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Do baseline glucocorticoids simultaneously represent fitness and environmental quality in a declining aerial insectivore?

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Glucocorticoids (GCs) are often interpreted as indicators of disturbance, habitat quality, and fitness in wild populations. However, since most investigations have been unable to examine habitat variability, GC levels, and fitness simultaneously, such interpretations remain largely unvalidated. We combined a quantification of two habitat types, a manipulation of foraging ability (feather-clipping just prior to nestling rearing), multiple baseline plasma GC measures, and multi-year reproductive monitoring to experimentally examine the linkages between habitat quality, GCs, and fitness in female tree swallows *Tachycineta bicolor*. Control females experiencing the higher early-season food resources of inland–pasture habitat laid larger clutches, but fledged an equal number but lower mass offspring compared to those in riparian–cropland habitat. Despite these differences in reproductive success, females nesting in the two habitat types did not differ in baseline GC levels at the early- or late-breeding stage. Feather-clipping reduced provisioning rate in both habitat types. However, baseline GC levels were affected in a habitat-specific way; only individuals in inland-pasture habitats showed an increase in GCs. Despite this difference in GC levels, the manipulation did not influence offspring mass, reproductive output, adult return rate (a proxy for survival) to the following year, or reproductive success in the subsequent year. Nonetheless, regardless of treatment, individuals with higher GC levels during the late breeding stage returned in the following year with higher GC levels at incubation, indicating a long-term effect on future GC levels. Our results indicate that environmental changes (e.g. foraging conditions) can have consequences for body condition, behaviour, and current and future baseline GC levels without concomitant influences on fitness, and that differences in fitness components between habitats may not be reflected in baseline GC levels. These results illustrate that baseline GCs may not simultaneously reflect environmental quality and fitness, potentially limiting their application in ecological and conservation settings.

Organisms are increasingly exposed to changing environmental conditions (Steffen et al. 2004), responding by altering their behaviour and physiology with potential consequences for performance and fitness (Clemmons and Buchholz 2002, Willmer et al. 2009, Sih et al. 2011). Determining the mechanisms by which changes in environmental quality translate to variation in fitness can provide ecological, evolutionary, and conservation-relevant insight into how and why populations may change in the face of further alteration (Carey 2005, Cockrem 2005, Wikelski and Cooke 2006, Angelier and Wingfield 2013). In particular, hormonal systems which constitute a vast array of traits involved in growth, metabolism, immune function, and reproduction are involved in modulating physiology and behaviour in response to internal state, social interactions, and environmental conditions (Ricklefs and Wikelski 2002). Glucocorticoids (i.e. GCs: cortisol and corticosterone) are often cited as strong potential candidates for mechanistically linking environmental variation and fitness due to their role in regulating energetics and their involvement in an integrated response to acute perturbations (Korte et al. 2005, Walker et al. 2005, Wikelski and Cooke 2006, Breuner et al. 2008, Bókony et al. 2009, Bonier et al. 2009, Busch and Hayward 2009).

The diversity of taxa in which GC levels of populations differing in habitat type have been compared (e.g. amphibians: Newcomb Homan et al. 2003, Hopkins and DuRant 2011; reptiles: Cash and Holberton 2005, French et al. 2008; fish: Blevins et al. 2013, Belanger et al. 2016; birds: Wasser et al. 1997, Leshyk et al. 2012; mammals: Allen et al. 2010, Rehnus et al. 2014) illustrates the desire of eco-physiologists and conservation biologists to understand organismal response to change, monitor disturbance levels, measure underlying habitat quality, and delineate areas, populations, or time periods for conservation endeavours. Often, investigations of GCs in relation to disturbance or habitat quality take the form of measuring two populations in differing habitat types and interpreting a difference (or lack of difference) in GCs as a proxy of disturbance or energetic challenge (Bonier et al. 2009). More specifically, higher GC levels are interpreted as indicators of a more disturbed or less healthy population or habitat (Bonier et al.

2009). This interpretation is rooted in a number of key characteristics of GCs: 1) their up-regulation in response to acute events such as predation threat, human presence or handling, inclement weather, social challenge and food shortage (Wingfield and Kitaysky 2002, Reeder and Kramer 2005, Bonier et al. 2009); 2) long-term over-activation of the HPA/HPI (hypothalamic–pituitary–adrenal/interrenal) axis leading to declines in growth rate, reproduction, cognitive function, and survival (Romero et al. 2009, Boonstra 2013); 3) levels sometimes correlating negatively with body condition indices (Wingfield et al. 1997, Moore et al. 2000, Romero and Wikelski 2001, Moore and Jessop 2003, Jenni-Eiermann et al. 2008, Williams et al. 2008, Husak and Moore 2008); and 4) the expectation that allostatic load (i.e. current and predicted energetic demands) should be higher in more disturbed populations or habitats, leading to higher GC levels (McEwen and Wingfield 2010, Bonier 2012). However, this supposition ignores that GC physiology is often context-dependent and can vary independently of environmental variation, a characteristic that is highly relevant to their interpretation (Millspaugh and Washburn 2004, Bonier et al. 2009, Dantzer et al. 2014, Madliger and Love 2014).

For example, levels of GCs can be elevated during energetically demanding, but not necessarily "stressful", points in the life history cycle such as breeding or migration (Romero 2002), potentially promoting foraging/food intake and locomotor activity rather than signalling environmental disturbance (Landys et al. 2006). This has important implications for the interpretation of GCs in the context of disturbance as comparison of two habitats or populations that differ in, for example, reproductive status, could lead to differing GC levels that are independent of disturbance level. Other contexts that can also differ across populations/habitats with influences on GC levels are demographic composition (i.e. ratio of young to old individuals, or males to females: Homan et al. 2003, Touma et al. 2003, Kern et al. 2005, Goymann 2012, Rector et al. 2012, Hämäläinen et al. 2015), weather conditions (Romero et al. 2000, Huber et al. 2003, Touma and Palme 2005, Baker et al. 2013), or time of day of sampling (Breuner et al. 1999, Tarlow et al. 2003, Touma and Palme 2005, Heintz et al. 2011). In addition, the interpretation of GC levels as informative indicators of disturbance requires validation of downstream fitness consequences at individual and population levels (Tarlow and Blumstein 2007, Breuner et al. 2008, Bonier et al. 2009, Busch and Hayward 2009, Cooke 2014, Madliger and Love 2014).

Unfortunately, few studies to date have been able to simultaneously measure environmental quality, GCs, and fitness consequences, leading to variable results that can be sex- (Strasser and Heath 2013), season- (Escribano-Avila et al. 2013), or scale- (i.e. individual versus population) specific (Riechert et al. 2014), or only evident when other measures of physiology are accounted for (thyroid hormone: Hayward et al. 2011) (but see Gobush et al. 2008, Sheriff et al. 2009, Kaiser et al. 2015). Overall, we currently lack information regarding the spatial scale over which differences in such relationships can occur, whether they change over different stages in the life cycle, and how they can be further impacted by additional environmental change (e.g. prolonged weather events, human activity or declines in food availability). In light of these complexities, experimental studies designed to alter key components of environmental quality are needed to demonstrate causal links between GC levels, disturbance and vital rates (Patterson et al. 2011, Arlettaz et al. 2014). Establishing if and when such linkages occur is necessary to interpreting GC levels as relevant biomarkers (i.e. meaningful in terms of organismal health, condition and fitness) of disturbance or environmental quality.

Here we combine both correlative and experimental techniques to investigate whether baseline GCs simultaneously reflect environmental quality and fitness in a wild vertebrate across two habitat types. Tree swallows *Tachycineta bicolor* are an aerial insectivorous passerine that, along with other members of the aerial insectivore guild of birds, are in decline in North America (Nebel et al. 2010) and one of the predominant hypotheses for drivers behind this decline is decreases in flying insect food resources (Ghilain and Bélisle 2008). As a result, investigations into the connections between habitat quality, physiology and fitness could provide insight into how environmental change may be spurring population decline, and how to best monitor and mitigate future changes.

We employed an experimental manipulation of foraging profitability (designed to decrease a female's ability to acquire food resources) initiated at the late incubation stage and relevant throughout offspring provisioning, to determine whether GCs and fitness metrics respond in parallel or differentially to a biologically-relevant increase in workload depending on the initial habitat type birds had chosen for breeding. We were careful to control for or eliminate multiple contexts that could potentially mask underlying patterns in GC levels (i.e. age, reproductive stage, time of day). We also measured multiple components of fitness (i.e. offspring mass, reproductive output, adult return rate) since these may be differentially sensitive to an interaction between habitat type and further environmental change (i.e. a decline in foraging profitability). Finally, we investigated potential inter-annual effects of the manipulation of foraging profitability on GC levels, breeding decisions, and fitness outcomes the following year to better explore the mechanisms by which environmental alteration during breeding could influence subsequent investment and success. Overall, our goal was to assess the relationship between environmental quality, baseline GC levels, and fitness. Combined with other explicit validations of GCs as ecological and conservation biomarkers (Madliger and Love 2014, 2015), determining if and when GC levels can be interpreted as simultaneous proxies of environmental change and fitness at the population-level will refine their position as a conservation monitoring tool, and draw attention to contexts that may be necessary for their interpretation across disciplines.

Material and methods

Study species and site

We studied a colony of wild tree swallows breeding in nest boxes in Cayuga, Ontario, Canada from 2011–2013. A total of 175 next boxes were distributed across two conservation areas located four kilometers apart: Taquanyah Conservation Area (42°57′N, 79°54′W) and Ruthven Park National Historic Site (42°58′N, 79°52′W). Boxes were clustered in fallow fields and along roadways, and bordered by varying habitat types including active cropland, riparian areas along the Grand River, wetlands, forest and cattle pasture. Here, we focus on data from a subset of 96 boxes (clustered in five groups) used for an experimental manipulation of foraging profitability.

Nest monitoring and blood sampling protocol

We monitored nest boxes on a daily basis from late April to early July. We recorded nest building, the date of the first egg laid (lay date), total number of eggs laid and incubated (clutch size), number of offspring hatched, and number of offspring that successfully left (i.e. fledged) the nest. Tree swallows reproduce once per season and, on average, females incubate eggs for 14–15 days followed by bi-parental provisioning for approximately 18–22 days (Robertson et al. 1992). We recorded the mass of each egg laid, as well as the mass of the chicks at day 6 and day 12 after hatch. In addition, we captured females twice at the nest box over the reproductive season to obtain blood samples for baseline corticosterone quantification: 1) day 10 of the incubation period; 2) day 12 of the nestling provisioning phase. We obtained blood samples in microcapillary tubes within 2 min (Romero and Reed 2005) of plugging the nest hole through puncture of the brachial vein. At each sampling period, we also recorded female mass, wing length, and age (second year or after second year through observation of plumage coloration). Females were marked on the chest and underside of the tail at the first sampling period with blue marker to allow for identification during subsequent trapping and provisioning rate observations. Unbanded females were given a numbered federal band (Canadian Wildlife Service Permit: 10808). Blood samples were stored on ice for up to four h, centrifuged, and then plasma was stored at –80°C until assay.

Experimental manipulation of foraging profitability

To induce an increase in energetic workload and a decline in foraging profitability, we used a feather-clipping manipulation on a subset (n = 33) of females in 2011 (control: n = 38). More specifically, we cut every other primary flight feather on each wing using scissors, leading to a reduction of eight total flight feathers (Winkler and Allen 1995, Ardia and Clotfelter 2007). We performed this manipulation at the first trapping/blood sampling event (i.e. 10 days into incubation). We only performed the manipulation on after-second year birds as identified by plumage (i.e. birds aged at least 2 years) (Hussell 1983) to control for potential age-related effects on GCs (Lanctot et al. 2003, Angelier et al. 2006, Riechert et al. 2012) and performance (de Steven 1978, Stutchbury and Robertson 1988) and due to comparatively small sample sizes of second-year birds (i.e. first time breeders). We temporally matched control and clipped females based on lay date and study site (Ruthven or Taquanyah). Tree swallows forage solely on the wing, bringing their offspring a mass (bolus) of flying insects (Robertson et al. 1992), and feather-clipping has been shown to decrease female provisioning rate in our population and others (Winkler and Allen 1995, Patterson et al. 2011, Madliger et al. 2015). We assessed nest provisioning rates using 1-h focal observations between 12:00 and 15:00 when the nestlings were 8–9 days of age (i.e. at a stage of high demand for parents due to high growth rate) (Quinney et al. 1986, McCarty 2001). One hour observations during mid-day in this species have been shown to be strong proxies of overall provisioning rate at this stage of nestling growth (Lendvai et al. 2015). Females remained feather-clipped for the duration of the reproductive season, with feathers being moulted and re-grown post-breeding (Stutchbury and Rohwer 1990).

Habitat types

We only briefly describe our quantification of the habitat metrics which were used to delineate habitat types herein since our approach has been outlined extensively elsewhere (Madliger et al. 2015). We used two primary means to assess habitat features known to be important to tree swallow nesting success: 1) a GIS-based approach to quantify landscape-level habitat features; 2) direct quantification of flying insect food resources. We quantified habitat features related to nest disturbance by con- and hetero-specifics, food resources, and road disturbance using a geographic information system (ArcGIS 10.1) for each nest box (Table 1). We then combined these six GIS-based metrics intro a principal component analysis, followed by grouping boxes using a cluster analysis. This resulted in the quantification of two

Table 1. Differences in average habitat metrics between two habitat types (inland–pasture and riparian–cropland) used by breeding tree swallows.

	Habitat type	
Habitat metric	inland–pasture (n = 42)	riparian–cropland (n = 29)
Distance to Grand River (m)	2605.3 ± 38.5	353.7 ± 42.0
Distance to forest (m)	130.9 ± 10.0	39.6 ± 12.0
Distance to road (m)	58.9 ± 7.7	260.5 ± 28.0
Distance to hedgerow (m)	127.3 ± 15.4	233.9 ± 18.5
% high insect landuse (200 m radius)	56.7 ± 3.6	25.5 ± 4.3
% high insect landuse (1 km radius)	47.3 ± 3.1	24.4 ± 0.9
Density (no. occupied boxes within 200 m)	19.1 ± 1.0	15.7 ± 0.6
Insect biomass at egg laying/incubation (mg day^{-1})	38.8 ± 4.1	25.9 ± 2.0
Insect biomass at offspring provisioning (mg day^{-1})	27.9 ± 2.6	30.4 ± 3.6

'clusters' of boxes that differed in structural habitat features, which we have labeled riparian–cropland and inland–pasture (Fig. 1). This is an arguably more biologically-relevant way to quantify habitat features than simply grouping nest boxes by site because it identifies features functionally important to tree swallows, rather than those that are merely similar due to shared location. Indeed, this type of analysis combined two of our nest box groups that are the farthest apart by on-the-ground distance, but did not split any groups. Riparian–cropland habitat is characterized by lower proximity to high insect availability landuse, lower nest disturbance, and greater access to the Grand River (Table 1). In contrast, nest boxes in inland–pasture are characterized by higher local food resources, higher nest disturbance, but less access to the Grand River (Table 1). These landuse types are similar to much of the breeding habitat available to tree swallows in the eastern United States and Canada. Samples sizes in riparian–cropland were 16 control and 13 feather-clipped birds and sample sizes in inland–pasture were 22 control and 20 feather-clipped birds.

Since availability of flying insect food resources is a major component of environmental quality for aerial insectivores, we directly quantified biomass across the breeding season using four-sided commercially-available malaise traps (110 × 110 × 110 cm SLAM traps) placed within clusters of boxes (five traps total). Tree swallows forage primarily within a 100–300 m radius of their nest location (Quinney and Ankney 1985, McCarty and Winkler 1999, McCarty 2001), making local food availability a highly relevant component of habitat quality in this species. Insect bottles were collected daily from 1 May – 1 July. We calculated the daily dry biomass of insect orders and size classes known to be consumed by tree swallows (see Madliger et al. 2015 for detailed methodology).

Hormone assay

Baseline levels of corticosterone (CORT – the primary GC in birds) were quantified using an enzyme-linked immunoassay (EIA; previously validated: Love and Williams (2008) and optimized for our species). We ran samples in triplicate at a 1:40 dilution and used a 1.5% steroid displacement buffer (SDB). Plates were run using a standard curve created by serially diluting a kit-provided corticosterone standard (20 000 pg ml^{-1} – 15.63 pg ml^{-1}). We used spiked laying hen plasma as a control. Assay plates were read at 405 nm using a spectrophotometer plate reader. Intra-assay variation was 8.0% and inter-assay variation was 13.3% in 2011. Intra-assay variation was 10.3% and inter-assay variation was 6.0% in 2012. In cases where concentrations fell below the detectable limit of the assay (0.74 ng ml^{-1}), samples were assigned this detection limit (< 5% of samples).

Statistical analyses

Comparison of food availability and breeding performance across habitat types

To determine the availability of insects in each of the two habitat types during the manipulation year (2011), we compared total daily dry biomass separately for the months

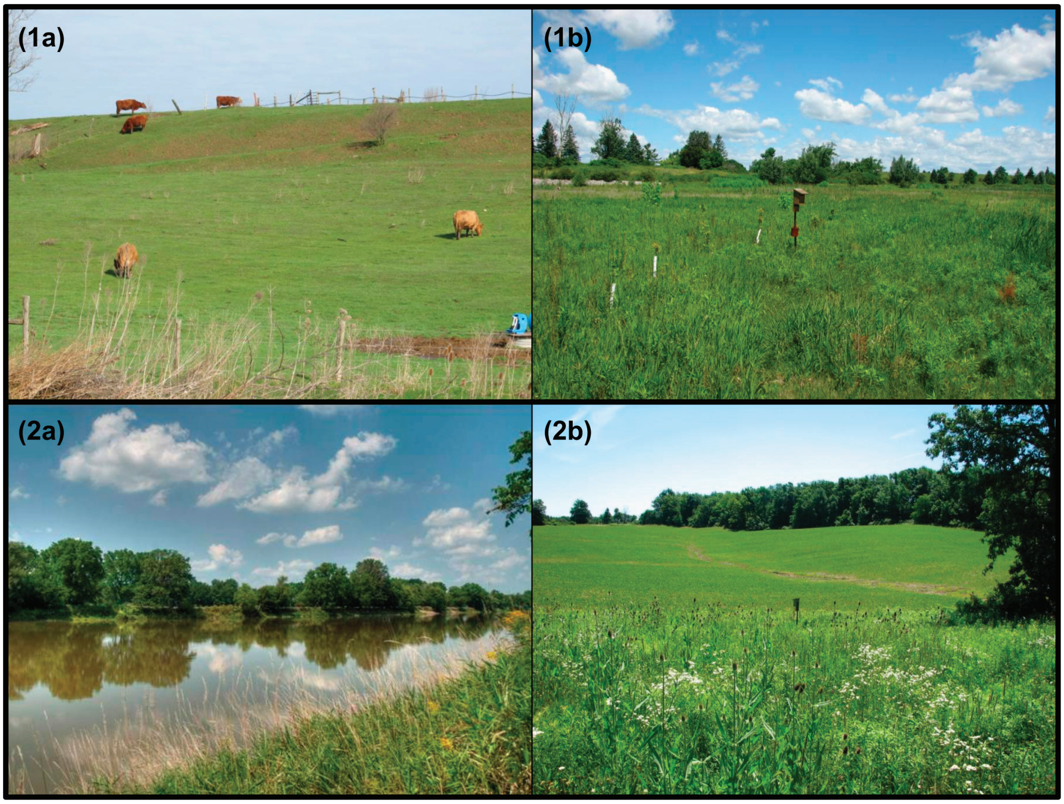

Figure 1. Characteristic landscape features (a) and representative nest box placement (b) in each of two habitat types of tree swallows used in this study: inland–pasture (1) and riparian–cropland (2).

of May (egg-laying/incubation stage) and June (nestling provisioning stage). We chose to split our analyses by month to address the possibility that the two habitat types may differ during one breeding stage, but not during the other. Splitting the analyses by month represents a valid proxy for reproductive stage in our population as mean lay date was May 13 ± 6 days, and mean mid-nestling provisioning stage was June 12 ± 7 days. Insect biomass data were heavily left-skewed and transformation did not alleviate non-normality. As a result, we used a Wilcoxon rank sum test (also known as a Mann–Whitney U-test) to compare insect biomass between habitat types in each month. We also compared the average CORT levels between habitat types at both the incubation (all individuals) and nestling provisioning stage (only control birds) using t-tests, with baseline CORT values log-transformed to achieve normality. To further quantify differences between the two habitat types from the perspective of female reproductive performance, we assessed four metrics related to female quality and investment: 1) lay date; 2) clutch size; 3) mean egg size; 4) size-corrected body mass at incubation. We compared each of these metrics between habitat types using separate t-tests. See section below for details regarding controlling for multiple comparisons.

Effects of foraging manipulation on within-season performance and fitness

We assessed the influence of the clipping manipulation on within-season GC physiology, breeding performance metrics, and fitness. Importantly, we simultaneously assessed the influence of treatment, habitat type, and the interaction between the treatment and habitat type to determine if individuals breeding in different habitat types had varying responses to the manipulation of foraging profitability. Our dependent variables included female provisioning rate, total provisioning rate (male and female combined), change in CORT over the reproductive season (late incubation to mid-nestling provisioning), change in female mass over the reproductive season (late incubation to mid-nestling provisioning), hatching success, offspring mass (a component of quality and reproductive output). It should be noted that we have previously broadly investigated female provisioning rate in relation to our manipulation (Madliger et al. 2015); however, those analyses did not consider habitat type or a potential interaction between habitat type and the manipulation. As such, we considered our current questions and analysis to warrant specific investigation to fully understand how the manipulation may manifest at the population-level.

We calculated residual provisioning rates for females and for both parents combined (i.e. total provisioning rate) by regressing provisioning rate against the number of chicks in the nest. Provisioning rates tend to increase with the total number of chicks being provisioned (Leffelaar and Robertson 1986), so the inclusion of residuals allows us to assess whether parents are foraging more or less than expected given the size of their brood. As a proxy of chick quality, we used the body mass of chicks at day 12, which represents the last day we were able to access nest boxes without pre-fledging offspring. The mass of chicks at this date is at a maximum and chick growth has been correlated with subsequent survival post-fledging in this species (McCarty 2001). There were two chick masses that were excluded from our analyses as they were beyond three standard deviations of the mean. We used multi-factor ANOVAs (ANCOVAs) for all analyses except when reproductive output was the dependent variable. In this case, we used a generalized linear model with a Poisson distribution and a log-link function.

Inter-annual effects of the foraging manipulation

We assessed longer-term consequences of the interaction between the foraging profitability manipulation and habitat type on female return rate and future investment, incubation CORT levels, and condition. For the analysis of return rate, we used a generalized linear model with a binomial distribution and a logit-link function with probability of return (1 = survived; 0 = died) as the dependent variable and treatment, habitat type, and the interaction between treatment and habitat type as independent variables. Female tree swallows display extremely high site fidelity, making return rates a strong proxy of survival in this species (Winkler et al. 2004). Given that we monitored 175 boxes within and surrounding our main study area for the three years subsequent to the manipulation year, our ability to detect the return of a female to the area was high, even if she switched breeding sites locally. Next, to assess potential impacts on future investment, we used lay date (an indicator of female quality in tree swallows), clutch size, and reproductive output the following year (2012) as dependent variables in separate analyses. We included lay date (except in the analysis with lay date as the dependent), prior treatment, habitat type, and the interaction between treatment and habitat type as independent variables. Only one individual switched habitat type from 2011 to 2012; therefore, controlling for habitat type in the manipulation year also controls for current habitat type in 2012. For both clutch size and output, which represent non-continuous dependent count variables, we used a generalized linear model with a Poisson distribution and log-link function. For the analysis with lay date as the dependent variable, we used a multi-factor ANOVA. To assess inter-annual effects on female condition, we used an ANCOVA with size-corrected female body mass at the incubation stage (day 10: the first day we captured individuals for blood sampling) as the dependent variable and prior treatment, habitat type, and a habitat by treatment interaction as independent variables. Finally, we assessed potential inter-annual effects of the manipulation on CORT levels using an ANCOVA with return (2012) baseline CORT level at incubation as the dependent and, as above, prior treatment, habitat type, and habitat by treatment interaction as independent variables. We also included previous year (2011) CORT level at the nestling provisioning stage as an additional covariate to control for prior physiological state, and to determine whether any longer-term effects were directly related to the manipulation, or to prior CORT levels in general.

Accounting for multiple comparisons

Given that we analyzed the influence of the clipping manipulation and habitat type on multiple traits using the same dataset, we controlled for false discovery rate (FDR) to account for multiple comparisons (Benjamini and Hochberg 1995). FDR refers to the expected proportion of tests that are declared significant when the null hypothesis is true

(Benjamini and Hochberg 1995). FDR control represents an alternative to Bonferroni correction that maintains considerably more power and is scalable with the number of tests performed, while simultaneously maintaining an acceptable error rate (Glickman et al. 2014). We used the classical one-stage method algorithm with a maximum false discovery rate (d) of 0.05 performed on overall model p-values. We performed two separate FDR control calculations, one which included all of the within-year analyses, and one which included the between year analyses because the datasets differed for each group of analyses. All significant results achieved p-values that were still considered significant after adjustment.

All analyses were completed using JMP 12 (SAS Inst.). All variables were assessed for normality using a Shapiro–Wilk test and transformed where relevant (stated within text). The homogeneity of variance assumption was met for all analyses (as indicated by Levene's tests). Data are presented as mean ± 1 SEM.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.qg630> (Madliger and Love 2016).

Results

Comparison of habitat types

Inland–pasture habitat was characterized by higher insect resources during the egg-laying/incubation stage (WRS: $n_{\text{Inland}} = 61$, $n_{\text{Riparian}} = 92$; $Z = 2.36$; $p = 0.02$; Fig. 2). However, the two habitat types did not differ in insect biomass during the nestling provisioning stage (WRS: $n_{\text{Inland}} = 66$, $n_{\text{Riparian}} = 98$; $Z = 0.65$; $p = 0.52$), indicating that inland–pasture habitat declined in insect biomass across stages (Fig. 2). Females nesting in inland–pasture habitat laid larger clutches (6.00 ± 0.15) compared to females nesting in riparian–cropland (5.41 ± 0.18) (t-test: DF = 69; t = –2.54; p = 0.01), although lay date (t-test: DF = 69; t = –0.03; p = 0.98), egg mass (t-test: DF = 69; t = –0.33; p = 0.74), and female body condition (t-test: DF = 68; t = –0.55; p = 0.59) did not differ between the two habitat types (Fig. 3). We did not take a body mass measure on one female, leading to the difference in sample size for that analysis compared to others. Baseline CORT levels of females nesting in inland–pasture habitat (2.51 ± 0.28 ng ml^{-1}) did not differ from those nesting in riparian–cropland habitat (2.90 ± 0.34 ng ml^{-1}) at the incubation stage (t-test: DF = 69; t = 1.10; p = 0.28; Fig. 3). Similarly, at the nestling provisioning stage, baseline CORT levels did not differ between inland–pasture (3.23 ± 0.31 ng ml^{-1}) or riparian–cropland (3.18 ± 0.37 ng ml^{-1}) habitats (t-test: DF = 69; t = –0.40, p = 0.69; Fig. 3).

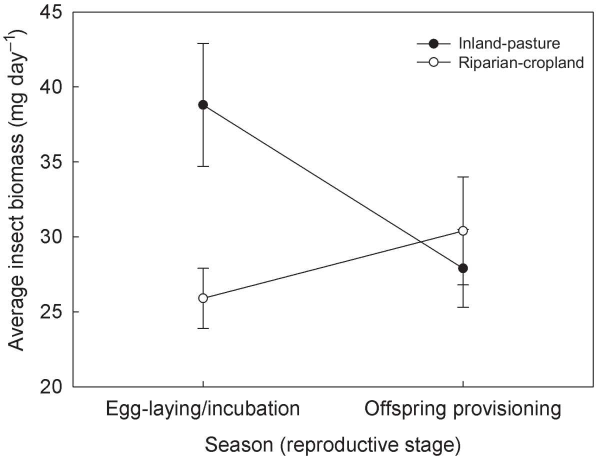

Figure 2. Average daily insect biomass in two tree swallow habitat types (inland–pasture and riparian–cropland) during the incubation and offspring provisioning stages of breeding. Insects were sampled using passive traps at three locations within riparian–cropland and in two locations within inland–pasture.

Effects of foraging manipulation on performance and fitness

Condition and performance

Female provisioning rate per chick was lower in clipped than control birds (ANCOVA: DF = 3,67, F = 9.24, $p < 0.0001$; treatment: t = 3.74, p = 0.0004; Fig. 4) and was lower overall in riparian–cropland as compared to inland–pasture habitat (ANCOVA: DF = 3,67, F = 9.24, $p < 0.0001$; habitat: t = 3.55, p = 0.0007; Fig. 4). Total provisioning rate/chick did not differ between control and clipped treatments, but was lower overall in riparian–cropland habitat compared to inland–pasture habitat (ANCOVA: DF = 3,26, F = 4.64, p = 0.005; habitat: t = 3.14, p = 0.003; Fig. 4). Clipped females lost a greater percentage of body mass between the late incubation and mid-nestling provisioning stage compared to controls, regardless of habitat type (ANCOVA: DF = 3,66, F = 8.89, $p < 0.0001$; treatment: t = –5.01, $p < 0.0001$; Fig. 5). However, we found a significant interaction between treatment and habitat quality on the change in baseline CORT levels (ANCOVA: DF = 3,67, F = 3.32, p = 0.025; trt × habitat: t = –1.96, p = 0.05). Baseline CORT levels were higher in birds clipped in inland–pasture habitat, but did not change in response to the manipulation in riparian–cropland habitat (Fig. 5).

Within-season fitness

We could not detect any relationship between treatment, habitat type or their interaction on reproductive output (i.e. the number of offspring fledged) (glm: DF = 3, $\chi^2 = 0.57$, p = 0.90). While chick mass differed between habitat types, with females in riparian–cropland habitat raising heavier chicks compared to females nesting in inland–pasture habitat (ANCOVA: DF = 3,67, F = 3.34, p = 0.02; habitat: t = –2.37, p = 0.02; Fig. 5), there was no influence of the foraging manipulation on chick mass (treatment: t = 1.86, p = 0.07).

Inter-annual effects of foraging manipulation

We found no effect of treatment, habitat type, or the interaction between habitat type and treatment on return rate (glm: DF = 3, $\chi^2 = 4.60$, p = 0.20), or lay date (ANCOVA: DF = 3,25,

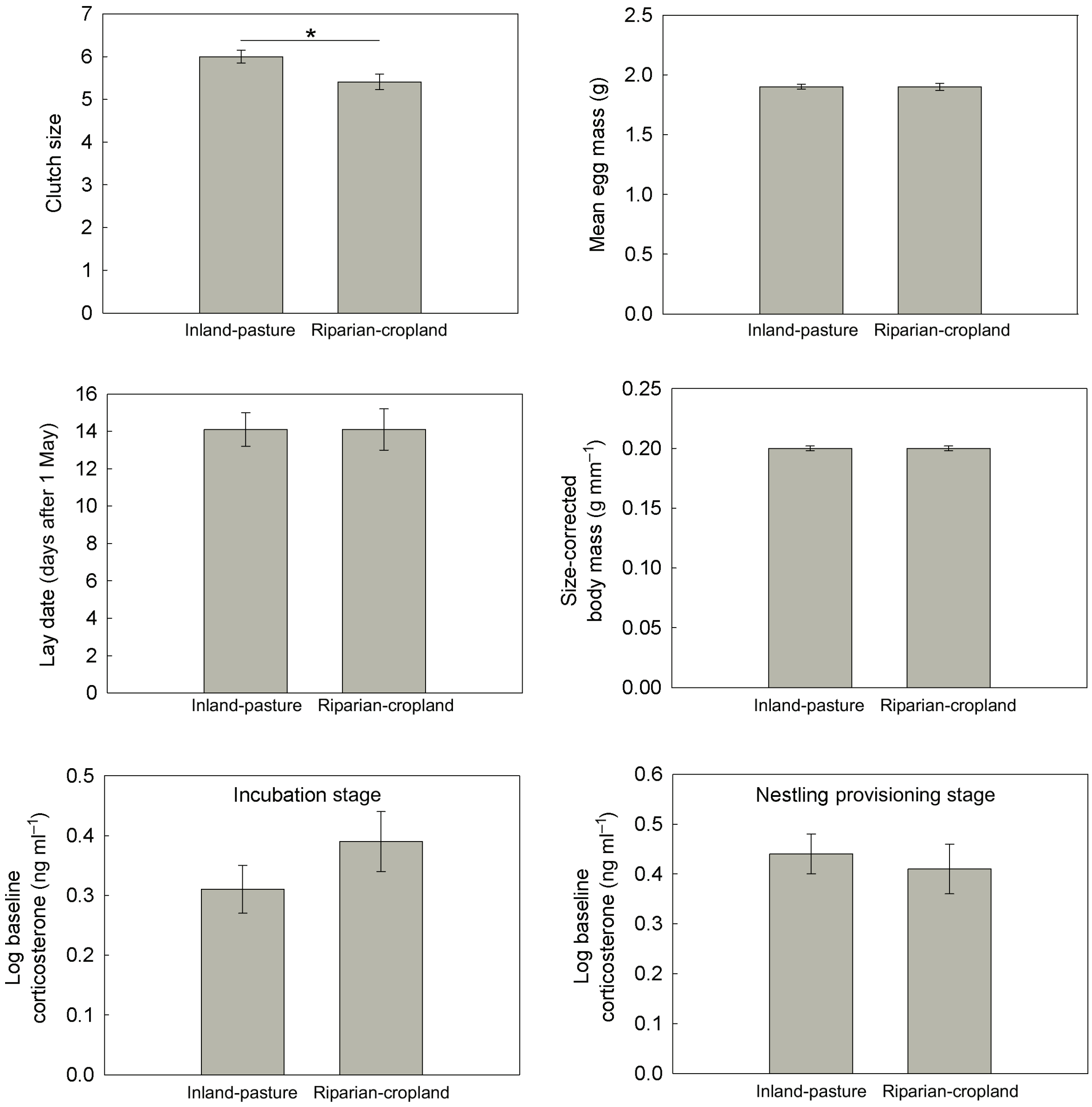

Figure 3. Female tree swallow investment (clutch size, egg mass, lay date) and condition (body mass, baseline corticosterone) between two habitat types of breeding tree swallows (inland–pasture and riparian–cropland). Untransformed baseline CORT levels (ng ml^{-1}) at incubation: inland–pasture (2.51 ± 0.28), riparian–cropland (2.90 ± 0.34); and at the nestling provisioning stage: inland–pasture (3.23 ± 0.31), riparian–cropland (3.18 ± 0.37).

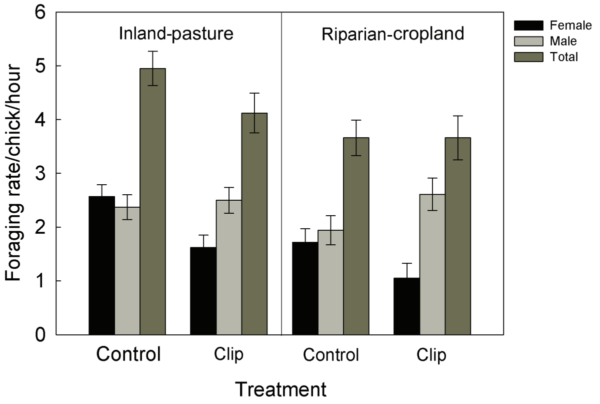

Figure 4. Female, male, and total tree swallow provisioning rates at control and manipulation (female feather-clipped) nests in two habitat types, inland–pasture and riparian–cropland.

F = 1.00, p = 0.41), female body condition at incubation (ANCOVA: DF = 4,24, F = 2.06, p = 0.12), clutch size (glm: DF = 4, $\chi^2 = 2.11$, p = 0.72), or reproductive output (glm: DF = 4, $\chi^2 = 1.03$, p = 0.90) the following year. Interestingly, females clipped in 2011 returned with significantly higher baseline CORT levels the following year than females that had been in the control group (ANCOVA: DF = 4,22, F = 4.89, p = 0.006; treatment: t = –2.90, p = 0.008; Fig. 6). We used a post hoc t-test analysis to determine how return clipped females compared to similarly aged birds that were not previously included in a manipulation (i.e. the average incubation CORT level for birds present in 2012). Returning birds that were previously clipped exhibited higher baseline CORT levels than unmanipulated birds (post hoc t-test: DF = 40, t = 1.75, p = 0.04; Fig. 6). Finally, regardless of previous treatment or habitat type, birds with higher CORT levels at the nestling provisioning stage in 2011 returned with higher

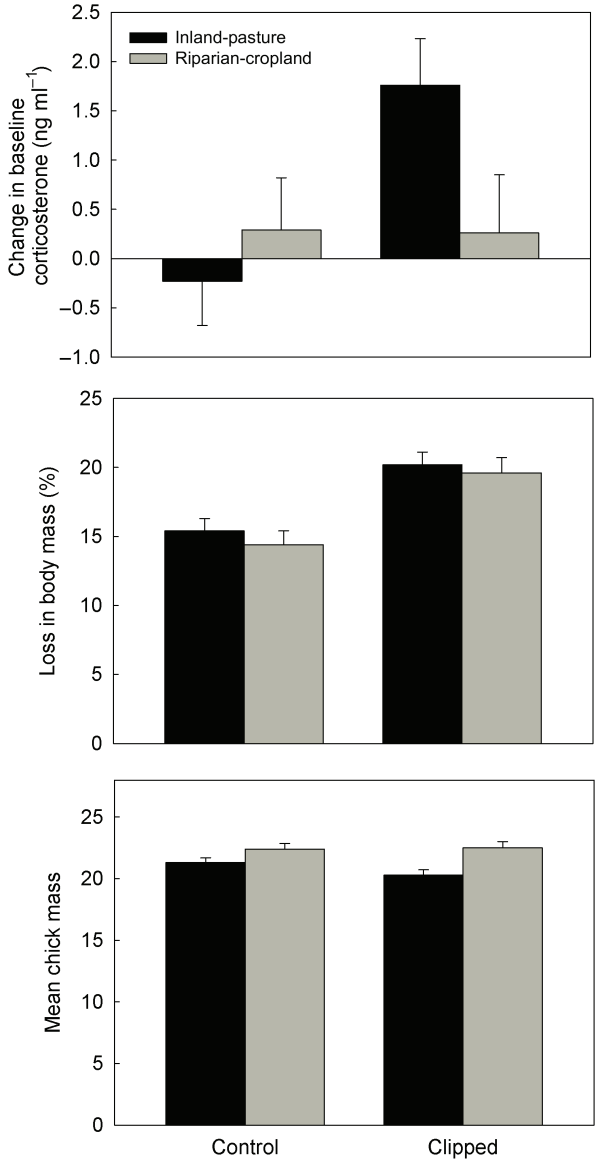

Figure 5. Change in baseline corticosterone over the breeding season, loss in body mass over the breeding season (late incubation to mid-nestling provisioning), and chick mass of control and manipulated (feather-clipped) female tree swallows in two habitat types (inland–pasture and riparian–cropland).

baseline CORT levels in 2012 (prior CORTlevel: $t = 2.56$, $p = 0.02$; Fig. 7).

Discussion

Despite habitat type differences in initial reproductive investment, provisioning rate, and offspring mass, baseline CORT levels at the incubation and nestling provisioning stage in female tree swallows did not differ between habitat types. An experimental decrease in foraging profitability (i.e. ability to acquire food resources resulted in lower female provisioning rates and greater losses in body mass from the late incubation to mid-nestling provisioning stage, but these effects manifested independent of habitat type. However, the foraging profitability manipulation resulted in a habitat-type specific increase in baseline CORT levels (Fig. 5), occurring only in the inland–pasture habitat. Despite this influence on baseline CORT levels, the manipulation did not concomitantly result in lowered offspring mass, output, or female survival (return rate) to the following year. Nonetheless, females returning to the breeding site the following year that had previously been feather-clipped returned with higher baseline CORT levels than birds previously in the control group or those breeding in the area for the first time, regardless of habitat type. Finally, across treatments and habitat types, females with higher levels of baseline CORT in 2011 returned with higher baseline CORT in the following year. Our results represent one of very few attempts to simultaneously investigate the linkages between habitat type, GCs, and fitness in conjunction with an experimental manipulation (but see Lanctot et al. 2003, Sheriff et al. 2009, D'Alba et al. 2011, Hayward et al. 2011), while also attempting to control for the broader contextual drivers of variation in baseline GCs. Overall, the important finding is that baseline GCs were not simultaneously associated with

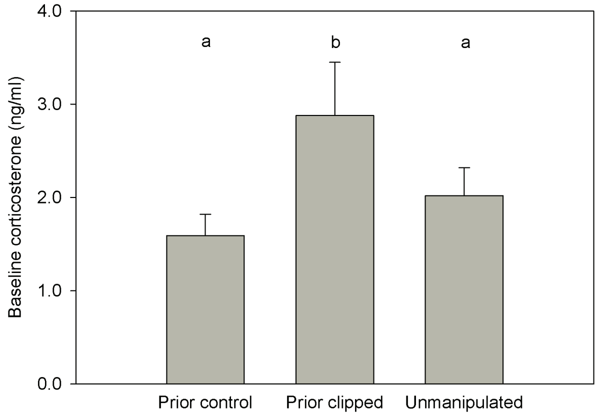

Figure 6. Baseline corticosterone levels of control ($n = 17$), manipulated (feather-clipped; $n = 12$), and previously unhandled (unmanipulated; $n = 30$) female tree swallows at the incubation stage in the year following the manipulation.

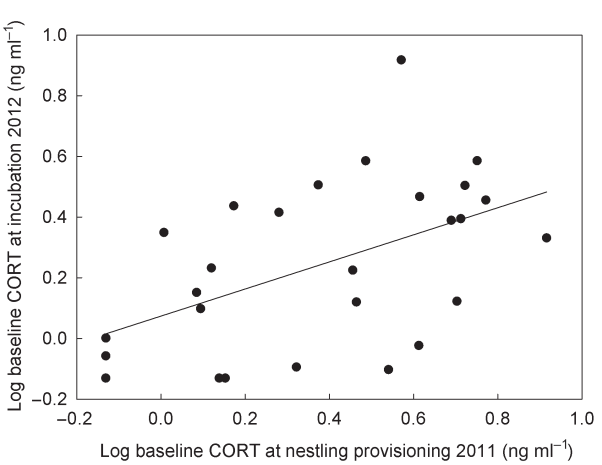

Figure 7. Relationship between baseline corticosterone (CORT) levels in 2011 (at the nesting provisioning stage) and baseline CORT levels in 2012 (at the incubation stage) ($n = 27$; overall model $R^2 = 0.47$).

fitness outcomes and variation in environmental quality in a representative aerial insectivore.

Variation in habitat type and female investment without variation in baseline glucocorticoids

Breeding female tree swallows in inland–pasture habitats had greater local availability of flying insect biomass (daily) during the egg-laying/incubation stage compared to females in riparian–cropland habitats. Tree swallows are classified as income breeders (sensu Drent and Daan 1980), acquiring all of their energetic resources for reproduction on the breeding grounds (Winkler and Allen 1995, 1996). In particular, insects in the days prior to egg laying are a strong predictor of clutch size in this species (Hussell and Quinney 1987). It is therefore fair to conclude that the quality of the habitat (i.e. availability of food resources) in inland–pasture likely resulted in a greater initial investment (larger clutches) compared to females in riparian–cropland habitat. Importantly, females in both habitat types initiated laying on similar dates, had similar incubation body masses, and laid eggs of the same size, indicating that the difference in clutch investment was likely due to a habitat-specific environmental effect rather than differences in underlying female quality. Females may anticipate food resources throughout the nesting attempt based on early availability, laying a clutch size that is expected to maximize their own individual recruitment of offspring in a given habitat (Perrins and Moss 1975). However, during offspring provisioning, while insect resources remained similar to egg-laying levels in riparian–cropland habitat, levels decreased from egg-laying/incubation levels in inland–pasture habitat. This, along with other features of the habitat, may have influenced female provisioning rates, as females raising offspring in riparian–cropland foraged at greater rates per chick than those in inland–pasture habitat. As inland–pasture is characterized by greater nest disturbance, parents may be less apt to leave their nests for extended periods of time without risking loss of chicks and may therefore adjust behaviourally (Fontaine and Martin 2006), taking shorter but more frequent foraging trips. In addition, birds nesting in riparian–cropland habitat have proximate access to a large water body (the Grand River) which may provide a buffer of food resources when weather conditions such as high wind or cooler temperatures decrease local insect availability at their nesting site. Indeed, insect boluses obtained from foraging females in riparian–cropland habitat had a greater proportion of *Ephemeroptera* mayflies, an order of insects which are associated with open water (Kriska et al. 1998), than those in inland–pasture habitat (Madliger unpubl.).

Although provisioning rates were lower in riparian–cropland habitat, chicks were approximately 10% heavier, on average, than those raised in inland–pasture habitat. Although we cannot quantify inter-annual realized chick survival due to high dispersal in juveniles (Winkler et al. 2004), since chick survival in this species is correlated with growth in the nest (McCarty 2001), females would appear to accrue a fitness benefit by nesting in the riparian–cropland habitat. Previous work has shown that the growth of insectivorous nestling birds is positively related to daily metabolized energy (Bryant and Bryant 1988). Therefore, potentially higher quality insect resources during the nestling provisioning stage in riparian–cropland habitat, particularly before chicks reach thermoregulatory capacity (4–5 days in average-sized broods; Dunn 1979), could have enabled females spend more time brooding, allowing chicks to gain greater mass during this time (Klaassen et al. 1994, Morbey and Ydenberg 2000). Moreover, chicks in inland–pasture habitat were being fed more heavy-bodied flies (*Diptera*) and less mayflies (*Ephemeroptera*) (Madliger unpubl.); as a result, although bolus size may be equivalent, amount of digestible protein, fat, micronutrients or total energy content could differ based on bolus composition (Bell 1990, Razeng and Watson 2015), potentially leading to differences in chick growth.

Despite differences between habitat types in terms of food availability, female investment, provisioning rate and chick mass in control birds, we could not detect any differences in average CORT levels between habitats at the incubation or nestling provisioning stage. Overall, our results in control birds across two relevant habitat types therefore reinforce the potential for a disconnect between habitat quality, GC levels, and fitness at the average level in some species. Similarly mixed findings have recently been reported in different populations of the long-lived black-legged kittiwake *Rissa tridactyla*. While Satterthwaite et al. (2012) reported stronger relationships between GCs and environmental indices as compared to relationships between productivity and environmental indices, Lanctot et al. (2003) found that baseline GCs were not consistently representative of forage availability and were not able to predict hatching or fledgling success. The authors concluded that counts of active nests or chicks could provide more reliable estimates of colony productivity than GC metrics. Unfortunately, regular reproductive monitoring in many species is not feasible and GCs may not represent an employable alternative for ascertaining population-level productivity or environmental quality. While it could be argued that in the tree swallow system GC levels earlier in the season (i.e. during the pre-laying or egg laying stage) may be more representative of habitat differences, logistically these samples are very difficult to obtain and capturing females at this time can cause abandonment (D. Hussell pers. comm.). Finally, without validation of fitness effects we could have falsely concluded that the lack of difference in average GC levels between habitat types was an indication that they are of equivalent quality for nesting tree swallows.

A decline in environmental quality alters baseline glucocorticoids in a habitat-specific manner without altering fitness

An extended period of decreased ability to acquire food resources via feather clipping caused lower female provisioning rates and greater losses in body mass compared to control females across both habitat types. While the pattern of lower female provisioning rate in riparian–cropland habitat compared to inland–pasture habitat was maintained across clipped groups, overall mass loss did not differ by habitat type indicating that there may be a physiological ‘ceiling’ where individuals are unwilling to lose additional somatic condition without risking abandonment of the brood (Chaurand and Weimerskirch 1994, Velando and Alonso-Alvarez 2003, Spée et al. 2010). Indeed, in our study, we did not record any brood abandonment as a result of the feather clipping manipulation; more likely, females lowered their provisioning rate and energy expenditure to maintain a certain level of investment in their brood. Overall, the greater mass loss in clipped birds is likely due to a combination of increased energetic demand during flight (Ardia and Clotfelter 2007) and an adaptive change in mass to maintain wing loading (Freed 1981, Norberg 1981, Neto and Gosler 2009, Boyle et al. 2012) in compensation for the loss of wing surface area. Importantly, even though total (male and female combined) provisioning rate to the nest differed between habitat types, it did not differ between clipped and control nests within a habitat type. As a result, it appears that males compensated for decreased foraging ability in clipped females (Patterson et al. 2011), likely leading to the lack of difference in offspring mass and output between control and clipped nests in a given habitat type.

Despite declines in provisioning rate and body mass in manipulated birds in both habitat types, baseline CORT only increased in females in inland–pasture habitat. In contrast, manipulated birds in riparian–cropland habitat showed very little change in CORT, with patterns similar to control birds in both habitat types. Ultimately, changes in baseline CORT were therefore not a consistent reflection of exposure to an environmental change, instead responding to the decrease in foraging efficiency in a habitat-specific manner. From a proximate (mechanistic) sense, there are a number of factors that may be contributing to this context-dependent pattern. First, while birds in inland–pasture habitat invested in larger clutches and hatched more offspring, they did not fledge a greater number of offspring, indicating that a larger relative amount of investment was lost subsequent to hatching in inland–pasture habitat. As a result, while females in inland–pasture habitat initially invested more in larger clutch sizes based on resources available during the laying period, these birds are ultimately raising this larger brood in a habitat that had a significant reduction in food resources during the chick provisioning stage. This disconnect between expected and realized resources may have increased total workload (Nilsson 2002) in feather-clipped females and led to the higher subsequent baseline CORT levels that we observed (Silverin 1982, Love et al. 2004, Bonier et al. 2011, Crespi et al. 2013). A second, but not mutually exclusive explanation, is that manipulated birds in inland–pasture habitat had higher provisioning rates than manipulated birds in riparian–cropland habitat, and the same provisioning rates as control females in riparian–cropland habitat despite having decreased flight efficiency, likely raising allostatic load and associated baseline CORT levels in comparison to other groups. This is likely especially evident in a species such as tree swallows with a high energetic requirement for flight (Williams 1988); females must continue to forage on the wing for their offspring and their own self-maintenance.

The manipulation in 2011 was reflected in baseline CORT levels a full year later, as returning birds in 2012 from the manipulated group had higher CORT levels than returning control birds. In addition, regardless of treatment, birds with higher baseline CORT during nestling provisioning in 2011 returned with higher baseline CORT levels at incubation the following year. While long-term repeatability of baseline GCs has been reported in some cases, a review of available studies found that high repeatability appears less likely over longer time periods (Ouyang et al. 2011, Pavitt et al. 2015). It is possible that individuals with higher baseline GCs are experiencing greater energetic demand in one breeding season and may also find the overwintering and subsequent breeding season similarly demanding (Angelier et al. 2010). However, this potentially greater allostatic load did not predict changes in current reproductive output or inter-annual return rate (a proxy of survival) in our population, or another population of tree swallows in New York, USA (Patterson et al. 2011). More specifically, despite an increase in CORT in females manipulated in inland–pasture habitat, we did not observe lowered chick mass, reproductive output, or return rate in this group. In addition, effects on fitness did not manifest in the following year as there was no influence of the manipulation, or habitat type, on subsequent timing of laying, initial investment (clutch size), body condition, or reproductive output. As a result, measuring CORT between habitat types in the face of a change in food availability, without the measurement of fitness consequences, would have led to the erroneous conclusion that individuals in inland–pasture habitat are more challenged by their environment (i.e. more disturbed) (Bonier et al. 2009) than individuals in riparian–cropland habitat, even though the consequences for downstream success were negligible. Crucially, these consequences were observed over a small spatial scale (all boxes are within an 8 km radius), interconnected by juvenile (and occasionally) adult dispersal (Madliger unpubl.), and well within the spatial range where females seek extra pair copulation (Dunn and Whittingham 2005). As a result, differences between habitat types in CORT response to the manipulation are not likely attributable to genetic differences in CORT physiology, or to selection against certain CORT phenotypes (Bonier et al. 2006, Bauer et al. 2013) which draws further attention to the importance of considering spatial scale when comparing GCs across habitat types.

Although we may predict from a life-histories point of view that tree swallows would be strongly affected by the habitat in which they breed and by the manipulation (i.e. they are short-lived, only reproduce once per season, and invest heavily within each breeding attempt), they may in fact possess a relatively high capacity to take on additional workload within the breeding season or recover well during wintering following a period of extra workload. For example, an experimental manipulation of increased brood size performed in three consecutive years in this species on the same females did not detect any changes in offspring size, parental survival, or future fecundity (Wheelwright et al. 1991). This further reiterates the importance of complementing investigations of GC–environment relationships with measures of fitness (Busch and Hayward 2009); while it may be expected that a certain severity of environment or increased workload would result in fitness effects, especially in conjunction with elevated GC levels, many species may be able to make physiological or behavioural adjustments. Studies across a diversity of habitat types representing greater disparity in food resources, or other aspects of environmental quality, would further clarify whether thresholds exist where GCs become stronger indicators of potential population dynamics (Romero and Wikelski 2001, Suorsa et al. 2003).

Additionally, our results call to attention the possible complexity of interpreting varying GC levels across sites due to unmeasured variables masking potential patterns. More specifically, although females across habitat types may have been experiencing different levels of challenge as indicated by CORT levels, flexibility in mate behaviour appeared to compensate for potential negative fitness effects. Therefore, underlying differences in mate quality or behaviour, or other aspects of social structure (e.g. helpers) that may not be readily observable can cause a disconnect between GC levels and fitness metrics if the sexes experience environmental effects differently (Wasser et al. 1997, Newcomb Homan et al. 2003, Bonier et al. 2006, Hayward et al. 2011, Strasser and Heath 2013, Riechert et al. 2014), or respond context-dependently to environmental alteration (e.g. adjusting investment based on sex ratio of offspring: Harding et al. 2009). It also remains unclear how year and site differences may interact to influence relationships between habitat quality, GCs and fitness (Lanctot et al. 2003, Riechert et al. 2014).

Conclusion

Moving forward, we urge those interpreting GC levels to validate environment–GC–fitness relationships at both the individual and average level; indeed, few studies have shown strong, experimentally-verified linkages. Notably, two different complications can arise at the average level: 1) differences in GC levels may not be representative of differences in fitness or disturbance level; 2) lack of differences in GC levels may not indicate lack of differences in fitness. Our finding of both circumstances occurring within the same population, the presence of complications under both natural conditions and after a change in environmental quality, and the mixed results across other species is particularly cautioning. The context-dependent nature of GCs is becoming increasingly apparent, with factors such as age, sex, life history stage other physiological traits, and environmental quality independently and/or interactively influencing GC levels. As evidence accumulates across species, it will be integral to attempt to delineate intrinsic characteristics such as lifespan, reproductive strategy, migratory propensity, social structure, etc. that may pin-point when baseline GCs may be most representative of environmental conditions and fitness outcomes. Our findings and the outlined considerations are also highly relevant from an applied perspective, in light of the desire of conservation biologists to apply GCs as conservation monitoring tools.

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