping (this study) vs. brood enlargement (Bonier, Moore & Robertson 2011)], so cort increases in response to unanticipated energetic challenges may be a general response by breeding aerial insectivores. As noted above, experimental investigations of the cort-adaptation hypothesis are

especially limited, so additional tests are needed to evaluate the extent to which this hypothesis is germane to additional taxa.

Our results with the violet-green swallow provide an interesting contrast to several studies that used feather clipping to increase the energetic costs of flight during breeding of the tree swallow, a closely related and ecologically similar species. Those studies were restricted to evaluating behavioural responses to feather clipping and found that, relative to controls, handicapped female tree swallows generally exhibited decreased feeding rates, produced smaller nestlings and had reduced reproductive output, (Winkler & Allen 1995; Nooker, Dunn & Whittingham 2005; Ardia & Clotfelter 2007; Hogle & Burness 2014). In contrast, our data suggest handicapped violet-green swallows maintained feeding rates, raised nestlings of similar size and had reproductive output that was similar to unmanipulated control females, although it is possible that large variation in effect size estimates may have reduced our ability to detect treatment effects for female provisioning rates and offspring body mass. Nevertheless, these results, if they are indeed robust, are unexpected because both swallow species are single-brooded and short-lived (Brown, Knott & Damrose 2011; Winkler *et al.* 2011), and life-history theory predicts that both species should respond with similarly to experimental changes in flight cost that arise from handicapping (Stearns 1992). Differences in annual survival between violet-green and tree swallows could be responsible for the divergent response to handicapping, but robust adult survival estimates are unavailable for testing this idea. Nevertheless, both swallow species breed together in our study population, and ongoing research has found noteworthy species-level differences in their physiological responses to capture and handling (J.W. Rivers, unpublished data). Thus, despite being congeneric, ecologically similar and sympatric throughout much of western North America, these two species may have subtle but important differences in how they respond to experimental feather clipping. This finding should serve as a caution against using one species as a surrogate for a close relative to understand the response to physiological stressors without first demonstrating that such similarities are actually present.

Our study found that the degree to which female swallows increased baseline cort was associated with the degree of handicapping, suggesting that swallows can modulate endogenous cort concentrations to match the degree of physiological challenge they experience during breeding. There is likely a threshold at which increases in baseline cort change from having a positive effect on fitness to having a negative effect (Romero 2004; Bonier *et al.* 2009); however, where such a threshold may lie, and how it varies relative to environmental conditions and individual differences in physiology remains unknown (Bonier *et al.* 2009; Ouyang *et al.* 2015). One potentially fruitful avenue for investigating this topic is to evaluate how variation in cort is linked to nest abandonment (Love *et al.* 2004; Ouyang,

Quetting & Hau 2011b). A subset of females in all three treatments abandoned their nests shortly after treatments were applied, including unmanipulated controls who did not undergo handicapping. Thus, additional study is warranted to test whether variation in baseline cort concentration or some other physiological parameter can predict when brood abandonment occurs, and whether there is a general threshold for ceasing reproductive behaviour and taking action to promote survival. Further study of this issue should bring better resolution regarding the circumstances that lead to increases in baseline cort that are adaptive and increase fitness, aligning with the cort-adaptation hypothesis, and the conditions that lead to fitness reductions because of elevated cort, aligning with the cort-fitness hypothesis (Bonier *et al.* 2009).

Acknowledgements

Financial support for this work was provided in part by the *Golondrinas de las Americas* programme at Cornell University (NSF-PIRE, OISE-0730180), the General Research Fund at Oregon State University, the Margaret A. Cargill Foundation, the North American Bluebird Society, the New York State Bluebird Society, the Oregon State University Honor's College, the Oregon State University URISC Program and the E.R. Jackman Internship Support Program. We are grateful to J. Allen, M. Bashaw, R. Heeter, B. Krahn, M. Meeker, T. Nichols, D. Robinson, R. Snyder and the numerous members of the *Tachycineta* field team for extensive logistical support. We thank several anonymous reviewers for valuable feedback that helped to improve this manuscript. The authors declare that they have no conflict of interest as it pertains to this work.

Data accessibility

Data associated with this paper have been deposited in the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.3bc3s> (Rivers *et al.* 2016).

References

- Ardia, D.R. & Clotfelter, E.D. (2007) Individual quality and age affect response to an energetic constraint in a cavity-nesting bird. *Behavioral Ecology*, **18**, 259–266.
- Bonier, F., Moore, I.T. & Robertson, R.J. (2011) The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. *Biology Letters*, **7**, 944–946.
- Bonier, F., Martin, P.R., Moore, I.T. & Wingfield, J.C. (2009) Do baseline glucocorticoids predict fitness? *Trends in Ecology and Evolution*, **24**, 634–642.
- Boyle, W.A., Winkler, D.W. & Guglielmo, C.G. (2012) Rapid loss of fat but not lean mass prior to chick provisioning supports the flight efficiency hypothesis in tree swallows. *Functional Ecology*, **26**, 895–903.
- Brown, C.R., Knott, A.M. & Damrose, E.J. (2011) Violet-green swallow (*Tachycineta thalassina*). *The Birds of North America Online*, Cornell Laboratory of Ornithology (ed. A. Poole), Ithaca, NY. Available online at <http://bna.birds.cornell.edu/bna/species/014> (accessed 12 April 2016).
- Cantarero, A., Lopez-Arrabe, J., Palma, A., Redondo, A.J. & Moreno, J. (2014) Males respond to female begging signals of need: a handicapping experiment in the pied flycatcher, *Ficedula hypoleuca*. *Animal Behaviour*, **94**, 167–173.
- Ebensperger, L.A., Ramirez-Estrada, J., Leon, C., Castro, R.A., Tolhuysen, L.O., Sobrero, R. *et al.* (2011) Sociality, glucocorticoids, and direct fitness in the communally rearing rodent, *Octodon degus*. *Hormones and Behavior*, **60**, 346–352.
- Ebensperger, L.A., Tapia, D., Ramirez-Estrada, J., Leon, C. & Soto-Gamboa, M. (2013) Fecal cortisol levels predict breeding but not survival of females in the short-lived rodent, *Octodon degus*. *General and Comparative Endocrinology*, **186**, 164–171.
- Ensminger, A.L. & Westneat, D.F. (2012) Individual and sex differences in habituation and neophobia in house sparrows (*Passer domesticus*). *Ethology*, **118**, 1085–1095.
- Escribano-Avila, G., Pettorelli, N., Virgos, E., Lara-Romero, C., Lozano, J., Barja, I. *et al.* (2013) Testing cort-fitness and cort-adaptations hypotheses in a habitat suitability gradient for roe deer. *Acta Oecologica*, **53**, 38–48.
- Garlick, N.W., Newberry, G.N. & Rivers, J.W. (2014) An assessment of nestling diet composition in the violet-green swallow (*Tachycineta thalassina*). *Northwest Science*, **88**, 49–54.
- Harding, A.M.A., Kitaysky, A.S., Hall, M.E., Welckere, J., Karnovsky, N.J., Talbot, S.L. *et al.* (2009) Flexibility in the parental effort of an Arctic-breeding seabird. *Functional Ecology*, **23**, 348–358.
- Hogle, N.C. & Burness, G. (2014) Sex-specific environmental sensitivity is transient in nestling tree swallows (*Tachycineta bicolor*). *Journal of Ornithology*, **155**, 91–100.
- Landys, M.M., Ramenofsky, M. & Wingfield, J.C. (2006) Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, **148**, 132–149.
- Leclaire, S., Bourret, V., Wagner, R.H., Hatch, S.A., Helfenstein, F., Chastel, O. *et al.* (2011) Behavioral and physiological response to male handicap in chick-rearing black-legged kittiwakes. *Behavioral Ecology*, **22**, 1156–1165.
- Lendvai, A.Z., Bokony, V. & Chastel, O. (2011) Coping with novelty and stress in free-living house sparrows. *Journal of Experimental Biology*, **214**, 821–828.
- Love, O.P. & Williams, T.D. (2008a) The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. *American Naturalist*, **172**, E135–E149.
- Love, O.P. & Williams, T.D. (2008b) Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. *Hormones and Behavior*, **54**, 496–505.
- Love, O.P., Breuner, C.W., Vezina, F. & Williams, T.D. (2004) Mediation of a corticosterone-induced reproductive conflict. *Hormones and Behavior*, **46**, 59–65.
- Magrath, R.D. (1991) Nestling weight and juvenile survival in the blackbird, *Turdus merula*. *Journal of Animal Ecology*, **60**, 335–351.
- Martin, J.G.A. & Reale, D. (2008) Temperament, risk assessment, and habituation to novelty in eastern chipmunks, *Tamias striatus*. *Animal Behaviour*, **75**, 309–318.
- Mathot, K.J., Nicolaus, M., Araya-Ajoy, Y.G., Dingemanse, N.J. & Kempenaers, B. (2015) Does metabolic rate predict risk-taking behaviour? A field experiment with a wild bird. *Functional Ecology*, **29**, 239–249.
- McCarty, J.P. (2002) The number of visits to the nest by parents is an accurate measure of food delivered to nestlings in tree swallows. *Journal of Field Ornithology*, **73**, 9–14.
- McEwen, B.S. & Wingfield, J.C. (2003) The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, **43**, 2–15.
- Mettke-Hofmann, C., Winkler, H. & Leisler, B. (2002) The significance of ecological factors for exploration and neophobia in parrots. *Ethology*, **108**, 249–272.
- Monaghan, P. (2008) Early growth conditions, phenotypic development, and environmental change. *Philosophical Transactions of the Royal Society of London Series B*, **363**, 1635–1645.
- Naef-Daenzer, B., Widmer, F. & Nuber, M. (2001) Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *Journal of Animal Ecology*, **70**, 730–738.
- Nemeth, Z., Bonier, F. & MacDougall-Shackleton, S.A. (2013) Coping with uncertainty: integrating physiology, behavior, and evolutionary ecology in a changing world. *Integrative and Comparative Biology*, **53**, 960–964.
- Nooker, J.K., Dunn, P.O. & Whittingham, L.A. (2005) Effect of food abundance, weather, and female condition on reproduction in tree swallows (*Tachycineta bicolor*). *Auk*, **122**, 1225–1238.
- Ouyang, J.Q., Quetting, M. & Hau, M. (2011b) Corticosterone and brood abandonment in a passerine bird. *Animal Behaviour*, **84**, 261–268.
- Ouyang, J.Q., Sharp, P.J., Dawson, A., Quetting, M. & Hau, M. (2011a) Hormone levels predict individual differences in reproductive success in a passerine bird. *Proceedings of the Royal Society Series B*, **278**, 2537–2545.
- Ouyang, J.Q., Lendvai, A.Z., Dakin, R., Domalik, A.D., Fasanello, B.G., Haussmann, M.F. *et al.* (2015) Weathering the storm: parental effort and experimental manipulation of stress hormones predict brood survival. *BMC Evolutionary Biology*, **15**, 219.

- Patterson, S.H., Winkler, D.W. & Breuner, C.W. (2011) Glucocorticoids, individual quality, and reproductive investment in a passerine bird. *Animal Behaviour*, **81**, 1239–1247.
- Pennyquick, C.J. (1989) *Bird Flight Performance: A Practical Calculation Manual*. Oxford University Press, Oxford, UK.
- Pravosudov, V.V. & Kitaysky, A.S. (2006) Effects of nutritional restrictions during post-hatching development on adrenocortical function in western scrub-jays (*Aphelocoma californica*). *General and Comparative Endocrinology*, **145**, 25–31.
- Rivers, J.W., Liebl, A.L., Owen, J.C., Martin, L.B. & Betts, M.G. (2012) Baseline corticosterone is positively related to juvenile survival in a migrant passerine bird. *Functional Ecology*, **26**, 1127–1134.
- Rivers, J.W., Newberry, G., Schwarz, C. & Ardia, D. (2016) Data from: Success despite the stress: violet-green swallows increase glucocorticoids and maintain reproductive output following experimental increases in flight costs. *Dryad Digital Repository*, <http://dx.doi.org/10.5061/dryad.3bc3s>.
- Romero, L.M. (2004) Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution*, **19**, 249–255.
- Romero, L.M., Dickens, M.J. & Cyr, N.E. (2009) The reactive scope model – a new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior*, **55**, 375–389.
- Romero, L.M. & Reed, J.M. (2005) Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology, Part A*, **140**, 73–79.
- Romero, L.M., Reed, J.M. & Wingfield, J.C. (2000) Effects of weather on corticosterone responses in wild free-living passerine birds. *General and Comparative Endocrinology*, **118**, 113–122.
- Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparatory actions. *Endocrine Reviews*, **21**, 55–89.
- Schoech, S.J., Rensel, M.A. & Heiss, R.S. (2011) Short- and long-term effects of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: a review. *Current Zoology*, **57**, 514–530.
- Slagsvold, T. & Dale, S. (1996) Disappearance of female pied flycatchers in relation to breeding stage and experimentally induced molt. *Ecology*, **77**, 461–471.
- Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford, UK.
- Verboven, N. & Visser, M.E. (1998) Seasonal variation in local recruitment of great tits: the importance of being early. *Oikos*, **81**, 511–524.
- Walker, B.G., Boersma, P.D. & Wingfield, J.C. (2005) Field endocrinology and conservation biology. *Integrative and Comparative Biology*, **45**, 12–18.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M. et al. (1998) Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *American Zoologist*, **28**, 191–206.
- Wingfield, J.C., Kelley, J.P., Angelier, F., Chastel, O., Lei, F., Lynn, S.E. et al. (2011) Organism-environment interactions in a changing world: a mechanistic approach. *Journal of Ornithology*, **12**, S279–S288.
- Winkler, D.A. & Allen, P.E. (1995) Effects of handicapping on female condition and reproduction in tree swallows (*Tachycineta bicolor*). *Auk*, **112**, 737–747.
- Winkler, D.W., Hallinger, K.K., Ardia, D.R., Robertson, R.J., Stutchbury, B.J. & Cohen, R.R. (2011) Tree swallow (*Tachycineta bicolor*). *The Birds of North America Online*, Cornell Laboratory of Ornithology (ed. A. Poole), Ithaca, NY. Available online at <http://bna.birds.cornell.edu/bna/species/011> (accessed 12 April 2016).

Received 13 November 2015; accepted 17 June 2016

Handling Editor: Ignacio Moore