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ASSESSING BASELINE GLUCOCORTICOIDS AS CONSERVATION BIOMARKERS IN A DECLINING AERIAL INSECTIVORE

By

Christine LaVern Madliger

A Dissertation
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Assessing baseline glucocorticoids as conservation biomarkers in a
declining aerial insectivore

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is the result of joint research, as follows: I am the sole author of the General Conclusion (Chapter 6). I am the principle author on all other chapters (Chapters 1 through 5). The General Introduction and the four data chapters are co-authored with my supervisor, Dr. Oliver Love. For all chapters, I primarily developed the major ideas and the experimental design, executed the field and hormonal data collection, and completed the data analysis, interpretation, and writing. For all chapters, Dr. Oliver Love contributed to the experimental design, interpretation, and editing, and provided funding and logistical support for the full scope of the research described therein. Chapter 2 is co-authored with Dr. Christina Semeniuk and Mr. Christopher Harris, who both contributed to experimental design, data analysis, and manuscript editing. Mr. Harris additionally shared in the field data collection and quantified the insect data contained in all chapters while conducting his own Master's research and as a research assistant under the supervision of Dr. Love.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

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II. Declaration of Previous Publication

This thesis includes material from four original papers that have been previously

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Thesis Chapter	Publication title/full citation	Publication status
Chapter 1 (key ideas, text passages, and figure)	The need for a predictive, context-dependent approach to the application of stress hormones in conservation	Published in *Conservation Biology*
Chapter 2	Assessing baseline stress physiology as an integrator of environmental quality in a wild avian population: Implications for use as a conservation biomarker	Published in *Biological Conservation*
Chapter 3	Employing individual measures of baseline glucocorticoids as population-level conservation biomarkers: Considering within-individual variation in a breeding passerine	Under review in *Animal Conservation*
Chapter 4	Conservation implications of a lack of relationship between baseline glucocorticoids in fitness in a wild avian population	Under review in *Ecological Applications*
Chapter 5	Do baseline glucocorticoids simultaneously represent fitness and environmental quality in a declining aerial insectivore?	Under review in *Oikos*
Chapter 6 (passage of text; less than one page)	The power of physiology in changing landscapes: Considerations for the continued integration of conservation and physiology	Published in *Integrative & Comparative Biology*

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ABSTRACT

Conservation biologists are increasingly incorporating a diversity of integrative approaches to monitor, manage, and mitigate the growing threats to biodiversity imparted by climate change and other anthropogenic pressures. Over the past 15 years, stress hormones (i.e., glucocorticoids: corticosterone and cortisol) have been gaining considerable attention as sensitive physiological biomarkers of wildlife disturbance. However, despite a substantial accumulation of studies citing glucocorticoids (GCs) as potential indicators of condition, health, or disturbance, comparatively little is known about their actual utility for conservation monitoring. This thesis aims to validate three key characteristics of baseline plasma GCs that are necessary to their employment as sensitive, predictive biomarkers of wildlife disturbance: 1) correlation with environmental quality; 2) consistency across individuals in response to environmental alteration; 3) relationship with fitness metrics at the individual and population level. I complete these validations across two different reproductive stages in female tree swallows (*Tachycineta bicolor*), a member of the aerial insectivore guild of birds that is in population decline in North America. My results indicate that baseline GCs may not reflect the natural variation in components of the internal and extrinsic environment that are associated with habitat quality or disturbance. In addition, baseline GCs show considerable within-individual variation across the breeding season, and display individually-specific responses to an experimentally-induced change in environmental quality (i.e., a decline in foraging profitability). Further, baseline GC levels do not relate to multiple metrics of fitness (offspring quality, reproductive output, or survival) despite the careful control of potentially confounding contexts such as age, reproductive stage,

time of day, and body condition. Finally, at the average level, my results indicate that an environmental perturbation (i.e., a decline in foraging profitability) can have consequences for body condition, behaviour, and current and future baseline GC levels in habitat type-specific ways without concomitant influences on fitness. Collectively, my findings suggest that baseline GCs may not be easily interpretable as individual or population-level indicators of disturbance or fitness. Importantly, these results indicate that GCs cannot be assumed to represent conservation biomarkers across species or time periods without careful validation.

DEDICATION

To my husband, Christopher,

My old, sweet Darwin, and

My rejuvenating spirit, Timber.

You are my every day.

ACKNOWLEDGEMENTS

happily performed behavioural observations and nest box checks in scorching farm fields in the middle of the summer, and carefully counted and characterized insect samples in the lab.

Prior to beginning my graduate degree, I had the rewarding experience of working at Ruthven Park National Historic Site. I am forever grateful for the field work and museum experience that I gained by being a part of their organization. A warm thank you to the board members of the Lower Grand River Land Trust Inc., especially Betsy Smith, for their support and encouragement as I tackled new projects. Through Marilynn Havelka's guidance, I developed independence, grant writing skills, creativity, and a great appreciation for the struggles and reward of working within a non-profit environment. My time banding with Rick Ludkin most certainly dictated the trajectory of my scientific career. He instilled in me a fascination of birds and the functional skills to identify and handle them, he was a member of the tree swallow field team, and he always made me feel like I would accomplish any goal I set for myself. I am similarly grateful for my friendship with Nancy Furber. Her emotional support, assistance with fieldwork, and genuine kindness have made my entire experience an even happier one. Finally, in Natalie Campbell I gained a lifelong friend whose patience and dedication to teaching is inspiring.

I extend a special thank you to my two closest friends. First, I cannot imagine my graduate experience without the presence of Meghan Vankosky. Through crafts, baking, shopping, dog walks, cheeseburgers, and long talks she has become my best friend and someone I admire professionally and personally. And second, I am grateful for my close friendship with Jessica Harris who always listened intently to my tales of frustration or excitement.

It is impossible to fully articulate my thanks to my family. My parents, Bonnie and Ken, have been unwaveringly enthusiastic about my academic choices. My mom is a beacon of love and support; everything I've accomplished is because she has stood beside me. My sister, Cheryl, is a constant reminder that finding an inner strength, determination, and passion fosters success and I am forever thankful that we've grown (and continue to grow) through this life together. Nearly 15 years ago I also gained a second family in Susan, Cliff, Jason, Jessica, and Amy Harris. My life is enhanced in every way because they are a part of it. In addition, I have to thank the canine members of my family. First, Timber, who reinvigorated me with his energy and carefree happiness, I am grateful for the laughter and love. And second, Darwin has represented my little oasis of calm throughout my entire undergraduate and graduate degree. He is, undoubtedly, the kindest soul I will ever know.

Finally, to my husband, Christopher: I completed this thesis only because you have been my field partner, my lab mate, and my best friend. You infuse each day with laughter, encouragement, and stability, and I will cherish all of the experiences of the past five years because I shared them with you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 - THE NEED FOR A PREDICTIVE, CONTEXT-DEPENDENT APPROACH TO THE APPLICATION OF STRESS HORMONES IN CONSERVATION*

*This chapter contains material that is the result of collaboration with O. Love that was published in *Conservation Biology* (doi: 10.1111/cobi.12185).

Conservation physiology: Goals and breadth

Due to the alteration of natural landscapes by anthropogenic disturbances and climate change, organisms are continually being exposed to new and enduring environmental challenges (Butchart et al., 2010; Corlett, 2015; Sih et al., 2011). Monitoring and mitigating the impact of environmental change on species or populations of concern is an important goal of conservation biologists (Van Dyke, 2008). While conservation biology was formally outlined in the mid-1980's as a "crisis discipline" (Soulé, 1985), the field has increasingly begun to appreciate proactive approaches to prevent further biodiversity loss (Brooks et al., 2006) and these types of endeavours can be cost-effective (Drechsler et al., 2011). More recently, there has been a call for a diverse conservation toolbox characterized by inter-disciplinary approaches, hypothesis-testing and experimental investigation, an appreciation of the underlying diversity of conservation ethics, and evidence-based conservation that can spur effective action (Tallis and Lubchenco, 2014). In particular, the relatively nascent field of conservation physiology (Wikelski and Cooke, 2006) has the potential to contribute a vast array of tools spanning toxicology, immunology, nutrition, sensory biology, genomics, endocrinology, and many others (Cooke et al., 2013).

The root of the conservation physiology approach is binary. First, physiological systems are sensitive to intrinsic and extrinsic environments, potentially providing earlier detection and greater predictive capacity in the face of environmental change than many

1

other approaches (e.g., demographics) (Ellis et al., 2011). Second, the mechanistic

approach imparted by the measurement of physiology has the power to provide insight

into cause-effect relationships (Carey, 2005). Growing from this basis, physiological

approaches to conservation are also proposed to generate knowledge of population

susceptibilities to various stressors, define particularly sensitive time periods for

disturbance, identify critical habitats, foster captive breeding, translocation, and

reintroduction success, and evaluate the effectiveness of conservation endeavours post

hoc (Besson and Cree, 2011; Carey, 2005; Chown and Gaston, 2008; Cooke and

O'Connor, 2010; Cooke and Suski, 2008; Cooke et al., 2013; Ellis et al., 2011; Kersey

and Dehnhard, 2014; Seebacher and Franklin, 2012; Stevenson et al., 2005; Wikelski and

Cooke, 2006). Finally, the incorporation of physiology into conservation also provides the

opportunity to interpret anthropogenic changes from the perspective of the organism,

rather than the researcher, thereby improving our understanding of which conditions can

constitute a disturbance (Homyack, 2010).

While the field of conservation physiology was only recently formally named,

physiological principles have been incorporated into conservation-relevant investigations

for decades (Cooke et al., 2013; Madliger et al., 2016; Wikelski and Cooke, 2006).

Importantly, successes in the field (i.e., endeavours that resulted in a change in

management, human behaviour, or policy) are characterized by a diverse complement of

physiological metrics (Madliger et al., 2016). Nevertheless, a recent review of

physiological investigations citing application to conservation discovered that

glucocorticoids (i.e., stress hormones such as cortisol and corticosterone) currently

comprise the majority of studies in the field (Lennox and Cooke, 2014). This pattern begs

the question of why such a vast accumulation of investigations of glucocorticoids citing

conservation implications have not led to on-the-ground implementation and subsequent

conservation success.

Glucocorticoid function and measurement

Physiological function

Glucocorticoids (GCs) are metabolic steroid hormones which are secreted as an end-

product of the hypothalamic-pituitary-adrenal (HPA) axis (or the hypothalamic-pituitary-

interrenal (HPI) axis in fish) (Barton, 2002; Sapolsky, 2002; Sapolsky et al., 2000). GCs

are pleiotropic, with influences on intermediary metabolism, immunocompetance, and the

reproductive, thyroid, and growth axes (Sapolsky et al., 2000). The HPA/HPI axis is

activated in the presence of environmental perturbations such as predators or weather

events, but also in response to internal conditions such as low blood glucose levels or

decreased energetic state (Wingfield and Kitaysky, 2002). At baseline levels (Figure

1.1a), GCs have the important, continuous role of maintaining energetic balance by

initiating gluconeogenesis and promoting foraging (Dallman et al., 1993). As a result,

variation in GCs allow individuals to meet both daily energy requirements and the

prolonged energetic expenditures associated with predictable life-history events (e.g.,

migration, rearing offspring) (Dallman et al., 1994; Landys et al., 2006). Indeed, baseline

GC levels show both diel (Figure 1.1a) and seasonal cycles, often peaking in the early

morning in diurnal animals, and showing increases during energetically-demanding points

in the life cycle (Dallman et al., 1993; Romero, 2002).

GCs are probably best known for their role in enabling individuals to respond to

unpredictable, acute events (i.e., over minutes to hours) through their involvement in the

stress response (McEwen and Wingfield, 2003; Romero, 2004; Wingfield et al., 1998).

By increasing within minutes of an acute environmental challenge (Figure 1.1b), GCs act to mobilize stored energy reserves (glycogen and lipids), enhance immune function, promote escape behaviours, and suppress non-survival activities such as courtship or copulation (Sapolsky et al., 2000; Wingfield, 2005; Wingfield et al., 1998). These stress-induced concentrations are often 5-50 fold higher than baseline levels (Cockrem, 2013). While short-term elevations of baseline and stress-induced GCs are thought to be beneficial for mediating energetic intake (Astheimer et al., 1992; Breuner et al., 1998; Lohmus et al., 2006; Wingfield et al., 1998), prolonged elevation of GCs over days to weeks (i.e., "chronic stress") at stress-induced levels stimulates the breakdown of lipid and protein stores and can negatively impact health, growth, and fitness by suppressing immune function and inhibiting the reproductive axis (Sapolsky et al., 2000).

The actions of GCs at baseline as compared to stress-induced levels have traditionally been thought to be mediated by two different intra-cellular receptor types with different affinity (Breuner and Orchinik, 2002; Funder, 1997). High-affinity (mineralocorticoid) receptors (MR) bind GCs at low (i.e., baseline) levels, while low-affinity (glucocorticoid) receptors (GR) were thought to bind only during acute activation of the HPA axis (Breuner and Orchinik, 2002; Funder, 1997; de Kloet et al., 1990, 1998). As a result, until just recently, baseline and stress-induced concentrations of GCs were thought to have separate physiological and behavioural consequences, and many authors suggested they should be viewed as two separate, but complementary hormonal systems (Bókony et al., 2009; Bonier et al., 2009a; Romero, 2004). However, recent evidence suggests that the physiological processes of GCs would be better considered on a continuous gradient, as low-affinity receptors are likely to also be involved in energy balance and baseline HPA activity (Breuner, 2011; Crespi et al., 2013; Herman et al.,

2003; Joëls et al., 2008; Marzolla et al., 2012). Overall, it is becoming increasingly clear that elevations in GCs well-below those associated with the acute stress response can have consequences for reproduction and survival (Breuner, 2011).

Sample media and relevance to conservation

Glucocorticoids can be measured in a variety of different media including at baseline and stress-induced levels in plasma and saliva, and over integrated time periods in feces, urine, and outer integuments such as claws, shed skin, feathers, and hair (Sheriff et al., 2011). Each type of GC measure represents HPA/HPI activity over different time periods, and requires different sampling, storage, and analysis protocols (Bortolotti et al., 2008; Sheriff et al., 2011; Touma and Palme, 2005). For example, plasma measures represent relatively pin-point indicators of hormone levels, fecal samples correspond to time periods of hours to days, and feathers and hair likely integrate HPA/HPI activity over the entire time period of their growth (Sheriff et al., 2011).

While stress-induced levels of GCs have been related to behaviour and fitness metrics in some cases (rev. in Breuner et al., 2008), making them potentially useful predictors of organismal response to environmental change (Angelier and Wingfield, 2013; Breuner, 2011; Romero, 2004), collection requires an extended (30-60 minute) restraint protocol and repeated sampling that can be difficult for conservation managers to justify, accomplish logistically, or afford to analyze. As a result, baseline blood and salivary sampling and non-invasive integrated measures from feces, hair, and feathers are the most likely target of conservation managers (Dantzer et al., 2014). Furthermore, from a biological relevance perspective, baseline GCs are thought to integrate an organism's intrinsic and extrinsic environment due to their primary role in energy regulation (Dantzer

et al., 2014; Wingfield, 2005). As the difference between an individual's energetic requirements and the energy available (termed "allostatic load") becomes larger, baseline GCs generally rise (McEwen and Wingfield, 2003). Both environmental and social perturbations (e.g., changes in food abundance, predator pressure, social dominance, parasite load) can increase allostatic load because they raise the costs of maintaining energetic balance (McEwen and Wingfield, 2003). Therefore, given that many conservation-relevant disturbances can influence general energy expenditures or the ability of organisms to acquire sufficient resources, baseline GCs should theoretically provide a powerful reflection of organismal state (see Figure 2.1 - Chapter 2).

It is important to consider how different types of ecological stressors may influence GC levels and which may be most relevant in the context of conservation monitoring. Figure 1.2a displays a hypothetical pattern of GCs over a one-week period. Diel variation occurs with baseline GC levels peaking prior to the onset of daily foraging. The first day is characterized by an acute stressor at 10:00 am (as indicated by the marked increase in GC levels) which could be representative of being chased by a predator or capture and handling. This increase in GCs is transitory and imparts short-term benefits to the animal in escaping and recovering from the stressor, but is not expected to result in long-term negative consequences to fitness. Figure 1.2b is a situation analogous to what many researchers term "chronic stress" (Dickens and Romero, 2013; McEwen and Wingfield, 2003; Romero et al., 2009; Sapolsky et al., 2000). In this scenario, a stressor occurs continuously or remains persistent in the environment and cannot be mitigated by the individual (e.g., a predator chase that results in severe injury or illness; a severe, long-term weather event). The maintenance of GCs at stress-induced levels leads to pathological consequences including inhibition of the reproductive axis, protein loss,

immune system depression, disruption of cellular function, and ultimately death (Sapolsky et al., 2000). In this case, the high levels of GCs themselves contribute to pathology and are as much a symptom as an indication of severe, rather than sub-lethal, effects. In this way, consequences may be too severe or too rapid to allow for proactive mitigation. In contrast to the above scenarios, the situations in Figure 1.2c and 1.2d are of much greater interest to conservation managers. First, in Figure 1.2c, a stressor (e.g., a predator chase that results in an injury or illness that necessitates increased daily energy expenditure over subsequent days to overcome) may result in stress-induced levels of GCs at onset, but is characterized by a gradual return to a new elevated baseline GC level as the individual adjusts to the new circumstances. Second, in Figure 1.2d, a more gradual environmental change (e.g., decreased food availability, increased competition) which is not abrupt or threatening enough to activate an acute stress response, but which increases the difficulty of survival, leads to a gradual elevation of GCs within baseline levels. In both cases (Figure 1.2c and d), organisms may suffer somatic or behavioural consequences as a result of a decrease in available energy, but the repercussions will be sub-lethal or of consequence over a longer timeframe. Additionally, it is unclear what the costs of the elevated GCs themselves might be as long as they remain below acute levels. As a result, GC levels may represent biomarkers of lowered fitness (e.g., decreased reproductive success, propensity to breed, survival probability) and therefore could provide an early warning signal of disturbance to allow for proactive mitigation measures.

Baseline glucocorticoids as biomarkers: What do we need to know?

Although the field of conservation physiology has grown rapidly over the past decade (Lennox and Cooke, 2014), researchers and practitioners still point to gaps in the

translation of physiological knowledge and data to successes in conservation (Cooke, 2014; Cooke and O'Connor, 2010; Lennox and Cooke, 2014). It is particularly interesting that, despite such a large complement of studies in GCs in the context of conservation physiology (i.e., 49% of all conservation physiology studies published since 2006; Lennox and Cooke, 2014), fewer success stories than predicted have emerged that employ this physiological marker (Madliger et al., 2016). I posit that this pattern is the result of a lack of directed validations regarding the utility of GCs in conservation monitoring prior to their widespread measurement.

Given the general perception of GCs as "stress" hormones, much of their application to conservation goals has been based on the overly-generalized assumption that increased levels are always indicative of lower quality or "stressful" environments (Baker et al., 2013; Bonier et al., 2009a; Busch and Hayward, 2009; Reeder and Kramer, 2005). Viewed in this way, the interpretation of changing GC levels and their application is relatively straightforward. However, mounting evidence suggests that GC physiology is much more complex and context-dependent (Baker et al., 2013; Bonier et al., 2009a; Romero, 2004; Romero et al., 2009), making this approach over-simplified and controversial, and likely accounting for some of the mixed results seen across studies. Instead, to effectively employ baseline GCs, conservation biologists must take a predictive, physiological approach, informed by validations of key characteristics of GCs necessary to their interpretation as biomarkers. Specifically, GCs must: 1) correlate with environmental quality; 2) change in response to environmental alteration consistently across individuals; and 3) relate to metrics important to population viability (i.e., fitness - reproductive output and survival) at individual- and population-levels. To satisfactorily

investigate each of these characteristics, the underlying ecological and life history contexts which can influence GC levels must be considered (Crespi et al., 2013).

Appreciating the context-dependency of glucocorticoid levels

Homeostasis occurs when a physiological trait is maintained at a given level or set point. In contrast, the concept of allostasis allows for the adjustment of physiology depending on life history demands or environmental conditions, and has been described as "maintaining stability through change" (McEwen and Wingfield, 2003; Wingfield, 2005). From the perspective of GC levels, allostasis allows for predictions by integrating the energetic expenditures related to predictable aspects of the life cycle (e.g., diel cycles, reproduction, migration, moult, hibernation) and unpredictable environmental events (e.g., injury or parasite load, changes in social structure, weather, food availability, competition) (Wingfield, 2005). Indeed, GCs have been shown to vary across a number of predictable contexts (see examples in Figure 1.3) and expectations for GC levels throughout the day, season, year, and lifetime of an individual, and across species with various life history strategies, must be adjusted to accommodate their physiological role in mediating energy availability. Therefore, by promoting an emphasis on the primary energetic role of baseline GCs, the lens of allostasis should allow conservation biologists to account for the context-dependent nature of GC levels and make informed predictions of how individuals will respond to an altered environment. As a result, I take an energetic perspective and a context-dependent approach to the interpretation of GC levels throughout this thesis.

Linking glucocorticoids to environmental variability

If GCs are to be employed as biomarkers of habitat quality or disturbance, they must reflect the components of the environment relevant to targeted wildlife. One of the most common approaches to investigating GC levels under the umbrella of conservation involves the comparison of GC levels across sites with (or less commonly, gradients of) differing environmental conditions or anthropogenic pressures. For example, investigations have compared GC levels of individuals or sites experiencing differing vegetation or habitat structure (Bauer et al., 2013; Cash and Holberton, 2005; Homan et al., 2003; Suorsa et al., 2004), forest practices (Leshyk et al., 2012; Suorsa et al., 2003), shelter (D'Alba et al., 2011), food availability (Jenni-Eiermann et al., 2008; Riechert et al., 2014), recreational or tourism pressure (Arlettaz et al., 2014; Creel et al., 2002; Müllner et al., 2004), predation risk (Clinchy et al., 2011; Hik et al., 2001), hunting pressure (Gobush et al., 2008), urbanization (Fokidis et al., 2009; French et al., 2008), road density (Butler et al., 2013), or other human-related presence (Ahlering et al., 2011; Strasser and Heath, 2013).

Despite the diversity of studies investigating baseline GCs and environmental variability, it can be difficult to garner the value of measuring GC levels as biomarkers of disturbance for a number of key reasons. First, as outlined above, GC levels can vary temporally across daily (Breuner et al., 1999; Heintz et al., 2011; Tarlow et al., 2003), seasonal (Goymann et al., 2006; Quispe et al., 2014; Reneerkens et al., 2002; Rubenstein and Wikelski, 2005; Wack et al., 2008), and lifetime scales (Angelier et al., 2006; Hämäläinen et al., 2015; Riechert et al., 2012; Sapolsky, 1992). As a result, differences in GC levels between locations could be attributable to differences in age ratios, time of day of sampling, or reproductive stage, for example (Baker et al., 2013; Tarlow and

Blumstein, 2007). Second, populations which lack inter-connectivity may differ in GC levels due to genetic differences, as opposed to extrinsic environmental conditions (Bauer et al., 2013). Third, it is currently unclear over which spatial scales differences in environmental conditions can influence GC levels and whether multiple aspects of environmental quality can be simultaneously reflected in GC levels, particularly across gradients. Furthermore, manipulations of environmental quality performed in the field across discrete habitat types or gradients are particularly rare (D'Alba et al., 2011; Hayward et al., 2011; Lanctot et al., 2003; Sheriff et al., 2009), leaving the question of whether GCs can integrate further environmental change unanswered. Finally, and most importantly, few investigations have included metrics of fitness. As a result, we have little information on the likelihood of linkages between environmental variability, baseline GCs, and fitness and how they may change over different stages of the life cycle (Baker et al., 2013; Gesquiere et al., 2008); however, this information is paramount to pin-pointing when GC levels may be most useful for population monitoring. Overall, these gaps in knowledge limit our ability to interpret differences in GC levels as biologically relevant to organismal health and population perpetuity. I address these gaps in Chapter 2 and Chapter 5 of this thesis.

Considering intra-individual variability

As mediators of energetic balance, life history trade-offs, and developmental transitions in vertebrates (Boonstra, 2005; Crespi et al., 2013; Romero et al., 2009; Wingfield and Sapolsky, 2003), baseline GC levels can show a large degree of intra-specific and intra-individual variation (Cockrem, 2013; Crespi et al., 2013; Romero, 2004). It has been assumed that the measurement of GCs from samples of individuals over time can provide

11

a reliable population-level indicator of disturbance, condition, and fitness (i.e., that monitoring the mean baseline GC values of sub-samples of individuals over time represents a proxy of the overall population trend). However, to my knowledge, no study has yet validated a key assumption of their use in that way: baseline GC levels must change in a similar (i.e., predictable) manner in response to the same environmental change across individuals. In contrast, individually-specific responses to environmental change can result in two complications for the interpretation of GC levels in the context of population condition or fitness: 1) a lack of difference in GC levels at the average (i.e., population level) may actually be underpinned by a high degree of change at the individual level that could be a sign of physiological "dysregulation" or disturbance; 2) changes at the average level may be difficult to observe if within-individual variation is high, particularly with small sample sizes (i.e., the population sizes likely to be monitored by conservation biologists). Studies which have addressed the repeatability of GC levels across various time spans (from days to years) have found highly variable results (rev. in Ouyang et al., 2011a), and to my knowledge, no study has approached this question from the perspective of examining GCs as potential conservation biomarkers (Cooke and O'Connor, 2010). I address this gap in Chapter 3 of this thesis.

Establishing the relationship between baseline glucocorticoids and fitness

At the core of assessing the applicability of GCs as conservation biomarkers is validating that a predictable relationship between GCs and the fitness metrics that drive population demographics exists (Busch and Hayward, 2009). Given the traditional and generalized assumption that higher GC levels are associated with an individual or population that is disturbed or in poorer condition (i.e., interpreted as "stress"), researchers have often

predicted these assumed linkages should then carry-over and be linked with reduced relative fitness (Bonier et al., 2009a; Busch and Hayward, 2009; Dantzer et al., 2014; Reeder and Kramer, 2005). This posited negative relationship between GCs and fitness has been coined the *Cort-Fitness Hypothesis* (Bonier et al., 2009a) and is principally based on the reasoning that high levels of GCs are indicative of individuals experiencing challenging conditions requiring reallocation of resources away from reproduction (e.g., Love et al., 2004; Silverin, 1982, 1986; Spée et al., 2011).

However, a growing body of work in free-living systems is indicating that the relationship between GC physiology and fitness is not nearly as simplistic (Bonier et al., 2009a; Busch and Hayward, 2009; Dickens and Romero, 2013; Romero, 2004; Romero et al., 2009). Specifically, just as with absolute baseline GC levels, the relationship between GCs and fitness can vary based on a number of contexts including sex, age, life-history stage, and environmental quality (Angelier et al., 2010; Bonier et al., 2009b, 2011; Ebensperger et al., 2013; Jaatinen et al., 2013). For example, elevated GCs during energetically-expensive life-history stages such as breeding and migration do not necessitate that an organism is disturbed by its environment, but instead can represent an adaptive response to promote beneficial foraging behavior, a phenomenon recently described by the *Cort-Adaptation Hypothesis* (Bonier et al., 2011). Indeed, during stages of high reproductive investment (e.g., offspring provisioning) a positive relationship between baseline GCs and both reproductive success and survival has been observed (Bonier et al., 2009b; Crossin et al., 2012; Escribano-Avila et al., 2013; Love et al., 2014; Ouyang et al., 2011b). Currently, it remains unclear whether the simultaneous consideration of additional contexts such as reproductive stage, fitness metric, environmental quality, and condition metrics (e.g., size-corrected body mass, fat stores,

species-specific condition indices) can improve our ability to predict fitness from baseline GC measures. However, characterizing the relationship between GCs and fitness is currently considered one of the highest priority questions in conservation physiology (Cooke, 2014). I contribute to addressing this gap in Chapter 4 and Chapter 5 of this thesis.

Study system

Tree swallows (*Tachycineta bicolor*) are a short-distance migratory passerine with a breeding range that extends from northern Canada and Alaska south to much of the United States, and an overwintering range spanning the southern United States, Mexico, and Central America (Robertson et al., 1992). The species is a member of a guild of birds known as aerial insectivores, which have been grouped due to commonality in their food source, flying insects. As a whole, aerial insectivores (swallows - *Hirundinidae*, swifts - *Apodidae*, flycatchers - *Tyrannidae*, nightjars - *Caprimulgidae*) in North America are in decline more than any other group of birds, particularly in the northeastern portion of their range, with some species' populations declining by up to 90% in the past 50 years (McCracken, 2008; Nebel et al., 2010). Even tree swallows, which are a relatively abundant and widespread member among the guild and which should have benefited by the provisioning of artificial nesting sites, showed a statistically significant 2.5% decline per year between 1986-2006 (McCracken, 2008). Currently, the causes of the decline in aerial insectivore populations remain unclear; however, phenological mismatches to food resources on the breeding grounds and intensifying agriculture, pollutants, and habitat loss on breeding and wintering grounds are all considered possibilities (Dunn et al., 2011; Fitzgerald et al., 2014; Fraser et al., 2012; Nebel et al., 2010; Nocera et al., 2012, 2014).

Tree swallows are a secondary cavity-nester (i.e., they nest in previously excavated cavities) that readily breed in artificial nest boxes and undergo a single reproductive attempt per year (Robertson et al., 1992). While not considered colonial in the strict sense, tree swallows can nest at relatively high densities in close proximity (10-15 meters apart) (Robertson and Rendell, 1990), but actively prevent conspecifics from nesting nearby and prefer farther differences when provided the choice (Muldal et al., 1985). The species shows high rates of extra-pair paternity (50-89% of nests contain at least one extra-pair offspring), with females actively seeking out extra-pair copulation opportunities (Barber et al., 1996; Dunn et al., 1994; Kempenaers et al., 1999; Whittingham and Dunn, 2001). Females lay approximately 5-6 eggs and solely incubate for approximately 14 days (Robertson et al., 1992). Laying date in this species is a strong predictor of reproductive success, with earlier nesting females generally considered to be of higher quality (Hasselquist et al., 2001; Stutchbury and Robertson, 1988; Wardrop and Ydenberg, 2003; Winkler and Allen, 1996). Following hatch, both parents forage for the offspring, and fledging occurs approximately 18-22 days later (Robertson et al., 1992). Parents feed their offspring a bolus of aerial (flying) insects, foraging primarily within a 100-300 meter radius of their nest location (McCarty, 2001; McCarty and Winkler, 1999; Quinney and Ankney, 1985); however, longer foraging trips are possible, especially during periods of inclement weather and low food availability (Blancher and McNicol, 1991; McCarty and Winkler, 1999). Tree swallows benefit reproductively from the presence of fallow fields and extensive (as opposed to intensive) forms of agriculture (e.g., cattle pastures) (Ghilain and Bélisle, 2008) and nesting success has been shown to be related to local insect availability (Nooker et al., 2005; Paquette et al., 2013; Winkler et al., 2013). The species is relatively short-lived, with an average lifespan of 2.7 years

(Butler, 1988). Females can be aged as second-year (SY - under two years of age) or after-second year (ASY - older than two years of age) based on plumage, while males obtain their definitive plumage prior to their first winter (Hussell, 1983).

Tree swallows are highly suitable for studying the relationships between habitat quality, physiology, and fitness given their: i) high accessibility for reproductive monitoring and hormone sampling (Jones, 2003); ii) settlement in a variety of likely suboptimal, human-disturbed habitats (Ghilain and Bélisle, 2008); iii) key component of habitat quality that can be assessed through measures of insect abundance (Dunn et al., 2011; Hussell and Quinney, 1987); iv) high rate of breeding site fidelity (philopatry) (Winkler et al., 2004). In addition, tree swallows are considered a model organism, having been heavily studied in the context of ecology, toxicology, and environmental quality (Jones, 2003; McCarty, 2001), providing a strong underlying framework of life history information. Finally, their obligate aerial foraging strategy allows for the manipulation of workload and foraging profitability through techniques such as the clipping of flight feathers (Ardia and Clotfelter, 2007; Nooker et al., 2005; Winkler and Allen, 1995). By decreasing access to food resources, this manipulation imparts a biologically-relevant adjustment of environmental quality from the perspective of food availability, especially during demanding stages of the reproductive season. Throughout this thesis I focus my research questions on females as they make the primary reproductive decisions of where and when to invest (i.e., laying phenology), as well as how much to invest (i.e., egg size and clutch size), and they are the sole incubators and thus the most accessible sex.

Research objective and thesis content

This thesis examines *the applicability of plasma baseline GCs as biomarkers of habitat quality and fitness*, with the goal of contributing to their refinement as monitoring tools in the conservation toolbox. I accomplish this objective using a member of the declining avian guild of aerial insectivores. Throughout the thesis, I have combined an experimental manipulation of foraging profitability (employed as a proxy for a decrease in habitat quality from the perspective of food availability), behavioural observations of foraging rate, a broad assessment of habitat features using a Geographic Information System (GIS), a fine-scale assessment of local food availability, detailed reproductive monitoring, and multi-year hormone analysis. By rooting each investigation in the physiological and ecological role of variation in GC levels, I aim to call attention to the importance of validation prior to application in conservation systems.

In **Chapter 2**, I investigate the ability of baseline GCs to reflect multiple components of tree swallow habitat quality, measured along a gradient, at two different reproductive stages. I do so under natural conditions and under the added constraint of a decline in food availability to investigate the first key assumption of GCs as conservation biomarkers: their capacity to integrate environmental variation. In **Chapter 3**, I assess the amount of within-individual variation in baseline GC levels occurring naturally across the reproductive season, and in response to a manipulation of food availability. By characterizing the within-individual patterns of baseline GC levels that may underlie changing average levels, I provide insight into the consequences of highly variable baseline GC levels for their interpretation as population-level indicators of environmental change. In **Chapter 4**, I examine the relationship between baseline GCs and multiple metrics of fitness under natural conditions and in the face of reduced food availability. I

17

consider whether the inclusion of additional contexts such as body condition, underlying

food availability, or reproductive investment can alter or improve the relationship

between GCs and fitness, or whether any of these metrics are better able to predict fitness

in my study system. In **Chapter 5**, I mimic the approach of many GC investigations

citing conservation application and investigate average-level differences in GC levels

across two habitat types that differ in early-season food availability. I incorporate a

manipulation of foraging profitability with observations of foraging rate and fitness

metrics to assess whether, and if so how, average levels of baseline GCs may represent

habitat type and fitness simultaneously. Finally, in **Chapter 6**, I concatenate my findings

to provide conclusions for baseline GCs as conservation biomarkers from an ease of use

and biological relevance perspective, and suggest future avenues of research.

While each chapter and validation is specifically aimed at conservation biologists

currently employing or considering GCs in their systems, an arguably rare approach

(Busch and Hayward, 2009), results are of interest and relevance to ecological,

evolutionary, and conservation physiologists simultaneously. To my knowledge, this is

the first attempt at a multi-faceted, experimental validation of baseline plasma GCs in the

context of conservation. By remaining cognizant of the logistical limitations of working

in systems of conservation concern, my approach is aimed at fostering the development of

a potentially powerful tool for evidence-based (Sutherland et al., 2004), proactive

conservation.

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Figures
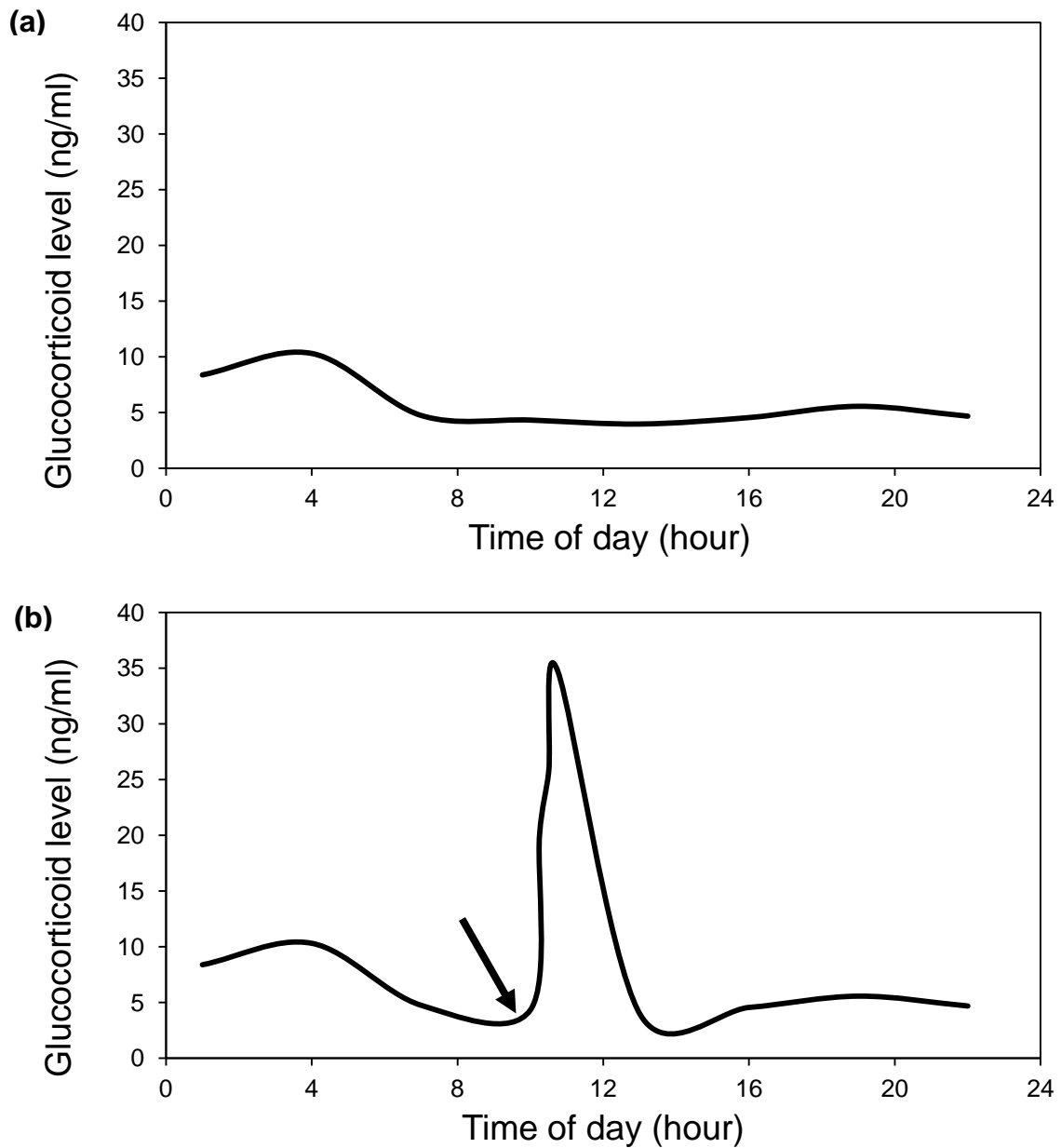
Figure 1.1 - Variation in glucocorticoid levels (modified from data from white-crowned sparrows, *Zonotrichia leucophrys gambelii*, Breuner et al., 1999 and tree swallows, *Tachycineta bicolor*, Franceschini et al., 2009): (a) diel baseline variation over a single day; (b) diel baseline variation with an acute stressor (e.g., capture and handling, predator chase) occurring at 10:00 am as indicated by the black arrow.

(d)
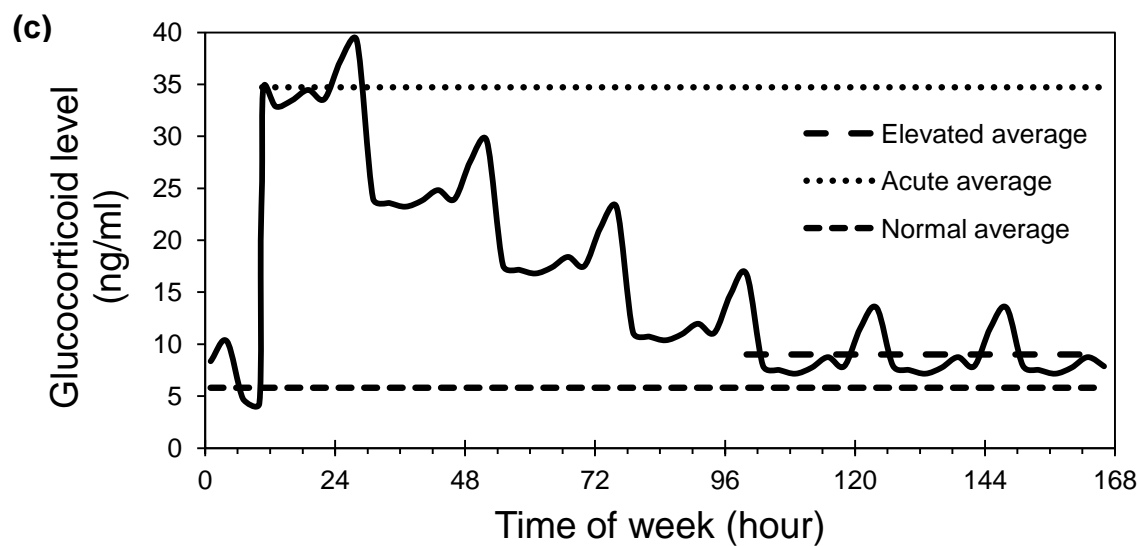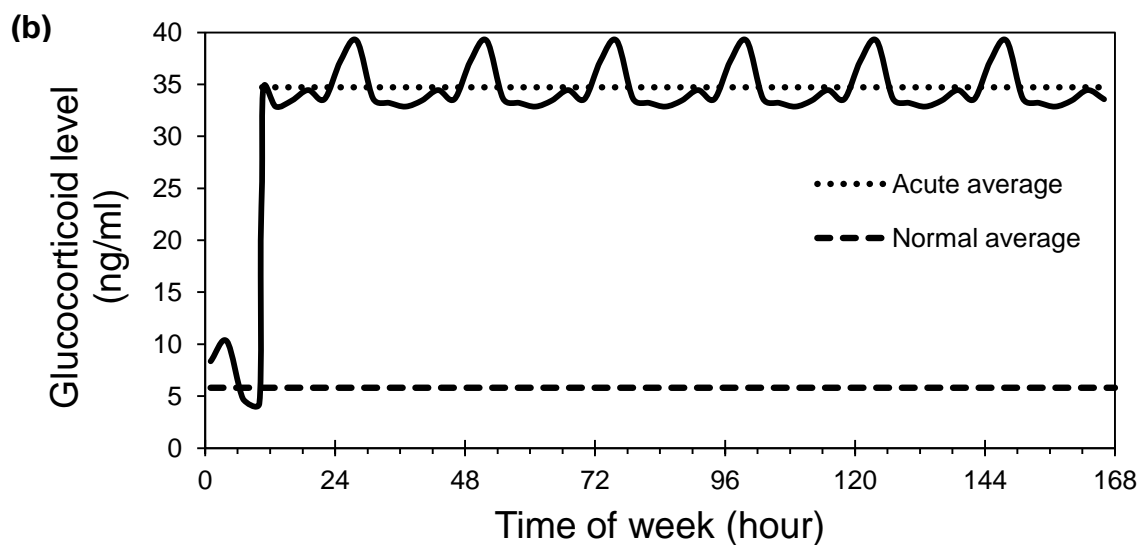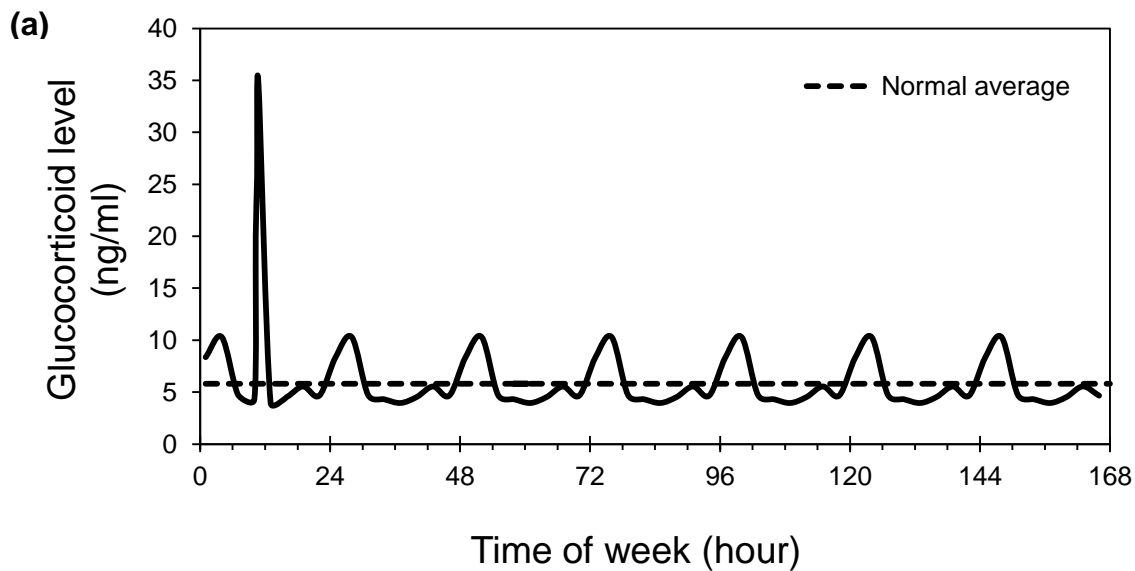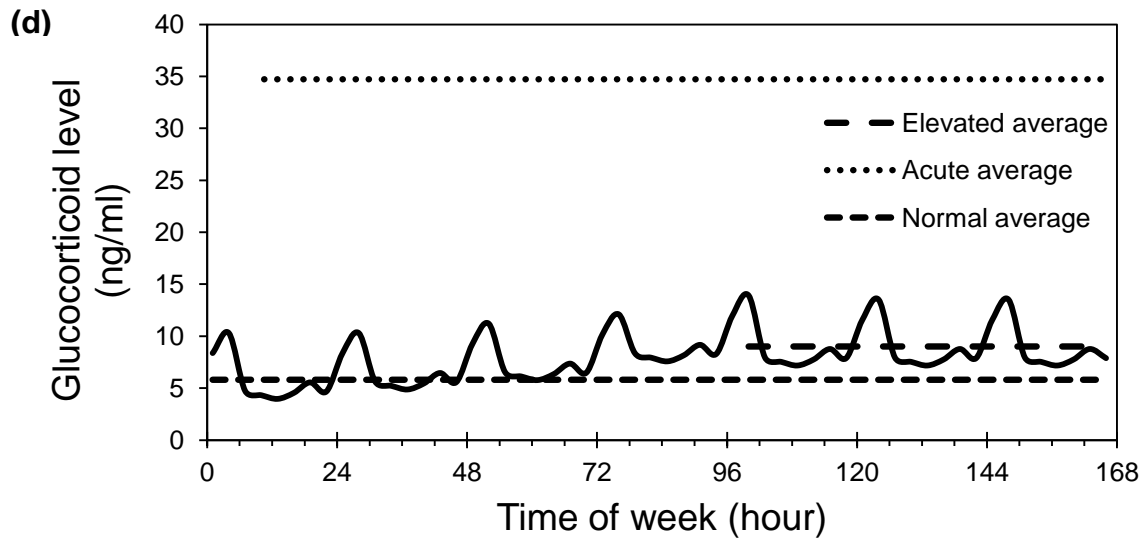
Figure 1.2 - Four scenarios in which glucocorticoid (GC) levels can become elevated. Each scenario occurs over a one-week timeframe and includes underlying diel variation. (a) A stressor (e.g., predator chase or capture and handling) occurs during the first day at 10:00 am and results in a marked, but transitory increase in glucocorticoid (GC) levels. Over the long-term (i.e., the full week) this increase does not affect the average baseline GC level. (b) A severe, extended weather event, injury, or illness results in prolonged elevations of GCs at stress-induced concentrations analogous to "chronic stress". (c) A stressor results in the temporary elevation of GCs to stress-induced levels, but subsequent elevation occurs within baseline levels, leading to a new, higher average baseline GC level. (d) A gradual or less severe alteration in environmental quality (e.g., decline in food resources or increased competition) leads to a moderate elevation of baseline GCs. Figures created using data modified from white-crowned sparrows (*Zonotrichia leucophrys gambelii*; Breuner et al., 1999) and tree swallows (*Tachycineta bicolor*; Franceschini et al., 2009).

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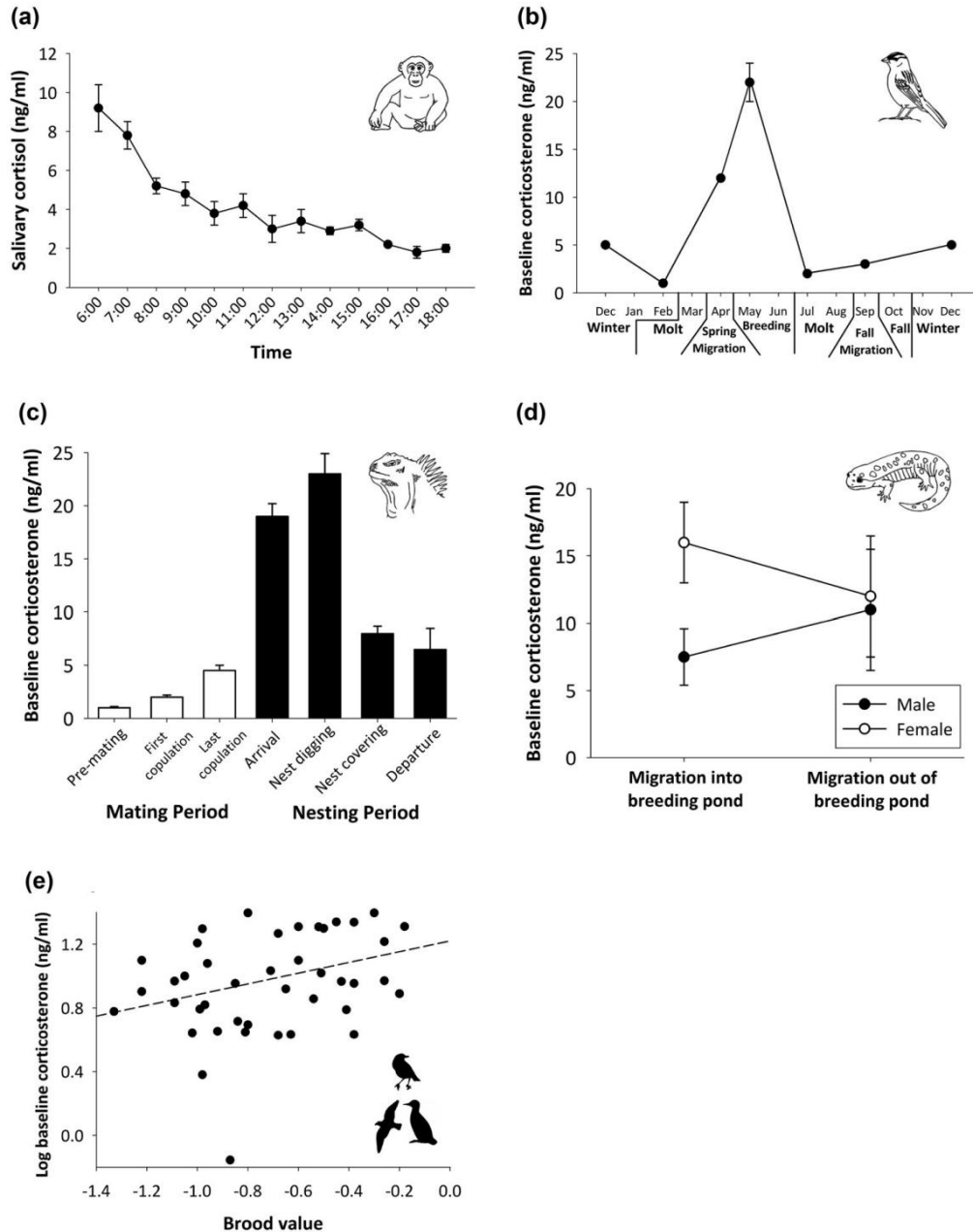
Figure 1.3 - Contexts that cause variation in baseline glucocorticoid levels: (a) diel cycle

(e.g., chimpanzee [*P.troglodytes*]; Heintz et al., 2011); (b) season (e.g., White-crowned

Sparrow [*Z. leucophrys gambelii*]; Romero and Wingfield, 1999); (c) life stage within

season (e.g., Galapagos marine iguana [*A. cristatus*]; Rubenstein and Wikelski, 2005); (d)

sex (e.g., spotted salamander [*A. maculatum*]; Homan et al., 2003); and (e) life history

(e.g., phylogenetic comparative analysis of 64 avian species; Bókony et al., 2009; brood

value, value of the current reproductive attempt relative to lifetime reproductive output for a given species). Graphs redrawn with permission from Heintz et al., 2011 (John Wiley and Sons), Romero and Wingfield, 1999 (Elsevier), Rubenstein and Wikelski, 2005 (Elsevier), Homan et al., 2003 (Elsevier), and Bokony et al., 2009 (University of Chicago Press).

CHAPTER 2 - ASSESSING BASELINE STRESS PHYSIOLOGY AS AN INTEGRATOR OF ENVIRONMENTAL QUALITY IN A WILD AVIAN POPULATION: IMPLICATIONS FOR USE AS A CONSERVATION BIOMARKER*

Introduction

The ability to detect anthropogenic disturbances in wildlife populations is of paramount importance to monitoring and conservation management (Nichols and Williams, 2006). As traditional demographic measures are often labour-intensive and require extended time spans to detect population trends, many conservation biologists have begun to employ more sensitive, labile physiological measures to monitor the health and condition of wildlife systems of interest (Cooke et al., 2013; Seebacher and Franklin, 2012; Wikelski and Cooke, 2006). The growing field of conservation physiology offers many potential traits spanning energetics, immune function, toxicology, reproductive biology and nutrition, each with their own optimal conditions for use and considerations for interpretation (Cooke et al., 2013). However, for a given physiological measure to be a sensitive biomarker, it must be reflective of the environmental changes that can influence condition, population health, and viability (Cooke and O'Connor, 2010). Glucocorticoids (i.e., cortisol, corticosterone) represent potential biomarkers due to their function in the maintenance of energetic balance (Landys et al., 2006), mediation of life history trade-offs (Crespi et al., 2013), and role in allowing individuals to respond behaviourally to perturbations in their environment (Wingfield, 2013).

Glucocorticoids (GCs) can be measured at baseline and stress-induced levels through blood samples (plasma or serum), and over more integrated time periods in feces

and keratinized outer integuments such as hair and feathers (Sheriff et al., 2011). Baseline measures are appealing because they are obtained less invasively when compared with the handling protocol required to achieve stress-induced samples and although still more invasive than fecal collection, blood samples always allow GC levels to be tied unambiguously to individuals and time periods. Perhaps most importantly, baseline GCs have been theoretically viewed as integrators of an individual's internal and external environment (Figure 2.1) due to their role in the maintenance of energetic balance through the promotion of foraging and the mobilization of stored energy reserves (Dantzer et al., 2014; McEwen and Wingfield, 2010; Shultz and Kitaysky, 2008). We would therefore expect an observable increase in baseline GC levels with any change in the environment that necessitates increased energetic expenditure or decreased access to food resources (i.e., increased energy expense or allostatic load; McEwen and Wingfield, 2010). Indeed, more energetically demanding life history stages are characterized by increased baseline GC levels (Romero, 2002), and on a finer temporal scale, more demanding stages of reproduction have been associated with higher baseline GC levels (e.g., Bonier et al., 2009; Reedy et al., 2014; Rubenstein and Wikelski, 2005).

Baseline GCs have also been shown to be representative of a variety of conservation-relevant variables (Busch and Hayward, 2009) including vegetation cover (Bauer et al., 2013; Janin et al., 2012; Stabach et al., 2015), parasite load (Bauer et al., 2013), urbanization (Bonier, 2012; French et al., 2008), predation pressure (Clinchy et al., 2011), forestry practices (Leshyk et al., 2012), pollution (Nordstad et al., 2012), traffic intensity (Strasser and Heath, 2013), tourism (French et al., 2010), and food availability (Fokidis et al., 2012). However, whether and in which direction GC levels change has not been consistent despite the predominant assumption that any disturbance will lead to an

increase in GC levels (Bonier et al., 2011; Dickens and Romero, 2013; Madliger and

Love, 2014). As a result, baseline GC levels may respond to environmental variation in a

context-dependent manner that necessitates the careful consideration of underlying

reproductive, demographic, or conditional parameters (Madliger and Love, 2014).

Investigating multiple aspects of environmental quality (both internal and

external) may help to determine which components most sensitively correlate to GC

levels. Unfortunately, few studies have investigated how multiple components of

environmental quality may influence baseline GC levels simultaneously, particularly

across gradients (Bauer et al., 2013; Grunst et al., 2014; Strasser and Heath, 2013; Zhang

et al., 2011). In addition, we currently have limited information on how baseline GCs may

integrate environmental contexts differently depending on reproductive stage. This is

particularly important given that timing of sampling could be highly relevant when

considering GCs as physiological biomarkers since even short time periods (e.g., those

separating different stages of reproduction) can have profound influences on underlying

baseline GC levels (e.g., Goymann et al., 2006; Kern et al., 2005; Pereyra and Wingfield,

2003; Rector et al., 2012; Williams et al., 2008). From a practical perspective, this type of

information is necessary for conservation managers to determine whether certain time

periods may be better-suited to the sampling of GCs, or whether contexts that vary within

a population (e.g., reproductive status) could influence the ability of GCs to represent

disturbances or environmental quality.

We combined three years of reproductive monitoring data, an experimental

manipulation of energetic demand, and an assessment of multiple components of habitat

quality to determine whether baseline GC levels represent a relevant biomarker of the

intrinsic state and extrinsic environmental quality experienced by breeding female tree

swallows (*Tachycineta bicolor*). Tree swallows are a member of the aerial insectivore guild which has been experiencing dramatic population declines in North America (Nebel et al., 2010); as a result, investigating how stress physiology relates to underlying variation in body condition and habitat quality can also contribute to our understanding of how future changes may influence this species and others in the guild. We specifically focused on two reproductive stages that differ in their parental energetic demand (Tatner and Bryant, 1993): incubation (lower demand) and offspring provisioning (higher demand). We chose environmental variables that represent major extrinsic and intrinsic factors that individuals of this species would experience during reproduction (Table 2.1), and that would therefore be expected to influence overall energetic management through changes in activity level or body reserves: 1) food availability; 2) inter-specific nest competition; 3) intra-specific nest competition; 4) reproductive investment (i.e., clutch size and brood mass); and 5) intrinsic state (i.e., body condition). We also experimentally increased energetic demand through feather clipping to test whether baseline GC levels are responsive to, and differentially influenced by, these environmental contexts when individuals are faced with an unexpected and prolonged disturbance while raising offspring (i.e., a decrease in foraging profitability and therefore the overall quality of their environment). Importantly, our manipulation forced individuals outside of preferred (optimal) investment decisions, but not past their capacity to successfully raise offspring. If baseline GCs are to be used as conservation-relevant biomarkers, we would predict that levels would be correlated with intrinsic and extrinsic environmental factors at both stages of reproduction. We also predicted that due to an increase in energetic demand (Tatner and Bryant, 1993), baseline GC levels would increase over the reproductive

period, and levels of birds facing an unexpected decrease in environmental quality (feather clipping) would be elevated in comparison to control individuals.

Methods

Study site

Our study was completed between April and July of 2010-2012 in a wild population of nest box-breeding tree swallows in Haldimand County, Ontario, Canada. Tree swallows represent an ideal study species for this investigation as they are easily accessible, settle in a variety of habitat types, and have been well-studied in the context of reproductive biology and ecological requirements (Jones, 2003). Our study area consists of 175 nest boxes in the Grand River watershed within Ruthven Park National Historic Site (42º58'N, 79º52'W) and Taquanyah Conservation Area (42º57' N, 79º54' W) (approximately four kilometers apart). The study area is a matrix of landuse types including riparian vegetation, fallow and livestock fields, active agricultural fields, Carolinian forest, and wetlands. Boxes are located in lines along roadways and in groups within fallow fields. For this study, we focused on the 96 boxes that were clustered in fallow fields to allow for quantification of food resources (see below). Boxes differed in terms of surrounding landuse type (which has been shown to influence food availability in this species; Paquette et al., 2013), intra-specific density, and distance to features that dictate the presence of primary nest site competitors such as house wrens (*Troglodytes aedon*) and house sparrows (*Passer domesticus*) (Table 2.1).

Nest monitoring and blood sampling

All experimental methods were approved by the University of Windsor's Animal Care
Committee (AUPP #10-10) and the Canadian Wildlife Service (Permit CA 0266). Over
the three years, we monitored 292 reproductive attempts of female tree swallows by
checking boxes once daily to record the date of the first egg (lay date), mass of each egg
on the day it was laid, clutch size, hatching success (number of chicks successfully
hatched), nestling mass at six and 12 days post-hatching, and breeding productivity
(number of offspring that successfully left the nest box). We focused on adult females
because they are the sole incubators and the more accessible sex overall, allowing us to
obtain necessary sample sizes for subsequent analyses. We captured females by plugging
the nest hole at two time periods during the reproductive season: 10 days after clutch
completion (incubation stage) and 12 days post-hatching (peak offspring provisioning
stage). At each capture, we obtained a small blood sample from each female representing
less than 10% of total blood volume (i.e., <150 µl) in heparinized microcapillary tubes
through puncture of the brachial vein. We collected all blood samples between 0800 and
1200 h to control for diel variation in baseline GC levels (i.e., birds had been actively
foraging for approximately two hours prior to sampling). We obtained all samples within
two minutes of covering the nest hole to ensure sampling of circulating baseline levels
(Romero and Reed, 2005). The amount of time required to trap a bird did not correlate
with GC levels at either reproductive stage (linear model: incubation: F=1.82, P=0.07;
nestling provisioning: F=-0.66, P=0.42). In addition, we recorded body mass, wing
length, and age and gave unbanded birds a numbered aluminum leg band (Canadian
Wildlife Service - Permit 10808).

Experimental manipulation

In 2011, we experimentally increased energetic demand via a primary feather-clipping manipulation (as per Ardia and Clotfelter, 2007; Winkler and Allen, 1995) on a random subset of females (n=33) temporally and spatially matched (i.e., on the same day and within the same site) to controls (n=38). More specifically, we cut off every other primary flight feather (four feathers per wing) at its base during the incubation stage capture (10 days after clutch completion). The manipulation creates an increase in the workload associated with flight and a decrease in foraging profitability in this species (Winkler and Allen, 1995) for the remainder of the breeding season until new feathers are naturally molted in the months following breeding (Stutchbury and Rohwer, 1990). As aerial insectivores, tree swallows catch all of their food resources for self-maintenance and offspring provisioning on the wing (Robertson et al., 1992) so this manipulation causes a decrease in realized habitat quality by increasing the effort required to access food resources. In addition, this handicap (Ardia and Clotfelter, 2007; Hasselquist et al., 2001; Winkler and Allen, 1995) was anticipated to cause a concomitant increase in baseline GC levels compared to control individuals.

Extrinsic habitat variables

We recorded geographic coordinates for each nest box and completed all spatial calculations in ArcGIS 10.1 (Esri) using a 2010 orthorectified SWOOP (Southwestern Ontario Orthoimagery Project - 30 cm resolution) satellite image as a base layer. We calculated three extrinsic habitat variables to represent intra-specific competition, inter-specific nest site competition, and food availability for each reproductive stage. At both the incubation and offspring provisioning stage, we calculated the number of occupied

nest boxes within a 200 meter foraging radius (McCarty and Winkler, 1999) for each nest box to represent breeding density. Female tree swallows display territoriality and prefer to nest as far as possible from conspecifics, most likely to decrease intraspecific brood parasitism and limit nest usurpation (Dunn and Hannon, 1991; Muldal et al., 1985), so we used density as a proxy of intra-specific competition. In addition, we calculated a proxy of inter-specific nest site competition at each reproductive stage. During the incubation stage, tree swallows compete for nest sites with native house wrens and must defend their nest to prevent their eggs from being pierced and removed (Quinn and Holroyd, 1989; Rendell and Robertson, 1990). As house wrens are associated with the edges of forests and hedgerows (Rendell and Robertson, 1990), we calculated the distance of each box from a wooded edge (forest or hedgerow) to provide a proxy of inter-specific nest site competition (i.e., house wren-associated risk) during incubation. At the nestling provisioning stage, tree swallows face strong competition from non-native house sparrows which can injure or kill adults and nestlings (Robertson et al., 1992; Robinson, 1927). As house sparrows are associated with human residences and outbuildings such as barns (Summers-Smith, 1963, 1988), we calculated the distance from the nearest building as a proxy for inter-specific nest site competition (i.e., house sparrow-associated risk) during offspring provisioning.

Finally, we quantified the daily availability of flying insects (i.e., the primary food resource of tree swallows; Hussell and Quinney, 1987) at each reproductive stage. Within each of five grid systems, we placed a centrally located four-sided malaise trap (110x110x110 cm SLAM traps, MegaView Science Co.), which caught insects passively in ethanol and was changed daily between 1300 and 1700h. Traps were raised 60 cm above the ground to better quantify a section of the air column frequented by foraging

tree swallows (McCarty and Winkler, 1999). Other passive traps at this height have been used previously to estimate food availability for this species during the breeding season (Hussell and Quinney, 1987; Mengelkoch et al., 2004; Paquette et al., 2013). We identified all insects to order, with the exception of Dipterans, which were further classified into sub-order *Nematocera* or *Brachycera* (midges and heavy-bodied flies, respectively) due to their large difference in size and mass. As per Hussell and Quinney (1987), we measured body lengths to place individual insects into 2 mm size categories. Within each order or suborder and each size class a sub-sample of randomly chosen, intact insects were used to determine biomass conversion factors for all other samples. We calculated the combined average daily biomass (mg) of six orders of insects that constitute the majority of tree swallow diet (*Diptera*, *Coleoptera*, *Hymenoptera*, *Hemiptera*, *Tichoptera*, and *Ephemeroptera*; Johnson and Lombardo, 2000; Quinney and Ankney, 1985). We limited our calculation based on size category to insects under 10mm based on previous findings that 99% of prey items are under this length and larger insects heavily bias biomass estimates (Madliger and Love, *unpublished data* and Quinney and Ankney, 1985). For the incubation stage, we calculated the average daily biomass over the 12 days prior to hatching (focal incubation period) for each female (similar to Nooker et al., 2005). For the nestling provisioning stage, we calculated the average daily biomass for each female over the time when nestlings were 5-10 days old (similar to Nooker et al., 2005) and therefore in their most demanding and fastest growth phase (McCarty, 2001) as this should represent an integral food availability period during parental care.

Hormone analysis

Blood samples were stored on ice for up to five hours prior to being centrifuged to separate plasma and then stored at -80 ˚C until analysis. We determined plasma levels of total baseline corticosterone, the primary GC in birds, in non-extracted plasma using a commercially-available Corticosterone Enzyme-linked Immunoabsorbent Assay (EIA - Assay Designs Inc., Michigan USA, catalog #901-097). We ran samples in triplicate at a total volume of 100 μl with 1:40 dilution and 1.5% steroid displacement buffer. Each assay plate contained a six-point standard curve created by serial dilution from 20 000 pg/ml to 15.63 pg/ml fitted with a four parameter logistic fit (Love and Williams, 2008). The detection limit of the assay was 0.74 ng/ml, calculated as per the manufacturer's method as the concentration of CORT that was two standard deviations from zero along our standard curves. Of a total of 291 samples, 12 fell below this limit and were therefore assigned the value of the detection limit. Intra-assay variation was 7.7%, 8.0%, and 10.3% in 2010, 2011, and 2012, respectively. Inter-assay variation was 6.7%, 13.3%, and 6.0% in 2010, 2011, and 2012, respectively.

Statistical analyses

We used separate linear mixed-effect models at each stage of reproduction (incubation and offspring provisioning) to determine the relationship between intrinsic and extrinsic environmental variables and baseline GC levels in female tree swallows. We log transformed GC values prior to analysis to achieve normality (GC levels prior to transformation were 0.74-9.47 ng/ml at incubation and 0.74-12.17 ng/ml at nestling provisioning). As baseline GC levels did not differ between our five grid sites (ANOVA: $P>0.05$), we pooled sites in all analyses. We had instances of the same individual being

present in our dataset for multiple years and therefore limited our dataset so that each female was included only once by randomly choosing one year. In addition, we excluded any individuals in their first year of reproduction (i.e., second-year birds) as we did not have a sufficient sample size to analyze this age class separately. This yielded a sample size of 127 females at the incubation stage and 93 females at the nestling provisioning stage across three years (2010-2012). Five environmental (independent), fixed-effect variables were included in each analysis to represent intra-specific competition, inter-specific nest site competition, food availability, reproductive investment, and intrinsic state (Table 2.1). None of the variables were highly inter-correlated as Variance Inflation Factors (VIFs) were all less than 1.30 (O'brien, 2007). Since we had strong *a priori* reasons why each of the five environmental variables could correlate with GC levels, and no reason to eliminate any specific combination of variables, we used an all sub-sets approach that yielded 32 models in each analysis. We did not have *a priori* reasons why any interaction terms would be more biologically relevant than others so to avoid model over-fitting we did not include any interaction effects in our models. We included year as a random effect and relative lay date (individual lay date relative to the intra-annual population mean) as a fixed effect in all models to take into account potential influences of seasonality or individual quality (Stutchbury and Robertson, 1988; Winkler and Allen, 1996) on baseline GC levels.

To determine the effect of the clipping manipulation performed in 2011, we compared GC levels of birds assigned to control (n=38) and treatment (n=33) groups prior to manipulation (incubation) using a t-test and after manipulation (offspring provisioning) using an ANCOVA to control for prior (incubation) GC level. We also performed a repeated measures ANCOVA to determine whether the two groups (control

and clipped) changed GC levels differently from the incubation to the nestling provisioning stage (i.e., to test for a time^{x}treatment interaction). We used general linear models to assess the influence of intrinsic and extrinsic variables on the GC levels of clipped and control birds separately at the offspring provisioning stage. As in the correlational analyses, five environmental (independent) variables were included (Table 2.1) using an all sub-sets approach. We did not detect any collinearity among variables (VIFs < 1.40). Lay date was included as a fixed effect in all models.

For all general linear model analyses, we used the Akaike Information Criterion corrected for small sample size (AIC_c) as a basis to perform model selection (Burnham and Anderson, 2002). We calculated AIC_c, ΔAIC_c (difference between each model's AIC_c and that of the lowest model), Akaike weights and cumulative weights for each model (Burnham and Anderson, 2002). Akaike weights can be viewed as the probability that a given model is the best approximating model to describe the data out of the full candidate set of models (Symonds and Moussalli, 2011). Cumulative weights allow for the determination of a 95% confidence set of models (i.e., a set of models in which we are 95% certain that the best model among the candidate set of models is included). Model uncertainty occurs when no single model can be identified as best (i.e., the Akaike weight of the top model is <0.90) (Burnham and Anderson, 2002). Where this was evident, we used multi-model inference based on the 95% confidence set to obtain model-averaged parameter estimates (β), unconditional standard errors and 95% confidence intervals (Burnham and Anderson, 2002; Johnson and Omland, 2004). Model-averaged β-values and unconditional standard errors are weighted by the Akaike weights of the models in the confidence set. All analyses were completed in JMP 10 (SAS Institute), except for the

calculation of marginal and conditional R^2 which was completed in R 3.1.1 (R

Development Core Team, 2014) with the rsquared.glmm package (Barton, 2015).

Results

Natural environmental variability

The top model in our analysis to determine which environmental variables explained

variation in baseline GC levels at the incubation stage included only body mass (*state*)

with a model weight of 0.77 (Table 2.2). However, there was some model uncertainty,

with four models comprising the 95% confidence set (Table 2.2). In other words, we can

be 95% certain that a model within this confidence set represents the AIC_c best model out

of the full candidate set. The null model (with lay date as a fixed effect and year as a

random effect) was also included in the confidence set. The parameter estimates and

unconditional error rates indicate that food availability (*food*) had a very weak positive

relationship with GC levels while reproductive investment (*invest*) and body mass (*state*)

had poor parameter estimation and 95% confidence intervals that cross zero (Table 2.3).

The marginal and conditional R^2 of the global model were 0.15 and 0.16, respectively. At

the nestling provisioning stage, the null model, (with lay date as a fixed effect and year as

a random effect), represented the best model with an Akaike weight of 0.98 (Table 2.2).

The marginal and conditional R^2 of the global model were 0.02 and 0.09, respectively.

Unexpected environmental challenge

Feather clipped females displayed a lower number of foraging trips based on a 1-hour

observation period at day 8 or 9 of offspring provisioning as compared to control females

while controlling for brood size and date (linear model: t_{71}=2.68, P=0.009; control (mean±SE)=9.7±0.7, clipped=6.8±0.8).

Birds assigned to control and treatment groups did not differ in baseline GC levels prior to the manipulation (t-test, t-ratio=-1.04; P=0.30; Figure 2.2). GC levels of control birds and those with experimentally decreased foraging profitability (via feather clipping) responded differently from the incubation to nestling provisioning stage (repeated measures ANCOVA, time^{x}treatment: t-ratio=2.95, p=0.004). Specifically, feather clipped birds increased baseline GC levels over the reproductive season and had significantly higher levels of baseline GCs at the nestling provisioning stage compared to control birds (ANCOVA, t-ratio=-2.69; P=0.009; Figure 2.2). When determining whether GC levels represented environmental variables in the control group, there was considerable model uncertainty with 21 models included in the 95% confidence set. Only the model with food availability (*food*) as the sole independent variable ranked higher than the null model. However, the R^2 of this model was 0.09 indicating a poor fit to the data overall. In addition, model-averaged unconditional standard errors and 95% confidence intervals of all environmental variables cross zero indicating poor precision in parameter estimation, coinciding with results from the multi-year analysis.

Within the clipped treatment, a single best model could not be resolved; 19 models comprised the 95% confidence set and all five environmental variables were found within the set (Table 2.4). Model-averaged parameter estimates and unconditional standard errors indicated that *state* (i.e., loss of body mass over the nestling provisioning period) was positively associated with GC levels (Table 2.5). In addition, the top three best supported models (with ΔAIC_c<2) all included *state* and displayed R^2 values greater than 0.20, with the top model having an R^2 of 0.27. All other environmental variables had

95% confidence intervals that crossed zero indicating poor parameter estimation and a lack of association with GC levels.

Discussion

Natural environmental variability

Under natural conditions, baseline GC levels did not reflect the external or internal environment at either stage of reproduction (incubation or offspring provisioning), indicating that baseline GCs were not representative of any component of habitat quality, individual condition, or reproductive investment that we measured, regardless of underlying parental demand. We do not believe that this is a consequence of the environmental gradients not representing sufficiently variable conditions, as similar environmental variation has been shown to result in fitness consequences in this species (e.g., proximity to wooded areas: Robertson and Jones, 2002; food availability: Ghilain and Bélisle, 2008). Additionally, our study eliminated other factors known to influence baseline GC levels such as sex (Homan et al., 2003; LormÉe et al., 2003; Rector et al., 2012), age (Angelier et al., 2006; Riechert et al., 2012), reproductive stage (Bonier et al., 2009; Rubenstein and Wikelski, 2005; Williams et al., 2008), and time of day (Breuner et al., 1999). More importantly, the variables we measured represent a broad assessment of the environmental variation faced by tree swallows during the reproductive season and are comparable to variables that practitioners are able to measure within their wildlife systems. While it is possible that unmeasured factors such as parasite load (Bauer et al., 2013; Raouf et al., 2006; St. Juliana et al., 2014) or predatory interactions (Clinchy et al., 2013) or a particularly harsh year with unexpected conditions (e.g., drought, excessive heat, excessive rainfall) could result in greater energy requirements and therefore greater

responsiveness of GC levels, our findings indicate that baseline GCs did not represent a reliable integrator of body condition and environmental quality under natural variability that is considered relevant for our study species.

It is also possible that the underlying extrinsic and intrinsic variation we measured constitutes a predictable component of an individual's environment to which baseline GC levels are relatively insensitive. During habitat selection, individuals may have established expectations of the features that will be present during the subsequent breeding season and may be able to maintain sufficient intrinsic resources to cope with expected challenges or adjust reproductive decisions accordingly (Doligez et al., 2003, 2008; Sih et al., 2011). As a result, small within-season changes in environmental quality may not be sufficient to cause pronounced changes in baseline GC levels, particularly in years with predictable conditions. This could also explain our finding that baseline GC levels did not increase from the incubation stage to the nestling provisioning stage in control females. While it is possible that HPA sensitivity may be modulated (down-regulated) during the most demanding stages of reproduction, allowing individuals to progress through the breeding season despite changes in environmental quality (Holberton and Wingfield, 2003; Love et al., 2004; Wilson and Holberton, 2004; Wingfield et al., 1995), GC levels may have maintained consistency between stages because they are representative of overall reproductive investment decisions when females are working within expected conditions (Love et al., 2014). Indeed, consistency (i.e., repeatability) in baseline GC levels between incubation and nestling provisioning stages has been shown previously in this species (Ouyang et al., 2011), with differences in GC levels only detectable when brood size is enlarged (Bonier et al., 2011). These

51

findings reinforce that it may be difficult to detect gradual alterations in environmental conditions with baseline GCs in some species.

Our findings are consistent with others that have found insensitivity in baseline GC levels in relation to intrinsic and extrinsic variation. For example, baseline GC levels in western fence lizards (*Sceloporus occidentalis*) did not vary based on temperature, humidity, or condition across a range of sites (Dunlap and Wingfield, 1995). In addition, there is growing evidence that baseline GCs may respond to environmental variation in a context-dependent manner; a recent review by Bonier (2012) indicates that avian responses to urbanization can result in increases, decreases, or no change in baseline GC levels, likely in part due to the differential ability of species to avoid, persist in, or exploit urban conditions. Moreover, Nordstad et al. (2012) found a positive relationship between baseline GC levels and concentrations of polychlorinated biphenyls (PCBs) at the pre-laying, but not other stages of reproduction in black-legged kittiwakes (*Rissa tridactyla*). Similarly, Clinchy et al. (2011) showed that greater predation threat can increase baseline GC levels in male, but not female song sparrows (*Melospiza melodia*). Finally, based on a meta-analysis of laboratory and field studies across diverse species, Dickens and Romero (2013) concluded that a generalized GC profile for chronically stressed wild animals is currently unsupported. Overall, these results indicate that baseline GC levels may respond to environmental variation in a context-dependent manner that necessitates the careful consideration of underlying reproductive, demographic, or conditional parameters (Madliger and Love, 2014), and suggests that there may be limited circumstances when baseline GCs are useful biomarkers of intrinsic condition or the external environment in some species.

Unexpected environmental challenge

The feather clipping manipulation led to a decrease in the frequency of feeding trips as compared to control females, likely due to a decrease in overall foraging efficiency (Patterson et al., 2011; Winkler and Allen, 1995). In contrast to natural underlying environmental variability, we found that this unpredictable environmental challenge increased the baseline GC levels of female tree swallows. Furthermore, baseline GC levels were also indicative of intrinsic state (change in body mass over offspring provisioning) when females were exposed to this environmental challenge, with individuals with higher baseline GC levels post-manipulation experiencing greater losses in body mass. Our manipulation represented a perturbation that extended over a two-week time period and it is therefore possible that this unexpected or elongated disturbance more easily manifested into an energetic deficit that led to a measureable change in baseline GCs. Indeed, baseline GCs have previously been found to be representative of other unexpected, or severe perturbations in habitat quality including oil spills (Wikelski et al., 2002), severe food restrictions (Romero and Wikelski, 2001), presence of a novel invasive species (Graham et al., 2012), and logging (Leshyk et al., 2012). Our findings reinforce the role of baseline GCs in the context of energetic management and provide further indication that environmental alterations that manifest as dramatic changes to resource availability or energy expenditure will more likely be represented by changes in baseline GCs (Madliger and Love, 2014).

In immediate response to the clipping manipulation, baseline GCs would likely have risen (i.e., a stress response would have been triggered). This initial stress response is adaptive in the face of short, acute stressors as it allows individuals to respond to a perturbation (e.g., predator, weather event) by causing short-term changes in behaviour,

53

immunity, and the mobilization of energy resources (Wingfield et al., 1998). However, if a perturbation is long-lived, this normally adaptive system can be pushed past its adaptive capacity (Dickens and Romero, 2013) and animals can experience chronically elevated GC levels that can lead to negative consequences for health, reproduction, or survival (Wingfield, 2003). Given that baseline GC levels of feather-clipped individuals were higher than controls two weeks following the initiation of the manipulation, it is likely that these individuals were experiencing a chronic elevation, albeit within baseline levels, of GCs over that time period. As a result, we would expect that the elevation in circulating GC levels in manipulated birds would impart fitness consequences, with trade-offs likely manifesting between current reproductive success and survival (Crossin et al., 2015). Moreover, it is likely that the brood value of clipped individuals may relate to how they responded to the manipulation. Specifically, individuals that more greatly value their current brood are expected to sacrifice self-maintenance in favour of increasing workload for their offspring and would therefore likely exhibit greater increases in baseline GC levels and therefore stronger negative longer-term (i.e., survival) consequences than individuals favouring somatic maintenance at the expense of reproductive output (Bókony et al., 2009). Although outside of the scope of the current study, future studies investigating the fitness costs of ecologically-relevant experimentally-manipulated baseline GCs have been cited as a pressing need for determining the usefulness of GCs as biomarkers (Madliger and Love, 2014) and to the field of conservation physiology as a whole (Cooke, 2014).

Implications for glucocorticoids as biomarkers

Our results suggest that baseline GCs may be more consistently useful in detecting

whether certain populations have been exposed to unexpected or more extended impacts,

rather than monitoring gradual changes in environmental quality that may be useful in

predicting future population changes. We have confidence that these negative results are

not due to low sample size as the associated confidence intervals for individual

environmental variables are relatively narrow and cross zero. Overall, baseline plasma

GCs may represent a trait that is too labile to easily be implemented as a sensitive

indicator of habitat quality or disturbance in many species. For example, recent

temperature, wind, precipitation, time since last feeding, or whether an interaction with a

conspecific or competitor has recently occurred may be more influential on short-term

baseline GC levels. If this is the case, baseline plasma GCs will be harder to interpret for

practitioners and a more integrative measure that takes into account processes occurring

over an extended time frame, such as fecal GCs, may be better (i.e., more consistently)

suited to this application in many species (Dantzer et al., 2014; Dickens and Romero,

2013; Sheriff et al., 2010).

It is possible that preparatory or consequential increases in baseline GCs during

reproduction may overshadow the ability of GCs to represent more gradual variation in

the environment. For example, the preparatory role of baseline GCs in mediating

investment in reproduction (Love et al., 2014) may be much more pronounced than the

changes associated with finer-scale environmental variation. It is therefore possible that

the non-breeding season may be better-suited to baseline GC measurements. However,

accessing individuals during the over-wintering season can be difficult in many species

(e.g., migratory or hibernating species) and may therefore limit the applicability of

baseline GC measures to some animal systems. There is therefore a need to determine which characteristics and contexts are most important to interpreting baseline GC levels as this will allow for the determination of which systems may be best suited to their use.

There is also a need for experimental approaches to better understand how GC levels can reflect changes in the environment, with particular attention to the changes most likely to be associated with climate change or other anthropogenic disturbances (e.g., agricultural intensification, urbanization, and resource extraction). Direct manipulation of environmental variables would be highly beneficial in determining these relationships. In addition, while outside the scope of this study, the most powerful experimental approaches will also include measures of fitness (productivity and survival) as this will allow the value of baseline GCs for predicting the demographic consequences that drive population viability to be determined. In this way it may also be possible to identify whether thresholds exist where baseline GCs become useful indicators of environmental quality and predictors of population change (Dantzer et al., 2014), allowing their application to be tailored to specific wildlife systems.

Conclusions

We found that baseline GCs were not representative of the internal and external environment of tree swallows, regardless of reproductive stage, indicating that baseline GCs may be limited in their ability to reflect gradients in habitat quality or disturbance in some species. Overall, we currently lack a complete picture of if and how baseline GCs may fit into the conservation toolbox. Most importantly, growing evidence indicates that the application of GCs will be highly context-dependent and the method will need to be

considered in light of its reliability, sensitivity, and ease of interpretation to determine

when it will be a useful tool for conservation biologists and wildlife managers.

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Tables

Table 2.1 - Descriptions of extrinsic and intrinsic environmental variables measured at incubation and offspring provisioning stages.

Abbreviations for each variable used in AIC_c models are provided.

Environmental Context	AIC Model Abbreviation	Description			
		Incubation Stage	Range	Nestling Provisioning Stage	Range
Intra-specific competition	*density*	Number of tree swallow pairs within 200m radius	7 - 29	Number of tree swallow pairs within 200m radius	7 - 29
Nest site competition	*disturb*	Distance to wooded area (forest or hedgerow) (m)	0 - 145	Distance to building (m)	20 - 604
Food availability	*food*	Average insect biomass over incubation period (mg)	7.6 - 71.3	Average insect biomass over days 5-10 of chick rearing (mg)	7.9 - 154.3
Investment	*invest*	Clutch size (number of eggs)	1 - 8	Brood mass (total mass of nestlings at day 12) (g)	19.7 - 147.9
Intrinsic state	*state*	Size-corrected body mass at blood sampling (g)	0.17 - 0.23	Percent loss in body mass over chick-rearing	3.9 - 27.1

Table 2.2 - Confidence set (95%) of linear mixed-effect models used to predict baseline glucocorticoid levels at the incubation (n=127) and nestling provisioning (n=93) stages. All models included year as a random effect and relative lay date as a fixed effect. Variables in each model, number of parameters (K), Akaike Information Criterion adjusted for small sample size (AIC_c), difference between each model and the model with the smallest AIC_c (ΔAIC_c), model weights (ω) and cumulative weights (cum. ω) are provided for each model.

Models	K	AIC_c	ΔAIC_c	ω	cum. ω
Incubation					
state	5	47.26	0.00	0.77	0.77
Null	4	51.96	4.70	0.07	0.85
state, food	6	52.11	4.85	0.07	0.92
state, invest	6	53.39	6.12	0.04	0.95
Nestling provisioning					
Null	4	51.79	0.00	0.98	0.98

Table 2.3 - Model-averaged parameter estimates, unconditional standard errors (SE) and

95% confidence intervals from linear mixed-effect models used to predict baseline

glucocorticoid levels at the incubation stage. Values were calculated with models

included in the confidence set by using Akaike weights as weighting factors (see

Methods).

Variable	Estimate	Unconditional SE	95% confidence interval lower, upper
state	-4.21	2.3	-8.72, 0.29
food	0.005	0.002	0.002, 0.01
invest	-0.16	0.65	-1.43, 1.11
lay date	-0.0004	0.003	-0.006, 0.005

Table 2.4 - Confidence set (95%) of general linear models used to predict baseline glucocorticoid levels at the nestling provisioning stage for clipped (n=33) and control (n=38) treatment groups (2011). All models included relative lay date as a fixed effect. Variables in each model, number of parameters (K), Akaike Information Criterion adjusted for small sample size (AIC_c), difference between each model and the model with the smallest AIC_c (ΔAIC_c), model weights (ω), cumulative weights (cum. ω) and R^2 are provided for each model.

Models	K	AIC_c	ΔAIC_c	ω	cum. ω	R^2
Clipped						
density, state	5	-94.00	0.00	0.18	0.18	0.27
state	4	-93.92	0.08	0.17	0.35	0.20
state, disturb	5	-92.89	1.11	0.10	0.45	0.24
state, invest	5	-91.99	2.01	0.06	0.51	0.22
density, state, disturb	6	-91.90	2.10	0.06	0.58	0.29
density, state, invest	6	-91.80	2.20	0.06	0.64	0.29
state, food	5	-91.43	2.57	0.05	0.68	0.21
density, state, food	6	-91.29	2.71	0.05	0.73	0.28
density	4	-90.90	3.10	0.04	0.77	0.13
state, disturb, invest	6	-90.62	3.38	0.03	0.80	0.26
state, food, disturb	6	-90.03	3.97	0.02	0.83	0.25
density, state, disturb, invest	7	-89.38	4.62	0.02	0.84	0.31
Null	3	-89.34	4.66	0.02	0.86	0.01
state, food, invest	6	-89.24	4.76	0.02	0.88	0.23
density, disturb	5	-89.22	4.79	0.02	0.89	0.16
disturb	4	-89.05	4.95	0.01	0.91	0.08
density, state, food, disturb	7	-88.86	5.14	0.01	0.92	0.29
density, state, food, invest	7	-88.81	5.19	0.01	0.93	0.29
density, food	5	-88.68	5.32	0.01	0.95	0.14
Control						
food	4	-89.55	0.00	0.19	0.19	0.09
Null	3	-89.23	0.32	0.16	0.36	0.02
food, invest	5	-87.23	2.33	0.06	0.42	0.10
state, food	5	-87.11	2.45	0.06	0.47	0.10
density, food	5	-87.02	2.53	0.05	0.53	0.10
food, disturb	5	-86.98	2.57	0.05	0.58	0.09
state	4	-86.98	2.57	0.05	0.63	0.03
invest	4	-86.95	2.60	0.05	0.68	0.03

density	4	-86.88	2.67	0.05	0.74	0.03
disturb	4	-86.83	2.72	0.05	0.78	0.02
state, food, invest	6	-84.86	4.70	0.02	0.80	0.11
state, invest	5	-84.76	4.79	0.02	0.82	0.04
food, disturb, invest	6	-84.50	5.05	0.02	0.84	0.10
density, food, invest	6	-84.49	5.06	0.02	0.85	0.10
density, state	5	-84.45	5.10	0.01	0.87	0.03
state, disturb	5	-84.43	5.12	0.01	0.88	0.03
density, invest	5	-84.40	5.15	0.01	0.90	0.03
density, state, food	6	-84.39	5.16	0.01	0.91	0.10
disturb, invest	5	-84.39	5.16	0.01	0.92	0.03
state, food, disturb	6	-84.38	5.17	0.01	0.94	0.10
density, disturb	5	-84.29	5.26	0.01	0.95	0.03

70

Table 2.5 - Model-averaged parameter estimates, unconditional standard errors (SE) and

95% confidence intervals from linear mixed-effect models predicting baseline

glucocorticoid levels in clipped and control treatment females at the nestling provisioning

stage. Values were calculated with models included in the confidence set using Akaike

weights as weighting factors (see Methods).

Variable	Estimate	Unconditional SE	95% confidence interval lower, upper
Clipped			
food	-0.0006	0.0010	-0.003, 0.002
invest	-0.001	0.0010	-0.004, 0.002
state	0.02	0.01	0.002, 0.05
density	0.01	0.007	-0.003, 0.03
disturb	0.0004	0.0003	-0.0003, 0.001
lay date	-0.004	0.008	-0.02, 0.01
Control			
food	0.003	0.002	-0.0006, 0.007
invest	-0.0008	0.002	-0.004, 0.003
state	0.006	0.01	-0.019, 0.03
density	-0.003	0.008	-0.02, 0.01
disturb	-0.0001	0.0003	-0.0007, 0.0005
lay date	-0.004	0.007	-0.02, 0.01

Figures
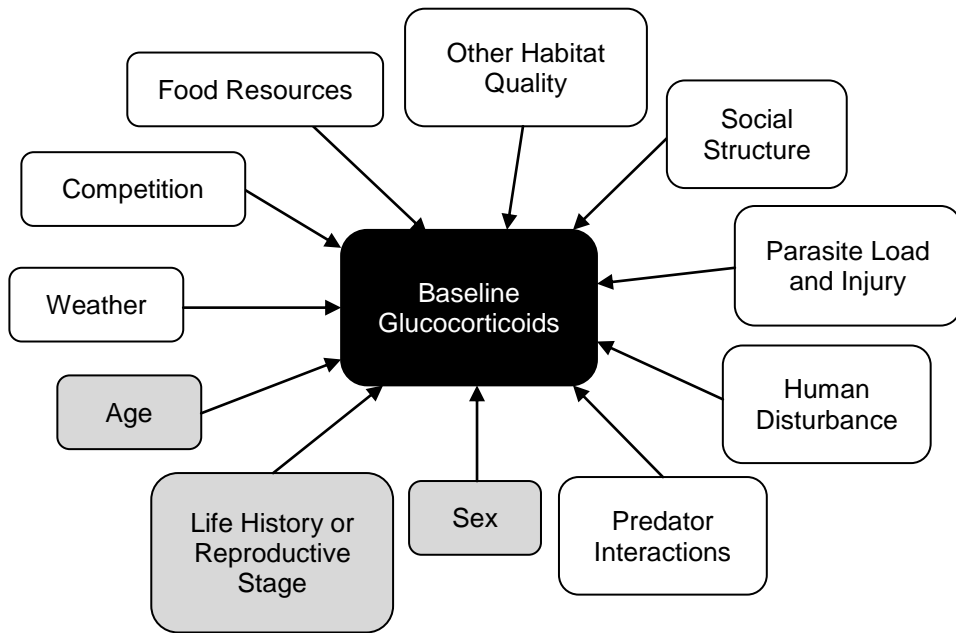
Figure 2.1 - Intrinsic (gray) and extrinsic (white) environmental variables expected to influence baseline glucocorticoid levels.
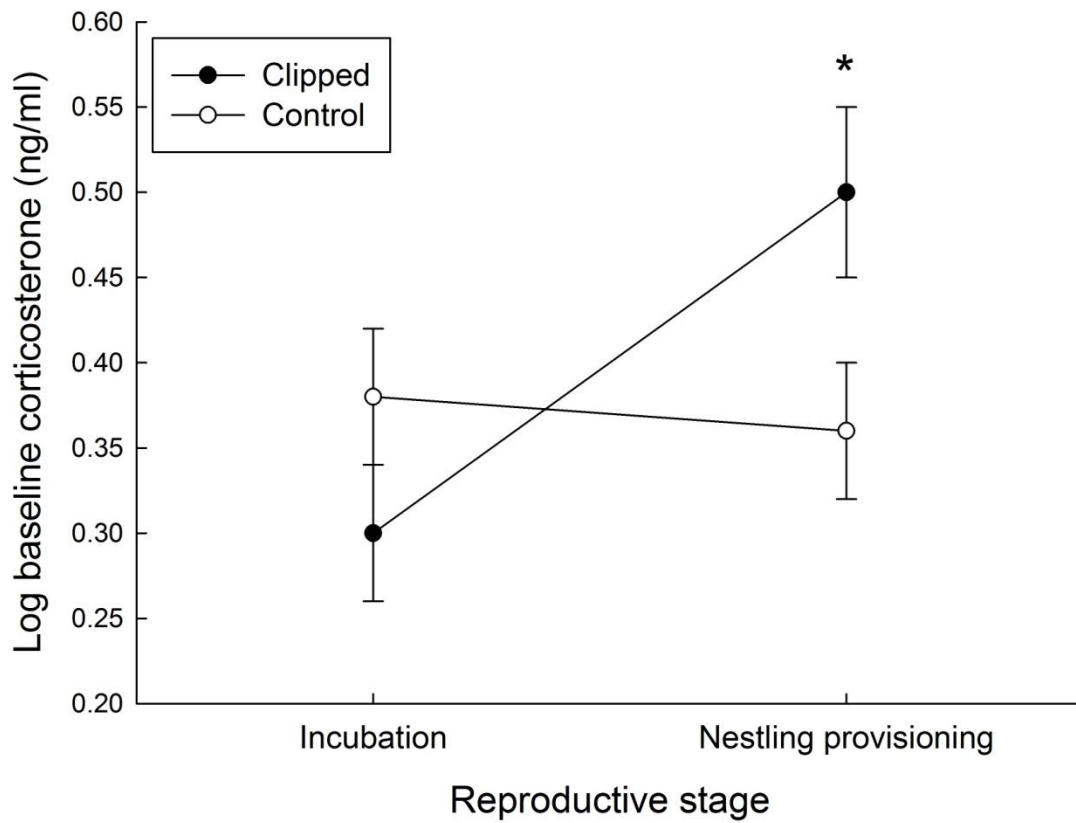
Figure 2.2 - Baseline glucocorticoid (corticosterone) levels (±SEM) of individuals in control (n=38) and clipped (n=33) treatment groups at the incubation (pre-clipping) and nestling provisioning (post-clipping) stage. Baseline glucocorticoids in clipped birds increased from the incubation to the nestling provisioning stage, while controls remained unchanged.

CHAPTER 3 - EMPLOYING INDIVIDUAL MEASURES OF BASELINE GLUCOCORTICOIDS AS POPULATION-LEVEL CONSERVATION BIOMARKERS: CONSIDERING WITHIN-INDIVIDUAL VARIATION IN A BREEDING PASSERINE*

*This chapter is the result of joint research with O. Love and is being revised after first review in *Animal Conservation*.

Introduction

With the increasing pace of habitat alteration and other anthropogenic influences on biodiversity, conservation biologists require a diverse toolbox to identify, ameliorate, and predict threats to wildlife, and to monitor the outcome of management initiatives (Bradshaw and Brook, 2010). The rapidly growing discipline of conservation physiology specifically focuses on documenting how organisms respond to changes in their environment, and potentially offers a unique set of predictive tools (Cooke et al., 2013; Wikelski and Cooke, 2006). In particular, the labile physiological processes related to metabolism, energetics, immune function, reproduction, and oxidative status can be highly sensitive to internal and external environmental factors (Carey, 2005; Cooke et al., 2013; Stevenson et al., 2005; Walker et al., 2005). As a result, physiology can change in response to disturbances or variations in habitat quality well in advance of behaviour or demographics, providing managers and practitioners with valuable predictive power (Carey, 2005; Ellis et al., 2011; Seebacher and Franklin, 2012). While many physiological traits are available as potential biomarkers, glucocorticoids (i.e., corticosterone and cortisol) have been widely employed for inferring disturbance across a variety of taxa (Busch and Hayward, 2009; Dantzer et al., 2014), largely because of their function in allowing organisms to acutely respond to unexpected perturbations in their

environment (Busch and Hayward, 2009; McEwen and Wingfield, 2003; Wingfield, 2005).

Glucocorticoids (GCs) are primarily metabolic hormones involved in the maintenance of energetic balance through their influences on glucose and lipid metabolism (Landys et al., 2006), and are most commonly associated with their role in the acute stress response (Sapolsky et al., 2000). In the face of an unexpected perturbation in the environment, GC levels rise to promote the mobilization of stored energy sources, regulate immune function, promote escape behaviour, and suppress non-essential activities such as reproduction in the minutes to hours following the challenge (Wingfield and Kitaysky, 2002). However, GCs also play a constant and essential role at baseline levels by promoting foraging and metabolism to maintain adequate glucose and fatty acid levels, leading to predictable variation over diel (Landys et al., 2006) and seasonal cycles (Romero, 2002). Specifically, baseline GCs increase during predictable periods of energetic demand when allostatic load increases (i.e., when energy required exceeds energy available; Wingfield, 2005), such as offspring provisioning (Romero, 2002). Baseline GCs and integrated measures of GCs such as those found in feces and outer integuments have also been shown to respond to changes in environmental quality (Baker et al., 2013; Busch and Hayward, 2009), further supporting their proposal as a monitoring tool for rapidly detecting disturbance in wildlife populations.

While the potential applicability of baseline GCs as conservation biomarkers is well-established (Baker et al., 2013; Busch and Hayward, 2009; Wingfield et al., 1997), there are a number of basic requirements that GCs must fulfill to be used easily and reliably in a management capacity (Cooke and O'Connor, 2010; Madliger and Love, 2014). Two major characteristics are being investigated extensively and include

establishing that baseline GCs respond to relevant environmental variability (Baker et al., 2013; Busch and Hayward, 2009; Madliger et al., 2015), and that they represent proximate indicators of fitness metrics (reviewed in Bonier et al., 2009). Establishing these properties will ensure that baseline GCs are sufficiently responsive to environmental change and that they will be predictive of the demographic parameters (e.g., growth rate, reproductive success, recruitment, survival) with which conservation managers are most concerned (Madliger and Love, 2014, 2015). However, a third characteristic that has been comparatively overlooked involves quantifying the amount of variation in baseline GC levels between and within-individuals (i.e., repeatability) and is necessary for determining whether measurements obtained at the individual level will be representative of population-level processes (Dantzer et al., 2014; Madliger and Love, 2014, 2015).

The most common approach to using GC levels to ascertain the influence of a disturbance or change in environmental quality on wildlife has been to compare the average hormone levels of populations at sites with differing exposure (e.g., pristine versus degraded; Figure 3.1 - upper panel). Drawing conclusions about the population from this type of average-based approach necessarily assumes that all individuals respond (or do not respond) to a given environmental change in a similar way (e.g., that all individuals will display an increase in GC levels in response to the habitat alteration; Figure 3.1a). However, it is possible that individuals may react in individually-specific ways to a change in environmental quality and ignoring this inherent possibility can lead to invalid interpretations of GC levels at the average (population) level (Dingemanse et al., 2010a; Williams, 2008). Specifically, an approach that only compares average, population-level GCs would conclude that scenarios a, b, c, and d in Figure 3.1 are

equivalent. However, if baseline GCs change in individually-specific ways (as in Figure 3.1b, c, d), the ability to measure sub-sets of individuals over time and consider them as representative of the population becomes more difficult, and the potential to sensitively detect a disturbance with baseline GC levels diminishes (Madliger and Love, 2014), especially when sample sizes are low (as can be the case in conservation situations). Additionally, limiting investigations to an average-based approach may lead to the conclusion that GC levels are stable (Figure 3.1e, f, g, h) despite a high level of within-individual variation (Figure 3.1f, g, h) that could be an indication of physiological disturbance, signalling important fitness consequences with implications for population health and persistence. Overall, experiments where the same individual is measured in both the control and altered environment (i.e., a repeated measures approach) are necessary to reveal whether we can have confidence that an average-based monitoring approach will be informative for the population (Dingemanse et al., 2010a).

Ecological and evolutionary ecologists have long been interested in quantifying between- and within-individual variation for the purpose of studying behavioural syndromes (Bell, 2007; Dingemanse and Dochtermann, 2013), quantifying the heritability and selective potential of a diversity of traits (Lynch and Walsh, 1998), and determining the fitness consequences of individual flexibility (Ghalambor et al., 2007; Nussey et al., 2007; Piersma and Drent, 2003). The consistency of traits (physiological and otherwise) is most often ascertained through the calculation of repeatability, which refers to the amount of variation in a trait that is attributable to between- rather than within-individual differences (Lessells and Boag, 1987). There are multiple ways to assess repeatability (Biro and Stamps, 2015; Nakagawa and Schielzeth, 2010), which can influence interpretations and which may be contributing to mixed findings regarding the

repeatability of baseline GCs (Ouyang et al., 2011a). For example, 'agreement repeatability' has most traditionally been applied in behavioural and physiological systems to determine whether individuals maintain the same trait value across time (Lessells and Boag, 1987; Nakagawa and Schielzeth, 2010). This type of repeatability is of paramount importance to assessing the existence of possible GC phenotypes and the resultant implications for the evolution of highly labile traits. In contrast, some studies have employed 'ranked repeatability' (e.g., Cook et al., 2012; Romero and Reed, 2008) which orders individuals from highest to lowest based on GC levels and assesses whether rank order changes over time. While this type of investigation will indicate whether individuals that have low or high concentrations of GCs relative to others maintain their rank over time, it is possible that a high amount of within-individual variability in GC levels can still underlie high estimates of ranked repeatability (e.g., the scenarios in Figure 3.1e and h would have equally high ranked repeatability estimates). However, a standardized form of repeatability known as 'consistency repeatability' can provide information on whether individuals show consistency in directional responses (i.e., if all individuals change similarly over time). With this type of repeatability, the scenarios in Figure 3.1a and e will both result in high repeatability estimates. Therefore, the analytical and statistical tools required to properly assess within-individual consistency are readily available and only need to be applied to the alternative goal of assessing whether individuals in a population respond to an environmental change (or lack of change) in the same way.

In this study, we quantified the average (population-level) response, amount of within-individual variation, and repeatability of baseline corticosterone (CORT) levels (the primary avian GC) in wild breeding tree swallows (*Tachycineta bicolor*): (1) across

breeding stages (from incubation to offspring provisioning), and (2) in response to a feather clipping manipulation during nestling provisioning that decreases adult foraging profitability. As the feather clipping manipulation can be viewed as creating a prolonged decline in realized habitat quality from the perspective of food availability (Madliger et al., 2015), it allowed us to determine whether birds responded in individually-specific ways to a standardized change in environmental quality. If baseline CORT levels represent a readily detectable indicator of environmental disturbance, we would predict that CORT levels would change at the population level, and across all individuals in the same manner in response to the clipping manipulation (i.e., we predict that consistency repeatability will be high).

Methods

Study species and sampling protocol

We monitored a nest-box breeding population of tree swallows from late April to early July during 2011. Tree swallows are a small migratory passerine that are a member of a group of birds known as aerial insectivores which are experiencing precipitous population declines in North America (Nebel et al., 2010). They readily nest in artificial boxes and are highly philopatric to their breeding grounds (Winkler et al., 2004). A total of 96 nest boxes were located across two sites in Haldimand County, Ontario, Canada located four kilometers apart: Taquanyah Conservation Area (42º57' N, 79º54' W) and Ruthven Park National Historic Site (42º58'N, 79º52'W). Boxes were grouped within fallow fields near active agricultural fields, wetlands, and riparian areas along the Grand River. We monitored boxes every two days during the nest building phase and daily following detection of the first egg to record date of the first egg laid (lay date), clutch size, egg

mass, hatch date, mass of chicks at days six and 12 post-hatching, and the number of

offspring that successfully left the nest (fledging success). In addition, 10 days following

clutch completion (late incubation) and 12 days following offspring hatch (peak nestling

provisioning), we captured adult females at the next box to record mass, wing length, and

obtain a blood sample (<150 ul) through puncture of the brachial vein. Females were

provided with a federal numbered aluminum band (Canadian Wildlife Service Permit

10808). Blood samples were obtained within two minutes of covering the nest hole to

ensure sampling of baseline levels of CORT (Romero and Reed, 2005) and between

0800h and 1200h to control for diel variation in hormone levels. Samples were stored on

ice for up to five hours until centrifuged to separate plasma and stored at -80 degrees C

until assay. All animal handling and experimental methodology was approved by the

Canadian Wildlife Service (Permit CA 0266) and the University of Windsor's Animal

Care Committee (AUPP #10-10).

Experimental manipulation

As tree swallows acquire all of their insect food resources on the wing for self-

maintenance and offspring provisioning (Robertson et al., 1992), we used a feather

clipping manipulation that alters flight performance and foraging profitability to induce

an extended decline of environmental quality on breeding females. Similar feather

clipping manipulations have been shown to result in a decreased ability to acquire food

resources in this species (Winkler and Allen, 1995), and the manipulation leads to a

decrease in the number of foraging bouts compared to control birds in our population

(Madliger et al., 2015). When females were captured for banding and blood sampling at

day 10 of incubation (just prior to hatching), we clipped every other primary flight feather

(four feathers on each wing) at the base of the wing with scissors (Ardia and Clotfelter, 2007; Winkler and Allen, 1995) on a subset of females (n=33). Control females (n=40) were handled identically, but their feathers were left intact. Control and manipulated females were matched spatially across habitat sites and temporally by date over the season. Feathers remain clipped until natural molt occurs following breeding (Stutchbury and Rohwer, 1990); therefore, this manipulation alters female foraging ability for the entire period of nestling provisioning.

Hormone analysis

We quantified baseline levels of CORT using a previously validated enzyme-linked immunoassay (EIA: Assay Designs, Ann Arbor, MI, USA) (Love and Williams, 2008). Briefly, samples were run in triplicate at a 1:20 dilution with 3% steroid displacement buffer (SDB). Plates were run using a standard curve created by serially diluting a kit-provided corticosterone standard (20,000 pg/ml - 15.63 pg/ml). Laying hen plasma was used as a control (Sigma-Aldrich, Oakville, Ontario, Canada). We read assay plates at 405nm using a spectrophotometer plate reader. Intra-assay variation was 7.9% and inter-assay variation was 11.2%. In cases where concentrations fell below the detectable limit of the assay (0.74 ng/ml), samples were assigned this detection limit (8 of 146 samples).

Quantifying habitat features

Nest boxes in our colony are surrounded by a variety of habitat types including fallow fields, riparian areas associated with the Grand River, roadways, active agricultural fields, wetlands, and forests. Because the boxes are spaced across this large heterogeneous expanse of landuses at both study sites, we characterized the surrounding habitat of each

individual box to allow for its assignment to a habitat "cluster". This allows for a more detailed quantification of variation in surrounding habitat types rather than simply including "site" as a covariate in subsequent analyses. Specifically, we used a geographic information system (ArcGIS 10.1; Esri) and a 2010 orthorectified SWOOP (South Western Ontario Orthography Project) satellite image (20cm resolution) to quantify the following habitat characteristics surrounding each next box: (1) distance to forest; (2) distance to hedgerow; (3) proportion of high insect (i.e., food) landuse types within a 200m radius; (4) proportion of high insect (i.e., food) landuse types within a 1 km radius; (5) distance to the Grand River; (6) distance to a roadway. We chose these variables based on tree swallow nest site preferences, requirements, and potential disturbances (Table 3.1). We performed a principal components analysis based on the correlation matrix of these six untransformed variables (James and McCulloch, 1990). Two principal components that explained 79% of the variance in the original variables were chosen based on examination of a scree plot (D'agostino and Russell, 2005) and were subjected to varimax rotation (Abdi, 2004) to produce two factor scores for each box. Variables associated with food availability loaded heavily onto factor 1, while variables associated with nest disruption loaded heavily onto factor 2 (Table 3.2). We subsequently performed a cluster analysis (James and McCulloch, 1990) using expectation maximization (normal mixtures) clustering (Nathiya et al., 2010) to create two categories (clusters) of boxes based on their factor scores. The final number of clusters was validated based on two characteristics obtained from a discriminant function analysis with cluster ID as the dependent variable and the original habitat variables as independent variables (Leimeister, 2010): (1) a highly significant Wilks' lambda (Wilks' lambda = 0.046; $P <$ 0.0001) indicating that over 95% of the total variance in the discriminant scores was

explained by differences between groups (clusters); (2) investigation of the number of errors the discriminant function analysis produced; two clusters produced the lowest number of classification errors (1%). Habitat cluster was then used in lieu of "site" in all subsequent analyses to better control for the environmental landscape characteristics associated with each nest box.

Statistical analysis

All statistical analyses were performed in JMP 12 (SAS Institute), unless otherwise stated. We used four analyses to characterize population-level and individual changes in CORT (i.e., to determine which scenario in Figure 3.1 best approximates our data) in control and feather-clipped birds separately to allow us to separate patterns between natural conditions and those associated with a change in environmental quality. Baseline CORT values were log-transformed prior to analysis to achieve normality (as indicated by Shapiro-Wilk test). First, we determined whether baseline CORT changed from the incubation to the nestling provisioning stage (i.e., over a two-week period) using a repeated measures ANCOVA with habitat cluster included as a random effect and laying date included as a fixed effect. This analysis determines whether there is a difference in baseline CORT at the average (population) level between the incubation and nestling provisioning stage, or in response to the feather clipping manipulation. It should be noted that we presented a similar analysis in a previous publication (Madliger and Love, 2014), but here we consider habitat type and re-present the data because it is integral to the interpretation of our subsequent analyses. Second, we tested for the equality of variances in baseline CORT between the incubation and nestling provisioning stages (control birds), and before and after the clipping manipulation (treatment birds), using a Bartlett test to

determine whether the spread of baseline CORT values increase, decrease, or remain the same over time. Third, we tested for differences in average baseline CORT levels between individuals (i.e., significant intercepts). This analysis determines whether, on average over the two sampling times, individuals differ in their baseline CORT level. For example, the individuals in Figure 3.1a and e would show significant between-individual variation in baseline CORT, while the individuals in Figure 3.1b and f would not. We tested this specifically by comparing two hierarchical models with the same fixed effect structure, but differing random effects structure using a likelihood ratio test (LRT). LRTs test for the significance of random effects by comparing the log-likelihoods of two nested models estimated with REML by using a χ^2 distribution (Pinheiro and Bates, 2006). Both of our models included baseline CORT as the dependent and habitat cluster (random) and lay date (fixed) as independent variables. In addition, one model included individual identity as a random effect to test for the significance of between-individual variance in baseline CORT levels. As variance components were bounded to be positive, we tested for statistical significance with an equal mixture of χ^2_0 and χ^2_1 distributions (as per Visscher, 2006).

Finally, we calculated the repeatability of baseline CORT in control and clipped groups separately. Repeatability is calculated as the variance between individuals divided by the total variance (the sum of between- and within-individual variance) (Lessells and Boag, 1987). Most estimates of repeatability refer to 'agreement repeatability', where high estimates indicate low within-individual variability in absolute measures of a trait (Biro and Stamps, 2015). We instead calculated 'consistency repeatability' which allows for high estimates of repeatability despite a change in a trait over time, as long as all individuals change in the same way (Biro and Stamps, 2015; Nakagawa and Schielzeth,

2010). As a result, consistency repeatability allows us to assess this key characteristic of baseline GCs in regards to changes in environmental quality that would not be observable with traditional agreement repeatability. We calculated consistency repeatability by centering baseline CORT values on their mean at each measurement time (incubation and nestling provisioning) (as per Dingemanse and Dochtermann, 2013). To allow for subsequent log transformation of the CORT data, we added a constant to the mean-standardized values so that the lowest value was 1.00. We then used linear-mixed effect models controlling for habitat cluster (random) and lay date (fixed) to determine adjusted repeatability in R 3.2.1 (R Development Core Team, 2015) using the package rptR (Nakagawa and Schielzeth, 2010). It is important to note that we did not employ a traditional random regression approach (e.g., Brommer et al., 2005; Dingemanse and Dochtermann, 2013; Nussey et al., 2007) to test for individually-specific responses in baseline CORT (i.e., slope or 'plasticity') for two primary reasons: (1) sample size requirements for the determination of statistically significant individual plasticity are outside of those easily obtained in many wild populations (e.g., 200 observations; Martin et al., 2011), particularly for physiological data requiring blood sampling; (2) such approaches are better-suited to experimental designs with more than two repeated measures per individual (Martin et al., 2011) and therefore do not fit with the goal of our current investigation. We have previously shown that metrics of reproductive workload (clutch size and brood size) do not relate to baseline CORT levels in our population (Madliger et al., 2015) and thus did not include these as covariates in the analyses presented here; importantly, the inclusion of both variables as fixed effects do not alter the outcomes of the analyses contained herein.

Results

There was no difference in average baseline CORT level between the incubation and nestling provisioning stage in control birds (repeated measures ANCOVA: F=0.48, P=0.49; mean ± SE ng/ml: incubation = 2.75 ± 0.29, nestling provisioning = 2.76 ± 0.29; Figure 3.2). In the clipped group, baseline CORT at nestling provisioning was significantly higher than during incubation (repeated measures ANCOVA: F=11.64, P=0.002; mean ± SE ng/ml: incubation = 2.50 ± 0.34, nestling provisioning = 3.67 ± 0.34; Figure 3.2) indicating that, on average, the clipping manipulation increased baseline CORT levels. The variance in baseline CORT levels at incubation and nestling provisioning were equal in both control (Bartlett test: F=0.87, df=1, P=0.35) and clipped (Bartlett test: F=1.54, df=1, P=0.23) birds. Control birds did not show significant individual differences in baseline CORT (LRT: χ^2=6.06, P=0.50) indicating that birds have similar average CORT levels (i.e., low between-individual variation in baseline CORT levels). Similarly, clipped birds did not show individual differences in baseline CORT (LRT: χ^2=1.36, P=0.38) in the average environment, also indicating the presence of low between-individual variation. Baseline CORT levels were not repeatable from the incubation to the nestling provisioning stage for both control (r=0.39, SE=0.13, CI=0.114, 0.63, P=0.50; Figure 3.3) and clipped birds (r=0.22, SE=0.15, CI=0, 0.51, P=0.40; Figure 3.3). See Table 3.3 for associated within- and between-individual variance components.

Discussion

We used a manipulation of realized environmental quality to quantify the population-level and within-individual response of baseline CORT levels. Under natural conditions, average baseline CORT values did not differ between the incubation and nestling

provisioning stages in free-living female tree swallows. In contrast, females that faced a decrease in foraging profitability (decreased realized habitat quality) via feather clipping had significantly higher average CORT levels at the nestling provisioning stage (post-manipulation) in comparison to the incubation stage (pre-manipulation). As this manipulation has been previously shown to result in a decreased number of foraging trips in relation to controls (Madliger et al., 2015; Patterson et al., 2011; Winkler and Allen, 1995), it represents a biologically-relevant proxy of a decrease in available food resources for females and their dependent offspring. As a result, a test of individual responses to this manipulation can provide insight into how females may respond to unexpected changes in environmental quality that manifest as decreases in food acquisition or other energetic constraints.

We found evidence of individually-specific responses in baseline CORT over the breeding season naturally and in response to a manipulation of environmental quality. Specifically, by quantifying average change in baseline CORT, equality of variances, individual differences in average baseline CORT, and repeatability, we determined that our repeated measures data for control birds most closely approximates the pattern in Figure 3.1f, while feather clipped birds most closely approximate the pattern in Figure 3.1b. The low repeatability estimates in both cases indicate that the amount of within-individual variation in baseline CORT was greater than the degree of between-individual variation. As a result, there is a relatively high degree of individually-specific changes in baseline CORT across breeding, and in response to a standardized manipulation of foraging profitability. In other words, birds showed a mixture of increases, decreases, and lack of change in baseline CORT levels across reproduction and in response to a decline in environmental quality. While we have relatively low samples sizes in comparison to

investigations of behavioural repeatability (Dingemanse et al., 2010b), they are comparable with other investigations that have been able to detect significant repeatability in baseline and stress-induced GCs in wild and captive populations (Cook et al., 2011; Ouyang et al., 2011a; Rensel and Schoech, 2011; Romero and Reed, 2008). We also combined a number of measurements to assess the shape of our repeated data, rather than employing the random regression methods that require large sample sizes (Martin et al., 2011). We believe that our data are much more representative of that which is available to managers, especially those working on sensitive species.

Previous repeatability estimates of baseline GCs have been mixed, differing depending on factors such as season, length of time between measurements, sampling conditions (e.g., wild versus laboratory settings), and other environmental factors (Ouyang et al., 2011a). For example, while high repeatability of baseline GCs has been found previously within the breeding season in tree swallows and great tits (*Parus major*) (Ouyang et al., 2011a), repeatability estimates have generally been low over longer time spans (months to years) in the same species (Ouyang et al., 2011a) and in largemouth bass (*Micropterus salmoides*) (Cook et al., 2011), Florida scrub jays (*Aphelocoma coerulescens*) (Rensel and Schoech, 2011), and garter snakes (*Thamnophis elegans*) (Sparkman et al., 2014). However, this pattern is not without deviation, as Angelier et al. (2010) found high repeatability of baseline GC levels over a one year period in breeding black-browed albatrosses (*Thalassarche melanophris*), and Pavitt et al. (2015) found high repeatability of fecal GC metabolites over a 10-year sampling period in wild red deer (*Cervus elaphus*), but only after accounting for age and season. Low estimates of repeatability are in line with baseline GCs' labile role in allowing individuals to respond to differing metabolic needs over time and this flexibility is considered adaptive (Bonier

et al., 2009b). For example, the ability to modulate baseline GC levels over short time frames has likely promoted range expansion in the invasive house sparrow (*Passer domesticus*) across Kenya (Martin and Liebl, 2014). More broadly, changes in baseline GCs may promote reallocation of resources during energetically demanding times of the life cycle, such as promoting foraging for offspring (Bonier et al., 2009a, 2009b; Escribano-Avila et al., 2013; Love et al., 2004; Ouyang et al., 2011b).

While an investigation solely at the average level in our study would have led to the conclusion that there is a high level of consistency in CORT levels over breeding (i.e., that energetic demands may be equivalent between incubation and nestling provisioning), there is indeed a great deal of underlying change at the individual level. In other words, the lack of change in non-manipulated birds at the population-level over the reproductive season was a simplification of a large amount of within-individual variation. Baseline CORT levels of individual nesting female tree swallows may have been changing based on the energetic demands imposed by breeding, investment decisions, inter- and intra-specific competition, food availability, temperature, or other weather conditions. Moreover, the lack of repeatability in response to the feather clipping manipulation is also important to the application of baseline GCs as conservation biomarkers as it indicates that individuals may respond in individually-specific ways to the same environmental perturbation. These results are in line with previous findings that a common GC profile of chronic disturbance does not exist (Dickens and Romero, 2013); baseline GC values do not appear to change in a predictable way in response to different types of prolonged perturbations and it may be much more important to document the presence of any change, rather than a change in a specific direction (i.e., an increase). Importantly, our results indicate that single measures of baseline GCs are unlikely to be broadly

representative of individual state, and it may be much more important to assess how flexibility in hormone levels over time may be allowing individuals to cope with environmental and life history demands (Bonier et al., 2009b, 2011; Love et al., 2014; Ouyang et al., 2011b). When sample sizes are low, or managers are not able to obtain repeated measures over time on the same individuals, the interpretation of changing baseline GC levels at the population (average) level may lack the full detail necessary to adequately draw conclusions about disturbance or health.

It is possible that other perturbations in the environment could cause more consistent responses in baseline GCs across individuals or that other times of the life cycle may be better-suited to measuring GCs in this way. For example, the underlying demands associated with breeding (or other stages such as migration) may impart difficulty in assessing baseline GCs as a biomarker of disturbance, while non-breeding seasons may show higher consistency in responses. However, individuals still cope with alternative demands, habitats, timing, and social interactions in the non-breeding season that can influence baseline GC levels (Baker et al., 2013; Garcia Pereira et al., 2006; Lindström et al., 2005; Marra and Holberton, 1998). It will therefore be important to investigate how individuals respond to perturbations of different intensities and durations across different seasons, sexes, and environments to fully ascertain the value of baseline GCs as a conservation biomarker. Overall, the use of baseline GCs may be limited in many wild systems, or may require repeated measures to fully determine how individuals are coping with disturbances (particularly long-term perturbations) in their environment. As a result, we encourage others to assess within-individual variation rather than relying on purely average-based approaches when interpreting GC (and other hormonal) data (Williams, 2008). As information accumulates on the consequences of this type of

variation for fitness and population persistence, we will be able to refine techniques to

better determine their relative role in the conservation toolbox.

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Tables

Table 3.1- Habitat variables quantified around each nest box and relevance of each feature to breeding tree swallows.

Habitat variable	Relevance to breeding tree swallows	Reference(s)
Distance to forest	Nest predators such as raccoons (*Procyon lotor*) and black rat snakes (*Elaphe obsoleta*), and the inter-specific nest competitor house wrens (*Troglodytes aedon*), which destroy tree swallow eggs, are associated with wooded areas.	(Dijak and Thompson, 2000; Durner and Gates, 1993; Finch, 1990; Parren, 1991; Rendell and Robertson, 1990; Weatherhead and Charland, 1985)
Distance to hedgerow	Inter-specific nest competitors (house wrens) are associated with wooded areas.	(Finch, 1990; Parren, 1991; Rendell and Robertson, 1990)
Proportion of high insect landuse type (200 m radius)	Fallow fields, wetlands, and cattle pastures (extensive landuse types) provide insect food resources. During nestling provisioning, tree swallows primarily forage within 200 meters of their nest box.	(McCarty, 1995; McCarty and Winkler, 1999; Robertson et al., 1992)
Proportion of high insect landuse type (1 km radius)	Fallow fields, wetlands, and cattle pastures (extensive landuse types) provide insect food resources. During incubation and nestling provisioning, tree swallows can travel longer distances to forage. One kilometer was chosen to quantify a landscape scale where the amount of extensive landuse has been associated with differences in reproductive success.	(Ghilain and Bélisle, 2008; Robertson et al., 1992)
Distance to Grand River	The Grand River represents a primary foraging location during periods of inclement weather.	Madliger, *pers. obs.*
Distance to roadway	Roadways represent a high-risk habitat feature to tree swallows (due to potential mortality or injury) and many passerine species are negatively influenced by roads indirectly (e.g., noise).	(Ashley and Robinson, 1996; Kociolek et al., 2011; Reijnen and Foppen, 2006)

Table 3.2 - Rotated factor loadings for habitat variables associated with tree swallow nest

boxes.

Habitat variable	Factor 1	Factor 2
Distance to road	0.14	0.86
Distance to forest	0.15	-0.55
Distance to hedgerow	0.37	0.70
Distance to Grand River	0.58	-0.67
% high insect landuse (200 m)	0.97	0.08
% high insect landuse (1 km)	0.88	0.02
Proportion of variance	0.40	0.39

Table 3.3 - Within-individual (residual) and between-individual (individual) variance components of mean-centred baseline corticosterone levels in breeding female tree swallows.

Analysis	Parameter/Variable		
Control		**Random effects variance**	**SD**
	Individual	0.024	0.089
	Site	0.000	-
	Residual	0.036	0.072
		Fixed effects estimates	**SE**
	Lay date	-0.001	0.004
Clipped		**Random effects variance**	**SD**
	Individual	0.010	0.073
	Site	0.000	-
	Residual	0.047	0.073
		Fixed effects estimates	**SE**
	Lay date	-0.005	0.006

Figures
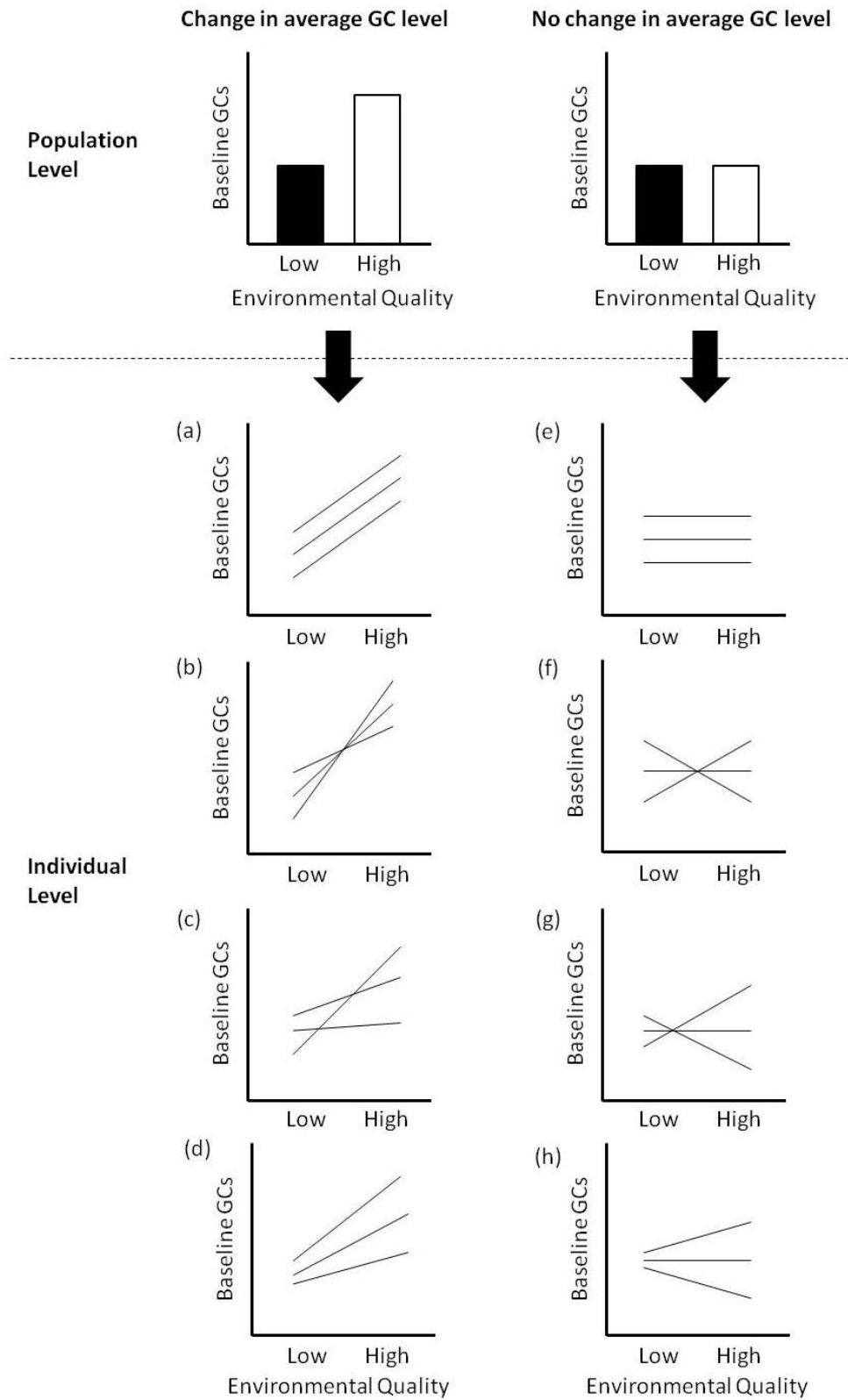
Figure 3.1 - Diagram showing the ways in which within-individual (i.e., repeated-measures) data can underlie patterns at the average (population) level. Scenarios a-d can occur when there is an average change in baseline glucocorticoid (GC) level at the population scale. Scenarios e-h can occur when there is no average change in baseline GCs at the population scale.
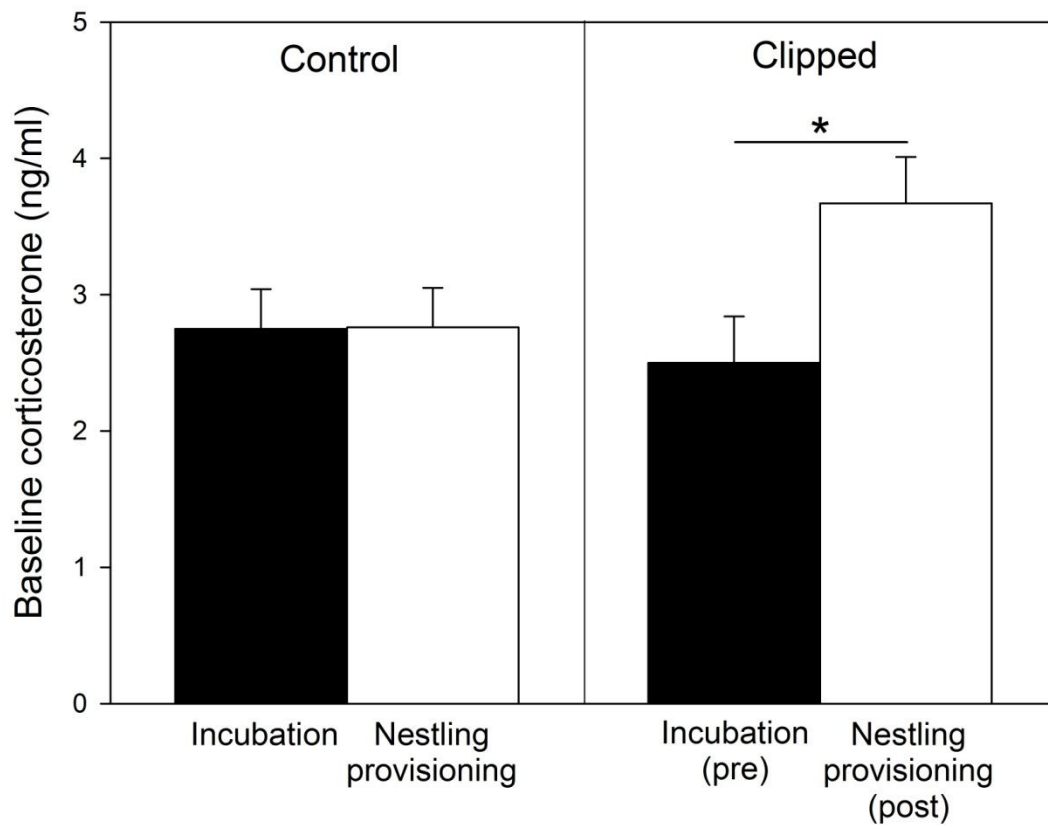
Figure 3.2 - Differences in baseline corticosterone between the incubation and nestling provisioning stage in control (n=40) and feather-clipped (n=33) female tree swallows. Birds were assigned to a treatment group immediately following the incubation sample.

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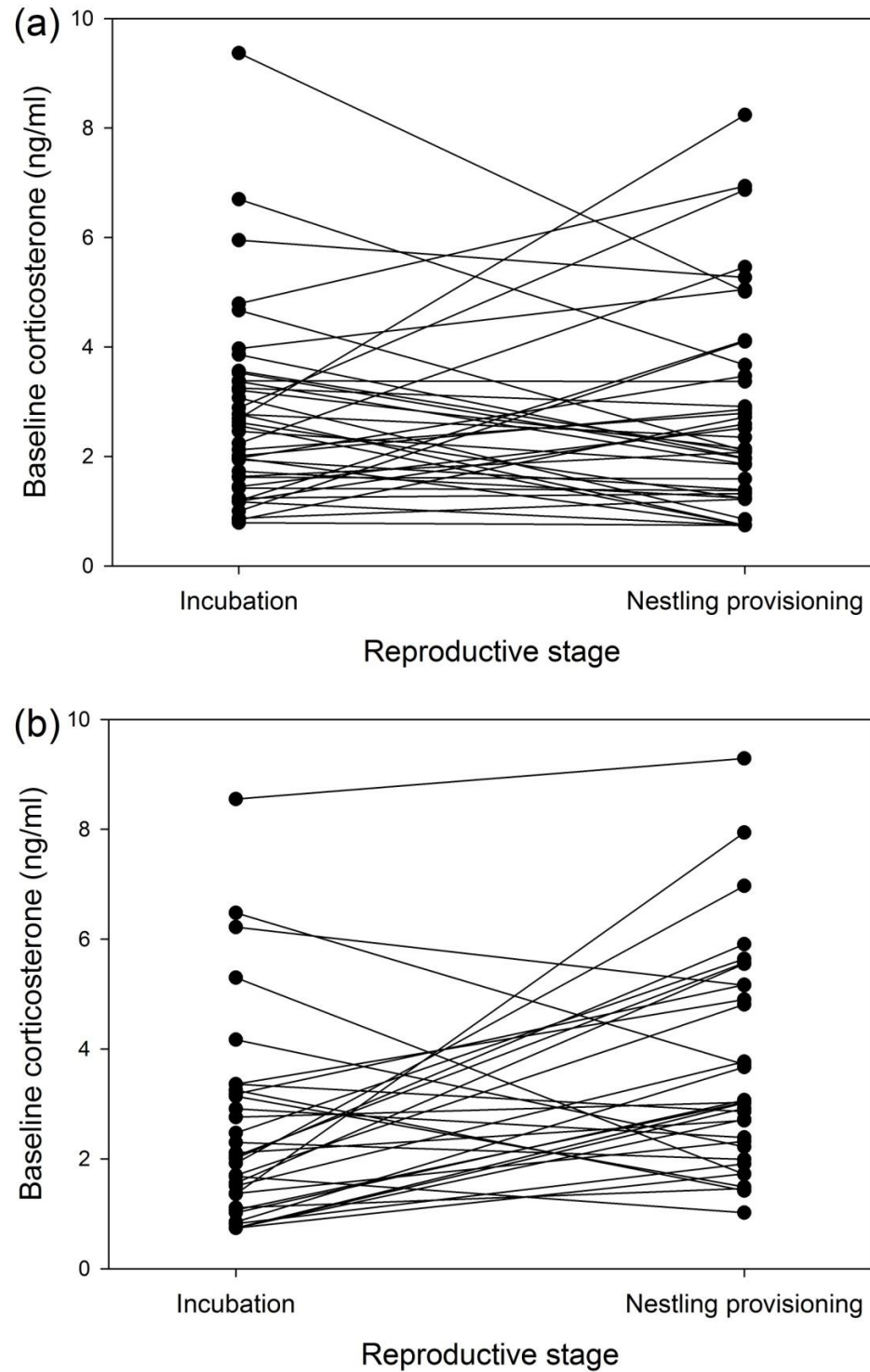
Figure 3.3 - Individual changes in baseline corticosterone from the incubation to the nestling provisioning stage in control (a) and feather-clipped (b) birds. Birds were assigned to a treatment group immediately following the incubation sample.

CHAPTER 4 - CONSERVATION IMPLICATIONS OF A LACK OF RELATIONSHIP BETWEEN BASELINE GLUCOCORTICOIDS AND FITNESS IN A WILD AVIAN POPULATION*

Introduction

Conservation biologists and managers are often tasked with urgently and accurately determining how wildlife populations respond to changing environmental conditions (Angelier and Wingfield, 2013; Wingfield, 2013). Traditionally, demographics have been employed to monitor changes in population persistence over time; however, these approaches provide little guidance on possible mitigation strategies and require large longitudinal datasets to ensure that changes in population numbers represent significant trends (Carey, 2005; Ellis et al., 2011; Wikelski and Cooke, 2006). Consequently, the measurement of physiological metrics such as hormones, metabolites, immune factors, etc. forms the foundation of the growing field of conservation physiology, which seeks to provide proactive insight into population health and condition (Cooke et al., 2013; Wikelski and Cooke, 2006). One of the essential requirements of employing any physiological metric as this type of biomarker is the presence of a predictable relationship with fitness (Busch and Hayward, 2009; Cooke and O'Connor, 2010; Madliger and Love, 2015). Without such a relationship, variation in physiology over time cannot be interpreted as a sensitive indicator of future population change; predictive capacity necessitates a linkage between physiology and the metrics that drive population persistence (Figure 4.1).

Although the physiological measures available to conservation managers (see Cooke et al., 2013 for an overview) and used successfully for conservation goals

(Madliger et al., 2016) are diverse, the field is currently dominated by measures of stress physiology (i.e., glucocorticoids (GCs): cortisol and corticosterone) (Lennox and Cooke, 2014). At baseline levels, GCs regulate feeding, promote regular activity patterns, and maintain energy metabolism (Landys et al., 2006). Unexpected acute perturbations result in a transient increase in GCs that mobilize energy reserves, temporarily suspend reproduction and other non-essential activities, promote subsequent foraging, and regulate immune function (Landys et al., 2006; Wingfield and Kitaysky, 2002; Wingfield and Sapolsky, 2003; Wingfield et al., 1998). If an organism is unable to overcome a stressful event (i.e., restore homeostasis), GCs can remain elevated for a prolonged period leading to allostatic overload with negative consequences for reproductive activities, foraging, immunity, vigilence, and/or survival (Korte et al., 2005; McEwen and Wingfield, 2010). Overall, GC levels are expected to parallel energetic demand (Wingfield, 2005), with recent evidence suggesting a causal link between baseline GCs and reproductive investment, likely mediated through behaviour or metabolism (Cook et al., 2011; Love et al., 2014; Ouyang et al., 2011, 2013a).

Much of the application of GCs in the context of conservation has been based on the assumption of a negative relationship between baseline levels and fitness (Bonier et al., 2009a; Busch and Hayward, 2009), with higher baseline levels correlating with lower condition, reproductive success, and/or survival probability (Bonier et al., 2009a). Although this relationship, formally known as the 'CORT-fitness hypothesis' (Bonier et al., 2009a), has been investigated relatively extensively by ecological and evolutionary physiologists, results have been extremely mixed (Angelier et al., 2010; Bonier et al., 2009a; Breuner, 2011; Crespi et al., 2013; Escribano-Avila et al., 2013; Sopinka et al., 2015). For example, Bonier et al. (2009b) found that the relationship between baseline

GCs and reproductive success in female tree swallows (*Tachycineta bicolor*) switched from negative during the early breeding (incubation) stage to positive during the offspring provisioning stage. This discrepancy is likely a reflection of the role of GCs in promoting foraging; when the fitness value of a reproductive attempt is high (e.g., late in a reproductive attempt), increases in GCs will promote allocation of resources towards the breeding attempt leading to a positive relationship between GCs and fitness (Bonier et al., 2009a, 2009b, 2011; Breuner, 2011). In contrast, when the fitness value of a reproductive attempt is low (e.g., early in reproduction), increases in GCs are more likely to interfere with successful reproduction (Bonier et al., 2009a, 2009b, 2011; Breuner, 2011). While some support for this hypothesis exists (Bókony et al., 2009; Bonier et al., 2011; Escribano-Avila et al., 2013), it is not yet clear how well it explains discrepancies across the entire range of GC-fitness investigations.

It is also possible that context-dependent GC-fitness relationships may be causing much of the ambiguity in results (Breuner, 2011; Crespi et al., 2013; Jaatinen et al., 2013; Madliger and Love, 2014). For example, Ebensperger et al. (2013) could not detect a relationship between fecal GCs and survival in degus (*Octodon degus*), but levels did predict whether females would produce a second litter, illustrating the importance of investigating multiple fitness metrics simultaneously. Other contexts, such as sex and environmental quality, have also proven to be important. For instance, Angelier et al. (2010) found a negative relationship between baseline GCs and 5-year reproductive output in male, but not female, black-browed albatross (*Thalassarche melanophris*). Similarly, D'Alba et al. (2011) found that a negative GC-fitness relationship was only evident in common eiders (*Somateria mollissima*) nesting in poorer, exposed sites compared to sheltered sites. Finally, the management of GC levels across demanding time

periods may better predict fitness outcomes than static (single-point) measures (Love and Williams, 2008; Love et al., 2014; Ouyang et al., 2011; Williams, 2008). For example, a manipulation of brood size in tree swallows indicated that the change in baseline GCs over reproduction, but not static measures, could positively predict maternal foraging rates and subsequent fledging success (Bonier et al., 2011). In other words, initial physiological state may influence the subsequent response to environmental constraint or reproductive demand (Arlettaz et al., 2014; Love et al., 2014; Ouyang et al., 2011, 2013b). This possibility has important consequences for management applications as it would necessitate the collection of two or more measurements of GCs across time to infer population health or persistence. Overall, context-dependent GC-fitness relationships also have important implications for employing GC levels in conservation, as levels will need to be interpreted differently depending on intrinsic and extrinsic factors, and in relation to different fitness metrics (e.g., current reproductive success versus survival probability) (Madliger and Love, 2014).

We used an explicit, context-dependent approach to investigate the relationship between baseline plasma GC levels and fitness in female tree swallows over three years. We measured baseline GCs at two different stages of reproduction (late incubation and mid-offspring provisioning) and assessed the relationship of each measure, as well as the change in GCs over the season, with three distinct metrics of fitness: i) reproductive output; ii) offspring quality; and iii) survival probability. Moreover, we determined whether the inclusion of additional contexts such as food availability, reproductive investment, or body mass could alter the GC-fitness relationship, or whether any of these contexts were able to better predict fitness than GC measures. Finally, we included an experimental manipulation of foraging profitability (feather clipping) to examine whether

the GC-fitness relationship changes when individuals are pushed outside of expected environmental conditions.

We predicted that the GC-fitness relationship would change from positive during incubation to negative during nestling provisioning in the control group (i.e., under natural conditions), in line with the increase in brood value over the reproductive period and the role of GCs in promoting allocation of resources to reproduction (Bonier et al., 2011). In contrast, we predicted that the GC-fitness relationship would remain negative at the nestling provisioning stage in the clipped group as we expected that the individuals with the highest GC levels would be those most negatively affected by the decline in foraging profitability, and also the least able to successfully raise offspring or survive to the following year. We also predicted that the change in baseline GCs over the reproductive season would be positively related to fitness in control birds. By examining contexts such as reproductive stage, GC metric, fitness metric, energetic contexts, and environmental quality, we can better assess when and how GC levels may predict population-level demographic consequences. Such an approach is essential to determining the applicability of GC levels as conservation biomarkers and is currently considered one of the highest priority questions in the field of conservation physiology (Cooke, 2014).

Methods

Study species

Tree swallows are small, migratory passerines that readily breed in artificial boxes. The species represents a model organism that has been heavily studied in the context of life-history tradeoffs, ecological requirements, toxicology, and immune responses (Jones, 2003). Tree swallows are aerial insectivores, a guild of birds which feed on flying insects

that has shown precipitous population declines in North America (Nebel et al., 2010).

Although they are an abundant, widespread member of this group, they have nonetheless

declined by 2.5% per annum over the past 20 years (McCracken, 2008), with some

populations declining more rapidly (Paquette et al., 2014). Our study population is

located in an area of southern Ontario, Canada that is characterized by agricultural

expansion and loss of wetland habitat similar to the alterations that this species would be

exposed to across a large proportion of its range. As such, it is an ideal species to

investigate relationships between physiology and fitness, and to draw conclusions about

how changes in environmental quality on the breeding grounds may lead to demographic

consequences for aerial insectivores.

Nest monitoring and sampling protocol

We monitored breeding attempts of pairs of tree swallows in a nest box colony located in

southern Ontario, Canada from 2010-2014. In total, our study area consists of 175 nest

boxes located across two conservation sites 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 are located adjacent to and within a variety of landuse types

including active agricultural fields, roadways, fallow fields, cattle pastures, and riparian

areas along the Grand River. We checked boxes once daily and recorded date of the first

egg laid, clutch size, egg masses, hatching success, and the number of offspring that

successfully left the nest (fledging success). We also recorded the mass of each nestling at

6 and 12 days of age. The nestling mass measurements taken at 12 days of age were

summed and used as a metric of brood mass at each nest box. At this 12-day age,

nestlings can have masses equal or greater to adults (McCarty, 2001; Quinney et al.,

1986) and nestling growth can influence post-fledging survival, with chicks with delayed or interrupted growth showing diminished long-term survival (McCarty, 2001).

We focused on females in our study because they are the sole incubators and the most accessible sex throughout reproduction. Females were captured at their nest box at two time periods: 1) late incubation (10 days after clutch completion); 2) mid-nestling provisioning (12 days post-hatch). Females were blood sampled through puncture of the brachial (wing) vein to obtain <150 ul of blood (i.e., less than 10% of total blood volume). Blood samples were obtained between 0800 and 1200 to control for diel changes in baseline CORT values, and within two minutes of trapping a bird in the nest box to ensure acquisition of baseline samples (Romero and Reed, 2005). We also recorded mass, wing length, and age, and visually scored fat and muscle condition. Female tree swallows in their first year are characteristically brown in colour, while females aged two years or older are iridescent blue-green (Hussell, 1983). We also gave unbanded birds a federal numbered band (Canadian Wildlife Service Permit: 10808). Finally, we determined return rates for each female to serve as a proxy for survival probability. Tree swallows live an average of 2.7 years and to a maximum of 8 years (Butler, 1988). Ninety-five percent of birds that fledge at least one offspring will return to the same breeding site in the subsequent year (many to the same nest box) (Winkler et al., 2004), and even after complete nest failure, females are still 72% likely to return to the same breeding site (Winkler et al., 2004). As a result, we considered return rates to be valid proxies for female survival in this species due to their extremely high level of philopatry (Winkler et al., 2004).

We also monitored food availability throughout the reproductive period using four-sided, commercially-available malaise traps. In total, we used 5 traps across our

study sites. Our sampling protocol has been described and justified for tree swallows in detail elsewhere (Madliger et al. 2015 - Chapter 2). Briefly, we calculated the average insect (dry) biomass (as per Hussell and Quinney, 1987) that was available over the most demanding stage of the nestling provisioning period (day 5-10) (McCarty, 2001) for each individual as our measure of food availability for subsequent analyses.

Manipulation of foraging profitability

In 2011, we used a feather clipping manipulation to decrease foraging profitability (Winkler and Allen, 1995) and therefore simulate an unexpected decline in habitat quality in the context of food availability (Madliger et al., 2015). We clipped four flight feathers at the base of the feather on each wing (i.e., every other primary flight feather) of 33 females (Ardia and Clotfelter, 2007; Winkler and Allen, 1995) (control: n=38). This level of feather clipping causes a handicap in this species by increasing the energetic cost of flight (Ardia and Clotfelter, 2007; Winkler and Allen, 1995), leading to decreased foraging rate (Madliger et al., 2015; Patterson et al., 2011; Winkler and Allen, 1995) and lower body condition compared to control birds (Ardia and Clotfelter, 2007; Patterson et al., 2011). In addition, this manipulation increased baseline GCs over reproduction and led to the clipped group having higher average levels of GCs compared to control birds at the nestling provisioning stage in our population (Madliger et al., 2015 - Chapter 2, 3). We performed this manipulation at the late incubation stage, immediately following acquisition of the first blood sample. All other birds were handled in the same way, but no flight feathers were clipped. Only females aged two years or older were included in the clipping manipulation due to a considerably smaller sample size of one-year-old females across our sites. Females faced this handicap for two weeks before the nestling

provisioning blood sample was obtained, and remained feather clipped until the natural

moult that follows breeding (prior to migration to wintering grounds) (Stutchbury and

Rohwer, 1990). All manipulation and monitoring protocols were approved by the

University of Windsor's Animal Care Committee (AUPP #10-10) and the Canadian

Wildlife Service (Permit CA 0266).

Corticosterone assay

We stored blood samples on ice for up to five hours and then centrifuged to separate

plasma. Plasma was stored at -80 ˚C until assay. We measured non-extracted levels of

baseline corticosterone (CORT: the primary GC in avian species) in plasma using a

Corticosterone Enzyme-linked Immunoabsorbent Assay (EIA - Assay Designs Inc.,

Michigan USA, catalog #901-097). Samples were run in triplicate at a total volume of

100 μl with 1:40 dilution and 1.5% steroid displacement buffer (SDB). We calculated the

detection limit of the assay as 0.74 ng^{-1} ml, (as per the manufacturer's method). Of 442

total plasma samples analyzed, 24 fell below that value and were assigned the value of the

detection limit. Intra-assay variation was 7.7%, 8.0%, and 10.3% in 2010, 2011, and

2012, respectively. Inter-assay variation was 6.7%, 13.3%, and 6.0% in 2010, 2011, and

2012, respectively.

Statistical analyses

We analyzed whether measures of baseline CORT could predict fitness metrics in clipped

and control birds separately to allow for conclusions about how the relationship may be

different when individuals are faced with an unexpected change in environmental quality

(i.e., feather clipping). Specifically, we used three metrics representing different

components of fitness as dependent variables, each analyzed separately. First, we calculated offspring quality as the residual of brood mass (calculated as the total mass (g) of all chicks in the nest at 12 days of age) on number of chicks. Residual brood mass provides a measure of offspring quality that is uncorrelated with the number of offspring (output), where individuals with heavier than average offspring for a given brood size can be discerned from those with smaller than average offspring. Second, we used the total number of offspring that successfully fledged from a nest as a measure of reproductive output. Third, adult survival was recorded as 0 for birds that were not subsequently recorded as returning to the breeding site, and as 1 for birds that returned to the breeding site in a subsequent year. We used three metrics of baseline CORT as independent variables: 1) late incubation CORT; 2) mid-nestling provisioning CORT; 3) percent change in CORT over the reproductive season, calculated as the absolute difference in CORT levels divided by the incubation CORT level. We used the percent change in CORT rather than the absolute difference, to better take into account the overall degree of change. We also included the percent change in body mass over the reproductive season as an energetic context. For within-season fitness metrics (reproductive output and offspring quality), we also included insect biomass over each female's peak offspring provisioning period as a measure of food availability. For the survival analyses, we included number of offspring fledged as an additional independent variable to represent previous reproductive investment. All analyses included habitat cluster (as described in Chapter 3; random) and lay date (fixed) as covariates. Tree swallow reproductive performance declines over the season (Stutchbury and Robertson, 1988), so including lay date allows us to control for the potential influences of timing on fitness outcomes. Lay date was standardized to represent a relative lay date within each year (by subtracting the

average) to make the timing of reproduction comparable across years. In addition, in control analyses (n=122), which included three years of data, we included year and individual ID as random effects. In contrast, analyses in the clipped group included only one year of data (n=33). We checked for collinearity of independent variables by calculating variance inflation factors (VIFs). Due to a high correlation between the change in CORT and the single time-point CORT measures (R>0.70), we did not construct any models with both variables included simultaneously. All other VIFs were below 1.40.

We used AIC (Akaike's Information Criterion) corrected for small sample size to perform model selection (Hurvich and Tsai, 1989) and determine which physiological or other contexts were best able to predict long and short-term fitness metrics. More specifically, we used AIC_c values to calculate ΔAIC_c, Akaike weights (ω), and cumulative weights, allowing us to determine the best-supported models from our candidate sets. Each fitness metric was analyzed separately in each treatment group (i.e., we performed a total of six analyses). Each candidate set included 20 models; we used an all-subsets approach, except (as outlined above) the percent change in CORT was never included in a model with either incubation CORT or nestling provisioning CORT due to high collinearity. We used cumulative weights to determine 95% confidence sets of models in each analysis. A confidence set represents a list of models in which we can be 95% certain the best model from our original candidate set is included (Symonds and Moussalli, 2011). Where model uncertainty was evident (i.e., when no single model could be identified as the top model), we performed multi-model inference using the 95% confidence set (Burnham and Anderson 2002). This allowed us to obtain model-averaged parameter estimates (β), unconditional standard errors, and 95% confidence intervals

(Johnson and Omland, 2004). Model-averaged β-values and unconditional standard errors were calculated by weighting them by the Akaike weights of the models included in the confidence set (Burnham and Anderson 2002).

Analyses with offspring quality as the dependent variable were completed using linear mixed effects models (LMMs), while analyses with reproductive output (i.e., count data) and survival probability (i.e., binary data) as dependent variables were completed using generalized linear mixed effects models (GLMMs). In the case of survival probability, we used a binomial distribution and a logit-link function. For reproductive output, we used a Poisson distribution and a log-link function. All continuous covariates were mean-centered (Bolker et al., 2009). Analyses were completed in R (R Development Team, 2015) using the lme4 package with the lmer and glmer function (Bates et al., 2015). Marginal and conditional R^2 of LMMs and GLMMs were calculated with the package MuMIN with the function rquared.glmm (Barton, 2015).

Results

In the analyses investigating which GC metrics and other energetic contexts (food availability, reproductive investment, mass loss) predicted fitness in control birds, the null model was among the best supported models in both the offspring quality and the survival analyses (Table 4.1). There was considerable model uncertainty with 11 and 15 models included in the 95% confidence sets of the offspring quality and survival analyses, respectively. For all GC and energetic variables, parameter estimates were very low and 95% confidence intervals cross zero, indicating a lack of association of the variables investigated with both offspring quality and adult survival (Table 4.2). In contrast, the model comprised of only percent change in body mass represented the best supported

117

model in the analysis of reproductive output (Table 4.1). More specifically, greater losses of body mass were associated with a greater number of successfully fledged offspring (Figure 4.2); however, the marginal R^2 of the relationship was only 0.05 indicating a large degree of unexplained variance. The 95% confidence set included 14 models, and all other variables investigated showed no association with reproductive output (Table 4.2).

In contrast to control birds, the top model for reproductive output in the clipped group was the null model (Table 4.3). The 95% confidence set included 14 models, and confidence intervals of all variables crossed zero, indicating poor precision of parameter estimation and a lack of association between energetic and GC metrics and the number of offspring produced (Table 4.4). In terms of offspring quality in the clipped group, the models containing single or multiple CORT metrics represented the best supported models (Table 4.3). However, there was considerable model uncertainty and all variables included in the 95% confidence set had confidence intervals that crossed zero (Table 4.4). Similarly, in the survival analysis, the best supported model contained only the percent change in CORT, and represented the only model in the candidate set ranking higher than the null (Table 4.3). Again, there was considerable model uncertainty with 13 models constituting the 95% confidence set. Model-averaged parameter estimates and confidence intervals indicate that all variables investigated show a lack of association with survival probability (Table 4.4).

Discussion

We used an integrative and environmentally-relevant, context-dependent approach to examine the relationship between baseline GC levels and multiple fitness metrics in an aerial insectivore undergoing population decline. Coupling this context-dependent

approach with a multi-year dataset and an experimental manipulation of environmental quality, we were able to assess whether GC measures may be useful as broader conservation biomarkers. We found no relationships between baseline GC metrics and any short- or longer-term fitness components that we measured in breeding female tree swallows. More specifically, baseline CORT at the incubation and at the nestling provisioning stage, and the change in CORT over the reproductive season, failed to relate to key components of fitness, namely offspring quality, reproductive output, and adult survival probability. Importantly, this was the case under both natural conditions and when females were faced with an unexpected, experimentally-induced decrease in foraging profitability during the nestling provisioning stage. In contrast, control birds that lost a greater percentage of body mass over the reproductive season raised more offspring.

Lack of GC-fitness relationships

Variation in circulating GCs is often proposed as a useful conservation biomarker of exposure to anthropogenically-induced stressors (Baker et al., 2013; Bonier et al., 2009a; Busch and Hayward, 2009; Dantzer et al., 2014). However, the lack of GC-fitness relationships we recorded adds to the already variable findings previously reported across populations and species (Bonier et al., 2009a). It has been proposed that such a high level of variability could, at least in part, be due to the presence of underlying context-dependency in GC-fitness relationships (Bonier et al., 2009a; Madliger and Love, 2014). In our investigation, the addition of energetic contexts such as food availability, reproductive investment, and somatic investment (i.e., decline in body mass) did not improve the capacity of baseline GC measures to predict fitness outcomes. We were also

119

rigorous in our work by: 1) limiting analyses by sex and broad age class; 2) controlling for factors such as habitat type and reproductive timing; and 3) explicitly investigating the potential of varying GC-fitness relationships across breeding stages and fitness metrics. Despite this attention to intrinsic and extrinsic context, both static measures and the change in baseline CORT over the reproductive season failed to be useful in predicting key components of fitness. It is possible that additional contexts may be necessary to link baseline GC levels and fitness. For example, as individuals are expected to alter their investment in their current brood based on their future reproductive potential, with baseline GCs potentially reflecting anticipated risks and demands during breeding, brood value may be particularly important in understanding the relationship between baseline GCs and fitness outcomes (Bókony et al., 2009). As would be the case in many conservation situations, we lacked knowledge on the specific age of each individual. We were therefore only able to partition birds into an age category of equal to or greater than two years, potentially encompassing ages of two to eight years (Butler, 1988) and as such, a range of potential brood values. While the possibility remains that age-related changes in baseline GCs (Angelier et al., 2006; Hämäläinen et al., 2015; Mateo, 2006; Riechert et al., 2012) could influence our ability to detect a GC-fitness relationship, if fine-scale age metrics are necessary (i.e., if pooling age categories will mask a relationship between GCs and fitness), this will limit the application of the technique in many species of concern that are not easily aged in a field setting.

As is often the case regardless of whether a study is evolutionary or applied in nature, the measurement of lifetime reproductive success rather than fitness components is preferable (Newton, 1989). This could therefore also be the case for properly assessing GC-fitness relationships. For example, Angelier et al. (2010) was able to predict five-year

reproductive success with breeding baseline CORT levels in black-browed albatross (*Thalassarche melanophris*). Nonetheless, baseline GCs have been useful in predicting similar fitness proxies to those that we measured across other species (rev. in Bonier et al., 2009b). Instead, given that circulating plasma baseline GC levels are a more instantaneous measure of current energetic demand or environmental conditions, if GCs at the time periods we measured are driven by social or environmental conditions that do not have downstream consequences on fitness, this could inhibit the ability to relate baseline GCs to reproductive outcomes or survival. One of the important known drivers of variation in baseline GCs is food availability (Astheimer et al., 1992; Corbel and Groscolas, 2008; Fokidis et al., 2012; Jenni-Eiermann et al., 2008; Kitaysky et al., 1999, 2010; Pravosudov et al., 2001). As a result, short-lived nadirs in food availability (e.g., due to temporary decreases in temperature) could have large consequences for variability in baseline GCs (Astheimer et al., 1992), but may not be detrimental enough on body condition, incubation behaviour, or nestling growth to confer downstream fitness effects. In addition, baseline GCs are known to vary in response to internal changes in state such as body condition and mass both within (Cabezas et al., 2007; Love et al., 2005; Romero and Wikelski, 2001; Schoech et al., 1997; Williams et al., 2008) and across species (Hau et al., 2010). When female passerine birds begin to drop body mass at the end of incubation, (most likely as an adaptive mechanism to decrease wing loading for the subsequent nestling provisioning period; Boyle et al., 2012; Freed, 1981; Neto and Gosler, 2009; Norberg, 1981), they may be experiencing changes in metabolic costs or may be adjusting food intake in preparation for chick hatching (Boyle et al., 2012; Portugal et al., 2007). If GCs act as mediators of this change (e.g., through mobilization of fat stores), small differences in the timing of when females initiate this adaptive

121

reduction in mass could lead to highly variable GC levels across individuals at this sampling time. This potential involvement of (or consequence on) GCs highlights the importance of also considering the metabolic role of GCs across fine temporal scales and illustrates that even small differences in sampling times could lead to altered relationships between GCs and fitness (Crespi et al., 2013).

Our results may differ from other findings in the same species (e.g., Bonier et al., 2009b, 2011) for a number of methodological reasons. Although we chose sampling windows to coincide with expected demands within individual reproductive stages, our baseline GC measures were obtained at a later stage in both incubation and nestling provisioning compared to previous work in this species (Bonier et al., 2009b, 2011). It is possible that the time periods we sampled are less sensitive to the environmental factors that may influence reproductive success, or that females are highly committed to nesting attempts very late in incubation (i.e., they may be more able to buffer extrinsic environmental changes due to increased body reserves; Boyle et al., 2012). It is also possible that relationships may differ between years or sites; it has been proposed that differences in the contribution of ecological factors and breeding effort to allostatic load may alter the relationship between GCs and fitness (Ebensperger et al., 2013). More specifically, when characteristics of the ecological or social environment are the main drivers of GC levels, as opposed to reproductive effort, a lack of relationship between GCs and fitness is expected (Bonier et al., 2009a; Ebensperger et al., 2013). Performing the feather-clipping manipulation in a different year may have led to alternative results given that our manipulation year (2011) was a reasonably stable one in terms of weather conditions as compared to others with harsher or unanticipated weather events (e.g., May snowfalls; Madliger, *pers. obs.*). Indeed, it has been proposed that GC-fitness

relationships may be more readily found when a portion of, but not all, individuals in a population are constrained by their environment (Angelier 2010). Overall, females may have been able to cope with the constraints of the manipulation (Patterson et al., 2011), potentially decreasing its total influence on GC-fitness relationships.

A final explanation for a general lack of GC-fitness relationships, particularly at the nestling provisioning stage when chicks begin to plateau in body mass (McCarty, 2001; Quinney et al., 1986), is the possibility that males could compensate for reduced foraging ability in females (Patterson et al., 2011), leading to unaltered nesting success despite alteration in female GC levels. This would be a particularly important factor for manipulated females, whose foraging rates decrease (Madliger et al., 2015 - Chapter 2), but whose breeding success was comparable to controls (Chapter 5). Indeed, the total number of foraging trips to manipulated and control nests was equivalent (Chapter 5), indicating that males did compensate to ensure a certain overall foraging rate for their brood (Patterson et al., 2011). In particular, this highlights the possibility that GC levels may not reflect fitness due to unmeasured variables (e.g., mate quality) and has implications for measuring GC levels during the breeding season in species with bi-parental care, particularly if environmental conditions affect the sexes differentially.

Loss of body mass as a predictor of reproductive success

Our results indicate that the loss in body mass over reproduction was a more sensitive predictor of within-season reproductive success than measures of baseline GCs. It is possible that the change in body mass is directly indicative of energetic investment, where individuals that invest the most in offspring are accruing the greatest fitness benefits, but are suffering from greater losses in somatic body condition (Bryant, 1988; Drent and

Daan, 1980; Neto and Gosler, 2009). Additionally, lower body masses are likely indicative of an adaptive change in body mass to increase flight efficiency during demanding stages of reproduction (Freed, 1981; Neto and Gosler, 2009; Norberg, 1981), particularly in species that forage solely on the wing (Boyle et al., 2012). It may therefore be the ability of females to adjust their body mass to foraging demands that may represent the most reliable indicator of individual quality (Boyle et al., 2012). Overall, the greater utility of a body mass metric compared to GCs is of interest from a management perspective, given the high monetary cost of analyzing GC levels, considerations for storage in field settings (Sheriff et al., 2011), and invasiveness of blood sampling. However, while a change in body mass did predict reproductive output statistically, the fit (R^2) of the relationship was weak, and was only evident in control birds. As a result, there is still a large amount of variation in reproductive output that is not well-captured with metrics of body mass, limiting the application of such a measure in conservation field settings. Nonetheless, our findings do reinforce the idea that, at some stages, loss in body condition can actually be an indication of a high quality individual investing heavily in reproduction, with concomitant fitness benefits (Breuner, 2011; Gillooly and Baylis, 1999; Golet and Irons, 1999; Hillstrom, 1995). This further draws attention to the importance of considering expected energetic demands of the organism of interest at the stage of sampling in conservation applications, and otherwise (Madliger and Love, 2014).

Implications for baseline GCs as conservation biomarkers and recommendations for future study

We have illustrated that measures of baseline GCs may fail to provide reliable biomarkers of reproductive success or survival probability in some populations at certain time points,

despite a robust experimental design, the measurement of multiple within-individual metrics, and careful investigation of the intrinsic and extrinsic contexts that may influence GC-fitness relationships. Most importantly, these results indicate the importance of validation prior to application of GCs in conservation settings, since varying GC levels may not always be indicative of population-level persistence. Additionally, examination of our results in comparison to others in the same species (e.g., Bonier et al., 2009b, 2011) provides additional evidence that GC-fitness relationships can change based on the time frame in which a GC measure is obtained (potentially in as little time as one week), or that the relationship could vary substantially by site or year. Finally, our results draw attention to the potential importance of measuring multiple time points per individual when investigating biomarkers of fitness. All of these considerations have important implications for the ease of use, costs, and time frames that may be necessary to monitor populations of conservation concern using physiological traits. Overall, our results demonstrate that GC measures, particularly those as labile as plasma baseline GCs, will likely need to be validated within specific populations prior to use as conservation monitoring tools. Indeed, mounting evidence is indicating that species- and context-specific studies are necessary before conservation managers can feel confident about the interpretation of changing GC levels in their systems (Sopinka et al., 2015).

Future studies should place emphasis on whether static measures of GCs are sufficient to infer disturbance, condition, or fitness. In addition, it is becoming increasingly clear that a GC-fitness relationship observed using one metric of fitness should not be expected to apply to other metrics (e.g., reproductive output versus survival probability). As a result, investigating the GC-fitness metric that is most important to the population of interest will be of paramount importance to properly interpreting changing

GC levels over time. This also has implications for seasonal changes in GC-fitness relationships, indicating that it will be important to determine when in the life cycle a predictable relationship exists. Finally, manipulative studies that compare how changes within normal baseline GC levels may change behaviour and fitness will further elucidate when GC-fitness relationships may exist, and, more broadly, how GC levels may mediate life history decisions (Crespi et al., 2013; Sopinka et al., 2015). In particular, habitat quality, disturbance, or GC manipulations that influence both sexes would likely be advantageous for determining how GC-fitness relationships may directly change in response to environmental alteration. Overall, there is still a great deal of validation necessary before baseline GC levels can be reliably utilized as conservation biomarkers in many species.

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Tables

Table 4.1 - Summary of confidence (95%) set of models predicting fitness metrics in
female tree swallows in control birds (n=122). All models included lay date as a fixed
effect, and female ID, habitat type, and year as random effects. The summary provides
AIC values corrected for small sample size (AIC$_c$), ΔAIC$_c$, Akaike weight (ω),
cumulative Akaike weights (cum. ω), and conditional R^2 for each model.

Model variables	AIC$_c$	ΔAIC$_c$	ω	cum. ω	R^2
(a) Offspring fledged (output)					
massΔ	476.63	0.00	0.28	0.28	0.05
massΔ, CORT1	478.73	2.10	0.10	0.37	0.05
CORTΔ, massΔ	478.77	2.14	0.10	0.47	0.05
massΔ, CORT2	478.86	2.23	0.09	0.56	0.05
massΔ, food	478.88	2.25	0.09	0.65	0.05
Null (lay date, ID, site, year)	479.19	2.56	0.08	0.73	0.01
massΔ, CORT1, CORT2	480.96	4.33	0.03	0.76	0.05
massΔ, food, CORT1	481.02	4.39	0.03	0.79	0.05
CORTΔ, massΔ, food	481.05	4.43	0.03	0.82	0.05
massΔ, food, CORT2	481.15	4.52	0.03	0.85	0.05
CORTΔ	481.34	4.72	0.03	0.88	0.01
CORT2	481.36	4.74	0.03	0.90	0.01
food	481.39	4.76	0.03	0.93	0.01
CORT1	481.40	4.77	0.03	0.95	0.01
(b) Offspring quality					
Null (lay date, ID, site, year)	874.82	0.00	0.30	0.30	0.25
CORTΔ	876.13	1.31	0.16	0.46	0.25
CORT2	876.53	1.71	0.13	0.58	0.22
CORT1	876.89	2.07	0.11	0.69	0.24
massΔ	877.62	2.80	0.07	0.76	0.28
CORT1, CORT2	878.57	3.75	0.05	0.81	0.22
CORTΔ, massΔ	879.01	4.19	0.04	0.85	0.28
massΔ, CORT2	879.40	4.58	0.03	0.88	0.25
food	879.50	4.68	0.03	0.91	0.31
massΔ, CORT1	879.71	4.89	0.03	0.93	0.27

CORTΔ, food	880.93	6.11	0.01	0.95	0.31
(c) Survival					
Null (lay date, ID, site, year)	174.54	0.00	0.22	0.22	0.01
massΔ	175.83	1.29	0.12	0.34	0.02
fledged	176.53	1.99	0.08	0.42	0.01
CORT1	176.56	2.02	0.08	0.50	0.01
CORTΔ	176.62	2.08	0.08	0.58	0.01
CORT2	176.68	2.14	0.08	0.66	0.01
CORTΔ, massΔ	177.95	3.41	0.04	0.70	0.02
massΔ, CORT1	177.95	3.42	0.04	0.74	0.02
massΔ, fledged	177.98	3.44	0.04	0.78	0.02
massΔ, CORT2	178.00	3.46	0.04	0.82	0.02
fledged, CORT1	178.59	4.05	0.03	0.85	0.01
CORTΔ, fledged	178.64	4.10	0.03	0.88	0.01
fledged, CORT2	178.70	4.17	0.03	0.90	0.01
CORT1, CORT2	178.72	4.19	0.03	0.93	0.01
CORTΔ, massΔ, fledged	180.14	5.61	0.01	0.95	0.02

massΔ is percent loss in body mass from incubation to nestling provisioning, CORT1 is baseline CORT at the incubation stage, CORT2 is baseline CORT at the nestling provisioning stage, CORTΔ is percent change in CORT from incubation to nestling provisioning, food is average insect biomass (food availability) during peak nestling provisioning, fledged is the number of offspring fledged (see Methods for details)

Table 4.2 - Model-averaged parameter estimates, unconditional standard errors, and 95% confidence intervals for models predicting fitness of control female tree swallows (n=122). All values were calculated using models included in the 95% confidence sets, weighting by Akaike weights.

Variable	Estimate	Unconditional SE	95% confidence interval (lower, upper)
(a) Offspring fledged (output)			
CORT1	0.002	0.01	-0.04, 0.06
CORT2	0.0008	0.01	-0.05, 0.04
CORTΔ	0.002	0.02	-0.08, 0.06
food	0.00004	0.001	-0.004, 0.005
lay date	0.008	0.006	-0.004, 0.02
massΔ	0.02	0.01	0.002, 0.05
(b) Offspring quality			
CORT1	-0.003	0.2	-0.89, 0.86
CORT2	-0.08	0.22	-1.05, 0.40
CORTΔ	-0.04	0.29	-1.39, 1.00
food	-0.005	0.02	-0.14, 0.02
lay date	-0.02	0.1	-0.21, 0.18
massΔ	-0.04	0.11	-0.56, 0.02
(c) Survival			
CORT1	0.007	0.05	-0.18, 0.25
CORT2	-0.0009	0.04	-0.18, 0.17
CORTΔ	0.006	0.06	-0.33, 0.26
fledged	-0.01	0.07	-0.29, 0.21
lay date	-0.02	0.02	-0.07, 0.03
massΔ	-0.01	0.03	-0.13, 0.05

massΔ is percent loss in body mass from incubation to nestling provisioning, CORT1 is baseline CORT at the incubation stage, CORT2 is baseline CORT at the nestling provisioning stage, CORTΔ is percent change in CORT from incubation to nestling provisioning, food is average insect biomass (food availability) during peak nestling provisioning, fledged is the number of offspring fledged (see Methods for details)

Table 4.3 - Summary of confidence (95%) set of models predicting fitness metrics in female tree swallows in feather-clipped birds (n=33). All models in included lay date as a fixed effect and site as random effect. The summary provides AIC values corrected for small sample size (AIC_c), ΔAIC_c, Akaike weight (ω), cumulative Akaike weights (cum. ω), and conditional R^2 for each model.

Model variables	AIC_c	ΔAIC_c	ω	cum. ω	R^2
(a) Offspring fledged (output)					
Null (lay date + site)	140.76	0.00	0.24	0.24	0.003
massΔ	141.52	0.76	0.16	0.40	0.06
CORT1	142.89	2.13	0.08	0.48	0.02
CORTΔ	143.24	2.49	0.07	0.55	0.01
CORT2	143.24	2.49	0.07	0.62	0.01
food	143.30	2.55	0.07	0.68	0.01
massΔ, CORT2	143.57	2.81	0.06	0.74	0.08
massΔ, CORT1	144.02	3.26	0.05	0.79	0.07
massΔ, food	144.15	3.39	0.04	0.83	0.07
CORTΔ, massΔ	144.27	3.52	0.04	0.87	0.06
CORT1, CORT2	145.66	4.90	0.02	0.89	0.02
food, CORT1	145.67	4.92	0.02	0.91	0.02
CORTΔ, food	146.00	5.24	0.02	0.93	0.01
food, CORT2	146.00	5.24	0.02	0.94	0.01
(b) Offspring quality					
CORT2	259.62	0.00	0.25	0.25	0.25
CORT1, CORT2	260.18	0.56	0.19	0.44	0.28
CORT1	261.21	1.59	0.11	0.55	0.29
CORTΔ	261.63	2.02	0.09	0.64	0.25
Null (lay date + site)	261.68	2.06	0.09	0.73	0.22
massΔ, CORT2	262.02	2.41	0.07	0.81	0.25
massΔ, CORT1, CORT2	262.75	3.13	0.05	0.86	0.27
massΔ, CORT1	263.32	3.70	0.04	0.90	0.29
massΔ	263.67	4.05	0.03	0.93	0.22
(c) Survival					
CORTΔ	47.34	0.00	0.31	0.31	0.19
Null (lay date + site)	48.83	1.49	0.15	0.45	0.0004
CORT1	49.80	2.46	0.09	0.54	0.06

CORTΔ, massΔ	49.91	2.57	0.08	0.62	0.21
CORTΔ, fledged	50.10	2.76	0.08	0.70	0.19
CORT2	51.06	3.71	0.05	0.75	0.02
massΔ	51.25	3.91	0.04	0.79	0.01
CORT1, CORT2	51.41	4.07	0.04	0.83	0.12
fledged	51.43	4.08	0.04	0.87	0.0006
massΔ, CORT1	52.49	5.15	0.02	0.90	0.07
fledged, CORT1	52.52	5.18	0.02	0.92	0.07
CORTΔ, massΔ, fledged	52.92	5.58	0.02	0.94	0.21
massΔ, CORT2	53.80	6.46	0.01	0.95	0.02

massΔ is percent loss in body mass from incubation to nestling provisioning, CORT1 is baseline CORT at the incubation stage, CORT2 is baseline CORT at the nestling provisioning stage, CORTΔ is percent change in CORT from incubation to nestling provisioning, food is average insect biomass (food availability) during peak nestling provisioning, fledged is the number of offspring fledged (see Methods for details)

139

Table 4.4 - Model-averaged parameter estimates, unconditional standard errors, and 95% confidence intervals for models predicting fitness of feather-clipped female tree swallows (n=33). All values were calculated using models included in the 95% confidence sets, weighting by Akaike weights.

Variable	Estimate	Unconditional SE	95% confidence interval (lower, upper)
(a) Offspring fledged (output)			
CORT1	-0.006	0.02	-0.13, 0.07
CORT2	-0.005	0.02	-0.12, 0.07
CORTΔ	0.001	0.02	-0.12, 0.14
food	0.0001	0.001	-0.005, 0.07
lay date	0.005	0.02	-0.03, 0.04
massΔ	0.01	0.02	-0.01, 0.07
(b) Offspring quality			
CORT1	-0.35	0.89	-3.39, 1.66
CORT2	-0.99	1.17	-3.89, 0.51
CORTΔ	-0.06	0.6	-3.78, 2.88
lay date	0.43	0.39	-0.4, 1.23
massΔ	-0.03	0.01	-1.27, 1.04
(c) Survival			
CORT1	0.06	0.15	-0.16, 0.72
CORT2	-0.02	0.1	-0.61, 0.28
CORTΔ	-0.31	0.41	-1.38, 0.09
fledged	0.007	0.1	-0.43, 0.5
lay date	0.01	0.07	-0.13, 0.16
massΔ	0.002	0.05	-0.22, 0.24

massΔ is percent loss in body mass from incubation to nestling provisioning, CORT1 is baseline CORT at the incubation stage, CORT2 is baseline CORT at the nestling provisioning stage, CORTΔ is percent change in CORT from incubation to nestling provisioning, food is average insect biomass (food availability) during peak nestling provisioning, fledged is the number of offspring fledged (see Methods for details)

Figures
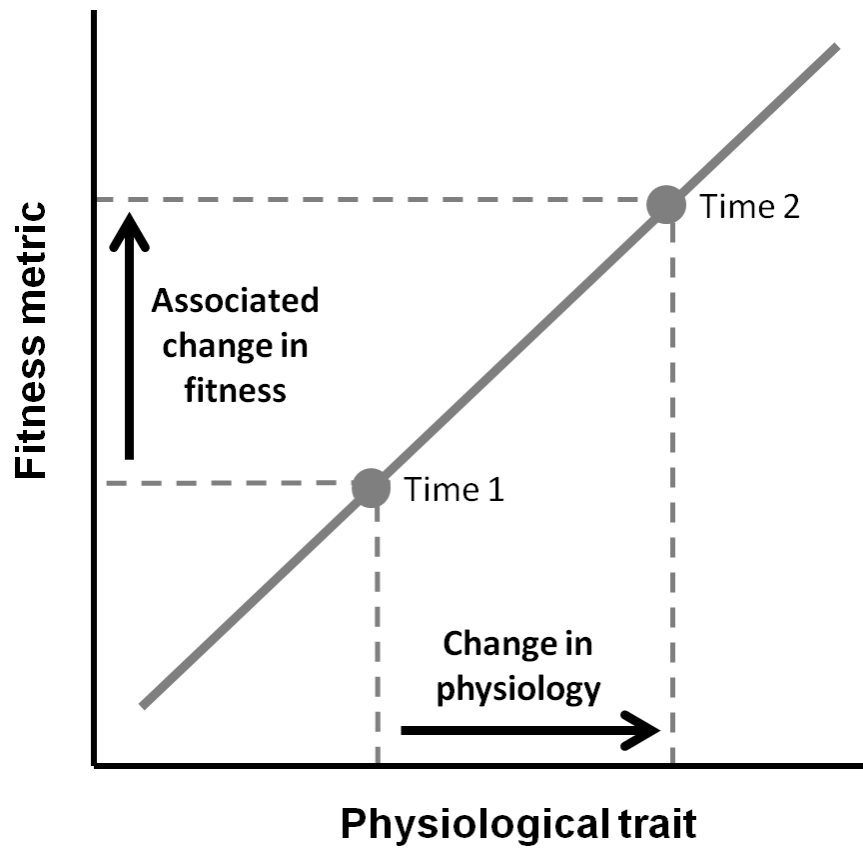
Figure 4.1 - Schematic displaying a hypothetical relationship between a physiological variable and fitness. Such a relationship is necessary to interpreting changes in physiology as predictive indicators of population health or persistence. It should be noted that negative, positive, and non-linear relationships would all be interpretable.
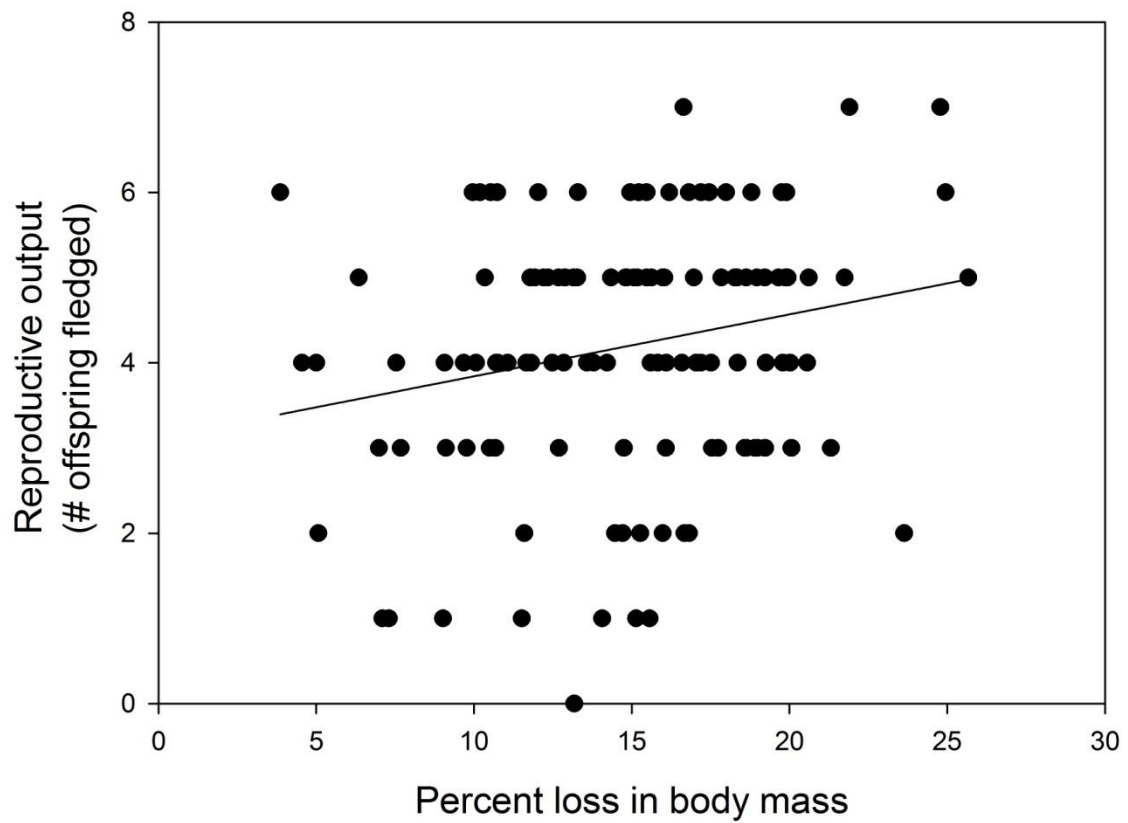
Figure 4.2 - Reproductive output (number of offspring fledged) in relation to percent loss in body mass over the reproductive season (from the late incubation to the mid-nestling provisioning stage) in female tree swallows ($R^2=0.05$).

CHAPTER 5 - DO BASELINE GLUCOCORTICOIDS SIMULTANEOUSLY REPRESENT FITNESS AND ENVIRONMENTAL QUALITY IN A DECLINING AERIAL INSECTIVORE?*

*This chapter is the result of joint research with O. Love and is under review at *Oikos*.

Introduction

Organisms are exposed to rapidly 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; Sih et al., 2011; Willmer et al., 2009). 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 (Angelier and Wingfield, 2013; Carey, 2005; Cockrem, 2005; Wikelski and Cooke, 2006). 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 (Bókony et al., 2009; Bonier et al., 2009; Breuner et al., 2008; Busch and Hayward, 2009; Korte et al., 2005; Walker et al., 2005; Wikelski and Cooke, 2006).

The diversity of taxa in which GC levels of populations in different habitat types have been compared (e.g., amphibians: (Hopkins and DuRant, 2011; Newcomb Homan et al., 2003); reptiles: (Cash and Holberton, 2005; French et al., 2008); fish: (Belanger et al.,

143

2016; Blevins et al., 2013); birds: (Leshyk et al., 2012; Wasser et al., 1997); mammals: (Allen et al., 2010; Rehnus et al., 2014)) illustrates the desire of eco-physiologists and conservation biologists to understand organismal response to environmental 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 different habitat types and then 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 (Bonier et al., 2009; Reeder and Kramer, 2005; Wingfield and Kitaysky, 2002); 2) long-term over-activation of the HPA/HPI axis leading to declines in growth rate, reproduction, cognitive function, and survival (Boonstra, 2013; Romero et al., 2009); 3) levels sometimes correlating negatively with body condition indices (Husak and Moore, 2008; Jenni-Eiermann et al., 2008; Moore and Jessop, 2003; Moore et al., 2000; Romero and Wikelski, 2001; Williams et al., 2008; Wingfield et al., 1997); and 4) the expectation that allostatic load (i.e., current and predicted energetic demands) should be higher in more disturbed populations/habitats, leading to higher GC levels (Bonier, 2012; McEwen and Wingfield, 2010). However, these suppositions ignore a number of key contexts within which GC physiology can vary independently of environmental variation that are thus highly relevant to their interpretation (Bonier et al., 2009; Dantzer et al., 2014; Madliger and Love, 2014; Millspaugh and Washburn, 2004).

First, levels of GCs can be elevated during energetically demanding, but not necessarily "stressful", points in the life history 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 similar influences on GC levels are demographic composition (i.e., ratio of young to old individuals, or males to females) (Goymann, 2012; Hämäläinen et al., 2015; Homan et al., 2003; Kern et al., 2005; Rector et al., 2012; Touma et al., 2003), weather conditions (Baker et al., 2013; Huber et al., 2003; Romero et al., 2000; Touma and Palme, 2005), or time of day of sampling (Breuner et al., 1999; Heintz et al., 2011; Tarlow et al., 2003; Touma and Palme, 2005). Second, a recent overview of studies measuring chronic stress suggests that any change in GCs is likely more representative of dysregulation than simply an elevation (Dickens and Romero, 2013), indicating that habitats or populations with lower GC levels could feasibly be the most disturbed. Finally, the interpretation of GC levels as informative indicators of disturbance requires validation of downstream fitness consequences at individual and population levels (Bonier et al., 2009; Breuner et al., 2008; Busch and Hayward, 2009; Cooke, 2014; Madliger and Love, 2014; Tarlow and Blumstein, 2007).

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 (e.g., thyroid hormone: Hayward et al., 2011), but see Gobush et al. (2008) and Sheriff et al. (2009). Overall, we 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 affected 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 (Arlettaz et al., 2014; Patterson et al., 2011). 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 habitat quality that can subsequently be used to delineate conservation priorities.

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, is in population decline in North America (Nebel et al., 2010). 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 during offspring provisioning designed to decrease access to food resources to determine whether GCs and fitness metrics respond in parallel or differentially to a

146

biologically-relevant change in environmental quality depending on the initial habitat type birds had chosen for breeding. We were careful to control for or eliminate multiple contexts that could mask underlying patterns in GC levels (i.e., age, reproductive stage, time of day). We also measured multiple components of fitness (i.e., offspring quality, reproductive output, and adult survival) 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 carryover 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 in a conservation-relevant species. Combined with other explicit validations of GCs as biomarkers for conservation (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.

Methods

Study species and site

We studied a colony of wild tree swallows breeding in nest boxes in Cayuga, Ontario, Canada from 2010-2015. The current study focused on data collected in the breeding seasons of 2011 and 2012. 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 a variety of 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 (see below).

Nest monitoring and blood sampling protocol

All manipulation and monitoring protocols were approved by the University of Windsor's Animal Care Committee (AUPP #10-10) and the Canadian Wildlife Service (Permit CA 0266). 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 two minutes 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 Sharpie© 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 4 hours, centrifuged, and then plasma was stored at -80˚C until assay.

Experimental manipulation of foraging profitability

To induce an energetic handicap and a decline in foraging profitability as a practical means of mimicing a decline in environmental quality, 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 8 total flight feathers (Ardia and Clotfelter, 2007; Winkler and Allen, 1995). We only performed the manipulation on after-second year birds as identified by plumage (i.e., birds aged at least two years) (Hussell, 1983) to control for potential age-related effects on GCs (Angelier et al., 2006; Lanctot et al., 2003; 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 foraging rate in our population and others (Madliger et al., 2015; Patterson et al., 2011; Winkler and Allen, 1995). We assessed nest foraging rates using 1-hour focal observations between 1200h and 1500h when the nestlings were 8-9 days of age (i.e., at a stage of high demand for parents due to high growth rate) (McCarty, 2001; Quinney et al., 1986). One hour observations during mid-day in this species have been shown to be strong proxies of overall foraging rate at this stage of

149

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). Overall, we interpret this manipulation as an unpredictable, prolonged decline in habitat quality from the perspective of food availability as females are no longer able to forage as efficiently as prior to the manipulation for the duration of the breeding season.

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 - Chapter 2; Chapter 3). 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 for each nest box using a geographic information system (ArcGIS 10.1; ESRI). We then combined these six GIS-based metrics in a principal component analysis (Chapter 4 - Table 4.1), followed by grouping boxes using a cluster analysis. This resulted in the quantification of two "clusters" of boxes that differed in structural habitat features, which we have labeled Riparian-Cropland and Inland-Pasture (Figure 5.1). This is an arguably more biologically-relevant way to quantify habitat features than simply grouping boxes by sites 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 grouped two of our box groups that are the farthest apart by on-the-ground distance into a single cluster.

Riparian-Cropland habitat is characterized by lower proximity to high insect availability landuse, lower nest disturbance, and greater access to the Grand River (Table 5.1). In contrast, boxes in Inland-Pasture are characterized by higher local food resources, higher nest disturbance, but less access to the Grand River (Table 5.1). These landuse types are similar to much of the breeding habitat available to tree swallows in the eastern United States and Canada. Sample 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 (110x110x110 cm SLAM traps, MegaView Science Co.) placed within clusters of boxes (5 traps total). Insect bottles were collected daily from May 1 - July 1. We calculated the daily dry biomass of insect orders and size classes known to be consumed by tree swallows (see Madliger et al., 2015 - Chapter 2 for detailed methodology).

Hormone assay

Baseline levels of corticosterone (the primary GC in birds) were quantified using an enzyme-linked immunoassay (EIA: Assay Designs, Ann Arbor, MI, USA; previously validated: Love and Williams, 2008). We ran samples in triplicate at a 1:40 dilution and used a 3% steroid displacement buffer (SDB). Plates were run using a standard curve created by serially diluting a kit-provided corticosterone standard (20,000 pg/ml - 15.63 pg/ml). We used spiked laying hen plasma as a control (Sigma-Aldrich, Oakville, Ontario, Canada). Assay plates were read at 405nm 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), samples were assigned this detection limit (<5% of samples).

<u>Statistical analyses</u>

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 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 indicated by Shapiro-Wilk test). 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 GC levels between habitat types at both the incubation (all individuals) and nestling provisioning stage (only control birds) using t-tests, with baseline GC values log-transformed to achieve normality (as indicated by Shapiro-Wilk test). 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: i) lay date; ii) clutch size; iii) mean egg size; iv) size-corrected body mass at incubation. We compared each of these metrics between habitat

152

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 different responses to the manipulation of foraging profitability. Our independent variables included female foraging (provisioning) rate, total foraging rate (male and female combined), change in GCs 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 quality (mass), and reproductive output. It should be noted that we have previously broadly investigated female foraging rate in relation to our manipulation (Madliger et al., 2015 - Chapter 2); however, those analyses did not consider habitat type or a potential interaction between habitat type and the manipulation.

We calculated residual foraging rates for females and for both parents combined (i.e., total foraging rate) by regressing foraging rate against the number of chicks in the nest. Foraging 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

153

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.

Carryover effects of foraging manipulation

We assessed longer-term consequences of the interaction between the foraging profitability manipulation and habitat type on female survival and future investment, glucocorticoid levels, and condition. For the analysis of survival, we used a generalized linear model with a binomial distribution and a logit-link function with survival probability (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 mutli-factor ANOVA. To assess carryover 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 carryover effects of the manipulation on GC levels using an ANCOVA with return (2012) baseline GC 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) GC level at the nestling provisioning stage as an additional covariate to control for prior physiological state, and to determine whether any carryover effects were directly related to the manipulation, or to prior GC 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 reported below achieved p-values that were still considered significant after adjustment.

All analyses were completed using JMP 12 (SAS Institute). 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).

Results

Comparison of habitat types

Inland-Pasture habitat was characterized by higher insect resources during the egg-laying/incubation stage (WRS: n_{Inland}=61, $n_{Riparian}$=92; Z=2.36; *P*=0.02; Figure 5.2). However, the two habitat types did not differ in insect biomass during the nestling provisioning stage (WRS: n_{Inland}=66, $n_{Riparian}$=98; Z=0.65; *P*=0.52), indicating that Inland Pasture habitat declined in insect biomass across stages (Figure 5.2). 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 (Figure 5.3). We did not measure the body of one female, leading to the difference in sample size for that analysis compared to others. Baseline GC levels of females nesting in Inland-Pasture habitat (2.51 ± 0.28 ng/ml) did not differ from those nesting in Riparian-

Cropland habitat (2.90 ± 0.34 ng/ml) at the incubation stage (t-test: df=69. t=1.10; P=0.28; Figure 5.3). Similarly, at the nestling provisioning stage, baseline GC levels did not differ between Inland-Pasture (3.23 ± 0.31 ng/ml) or Riparian-Cropland (3.18 ± 0.37) habitats (t-test: df=69, t=-0.40, P=0.69; Figure 5.3).

<u>*Effects of foraging manipulation on performance and fitness*</u>

Condition and performance

Female foraging (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; Figure 5.4) 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; Figure 5.4). Total foraging 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; Figure 5.4). Female body mass also decreased to a greater extent in clipped birds compared to controls, regardless of habitat type (ANCOVA: df=3,66, F=8.89, P<0.0001; treatment: t=-5.01, P<0.0001; Figure 5.5). However, we found a significant interaction between treatment and habitat quality on the change in baseline GC levels (ANCOVA: df=3,67, F=3.32, P=0.025; trt x habitat: t=-1.96, P=0.05). Baseline GC levels were higher in birds clipped in Inland-Pasture habitat, but did not change in response to the manipulation in Riparian-Cropland habitat (Figure 5.5).

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, χ^2=0.57, *P*=0.90). While chick mass differed between habitat types, with females in Riparian-Cropland habitat raising larger 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; Figure 5.5), there was no influence of the foraging manipulation on chick quality (treatment: t=1.86, *P*=0.07).

Carryover effects of foraging manipulation

We found no effect of treatment, habitat type, or the interaction between habitat type and treatment on survival (glm: df=3, χ^2=4.60, *P*=0.20), lay date (ANCOVA: df=3,25, 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, χ^2=2.11, *P*=0.72), or reproductive output (glm: df=4, χ^2=1.03, *P*=0.90) the following year. Interestingly, females clipped in 2011 returned with significantly higher baseline GC 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; Figure 5.6). 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 GC level for birds present in 2012). Returning birds that were previously clipped exhibited higher baseline GC levels than unmanipulated birds (post-hoc t-test: df=40, t=1.75, *P*=0.04; Figure 5.6). Finally, regardless of previous treatment or habitat type, birds with higher GC levels at the nestling provisioning stage in 2011

returned with higher baseline GC levels in 2012 (prior GC level: t=2.56, *P*=0.02; Figure 5.7).

Discussion

Determining the relationships between habitat quality, GCs, and fitness has the potential to illuminate mechanisms behind population decline and is necessary for validating GCs as biomarkers for conservation monitoring. Despite habitat type differences in initial reproductive investment, foraging (provisioning) rate, and offspring quality, baseline GC levels at the incubation and nestling provisioning stage in female tree swallows did not reflect habitat type. An experimental decrease in environmental quality (i.e., foraging profitability) resulted in lower female foraging rates and greater losses in body mass, but these effects manifested independent of habitat type. However, the foraging profitability manipulation resulted in a habitat-type specific increase in baseline GC levels, occurring only in the Inland-Pasture habitat. Despite this influence on baseline GC levels, the manipulation did not concomitantly result in lowered offspring quality, output, or female survival to the following year. Nonetheless, females returning to the breeding site the following year that had been feather-clipped returned with higher baseline GC 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 GCs in 2011 returned with higher baseline GCs 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 a manipulation of environmental quality (D'Alba et al., 2011; Hayward et al., 2011; Lanctot et al., 2003; Sheriff et al., 2009), 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 able to simultaneously represent fitness outcomes and responses to variation in environmental quality in an aerial insectivore.

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

Breeding female tree swallows in Inland-Pasture habitats had access to greater daily flying insect biomass 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

foraging 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 nestlings 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 mayflies (*Ephemeroptera*), an order of insects which are associated with open water (Kriska et al., 1998), than those in Inland-Pasture habitat (*Madliger, unpubl. data*).

Although foraging 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 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 to 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 fewer mayflies (*Ephemeroptera*) (*Madliger, unpubl. data*); 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, foraging rate, and chick quality in control birds, we could not detect any differences in average GC 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 (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 of concern 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 (*pers. comm.*, D. Hussell). The

latter complication would be deemed high-risk for most populations of conservation concern. Furthermore, from the perspective of a conservation-relevant biomarker, 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 food availability via feather clipping caused lower female foraging rates and greater losses in body mass compared to control females across both habitat types. While the pattern of lower female foraging 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; Spée et al., 2010; Velando and Alonso-Alvarez, 2003). Indeed, in our study, we did not record any brood abandonment as a result of the feather clipping manipulation; more likely, females lowered their foraging 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 (Boyle et al., 2012; Freed, 1981; Neto and Gosler, 2009; Norberg, 1981) in compensation for the loss of wing surface area. Importantly, even though total (male and female combined) foraging 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 compensate for decreased foraging ability in clipped females (Patterson et al., 2011), likely leading to the lack of difference in offspring quality and output between control and clipped nests in a given habitat type.

Despite declines in foraging rate and body mass in manipulated birds in both habitat types, baseline GCs only increased in females in Inland-Pasture habitat. In contrast, manipulated birds in Riparian-Cropland habitat showed very little change in GCs, with patterns similar to control birds in both habitat types. Ultimately, changes in baseline GCs were therefore not a consistent reflection of exposure to a decline in environmental quality, 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) and led to the higher subsequent baseline GC levels that we observed (Bonier et al., 2011; Crespi et al., 2013; Love et al., 2004; Silverin, 1982). A second, but not mutually exclusive explanation, is that since manipulated birds in Inland-Pasture habitat had higher foraging rates than manipulated birds in Riparian-Cropland habitat, and the same foraging rates as control females in Riparian-Cropland habitat despite having decreased flight efficiency, this may have raised

allostatic load and associated baseline GC levels in comparison to other groups. This is likely especially evident in a species such as tree swallows with a high energetic cost of flight (Williams, 1988); females must continue to forage on the wing for their offspring and their own self-maintenance.

Interestingly, the manipulation in 2011 was reflected in baseline GC levels a full year later, as returning birds in 2012 from the manipulated group had higher GC levels than returning control birds. In addition, regardless of treatment, birds with higher baseline GCs during nestling provisioning in 2011 returned with higher baseline GC 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, of importance to the use of baseline GCs as conservation biomarkers is that this potentially greater allostatic load did not predict changes in current reproductive output or inter-annual survival in our population, or another population of tree swallows in New York, USA (Patterson et al., 2011). More specifically, despite an increase in GCs in females manipulated in Inland-Pasture habitat, we did not observe lowered chick quality, reproductive output, or survival 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 GCs 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, pers. obs.*), and well within the spatial range where females

seek extra pair copulation (Dunn and Whittingham, 2005). As a result, differences

between habitat types in GC response to the manipulation are not likely attributable to

genetic differences in GC physiology, or to selection against certain GC phenotypes

(Bauer et al., 2013; Bonier et al., 2006) which draws further attention to the importance of

considering spatial scale when comparing GCs across habitat types.

Although we may predict from a life-history 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 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 different 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 environmental challenge due to the manipulation as indicated by GC levels, flexibility in mate behaviour appeared to compensate for potential negative fitness effects. This has implications for applications of GCs as biomarkers as it indicates that 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 (Bonier et al., 2006; Hayward et al., 2011; Newcomb Homan et al., 2003; Riechert et al., 2014; Strasser and Heath, 2013; Wasser et al., 1997), 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).

Conclusions

We urge those interested in interpreting GCs in the context of conservation to validate environment-GC-fitness relationships at both the individual and average level. 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. It is possible that certain populations may be better-suited to the monitoring of GC levels and it is becoming apparent as researchers increasingly appreciate the context-dependent nature of GCs that factors such as age, sex, life history stage, other physiological traits, and environmental quality may independently and/or interactively influence 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 useful in a conservation setting.

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Tables

Table 5.1 - Differences in average habitat metrics between two habitat types (Inland-Pasture and Riparian-Cropland) used for breeding by tree swallows. See Chapter 3 for justification of each metric's relevance to breeding tree swallows.

Habitat Metric	Habitat Type	
	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 (200m radius)	56.7 ± 3.6	25.5 ± 4.3
% high insect landuse (1km radius)	47.3 ± 3.1	24.4 ± 0.9
Density (# occupied boxes within 200m)	19.1 ± 1.0	15.7 ± 0.6
Insect biomass at egg laying/incubation (mg/day)	38.8 ± 4.1	25.9 ± 2.0
Insect biomass at offspring provisioning (mg/day)	27.9 ± 2.6	30.4 ± 3.6

Figures
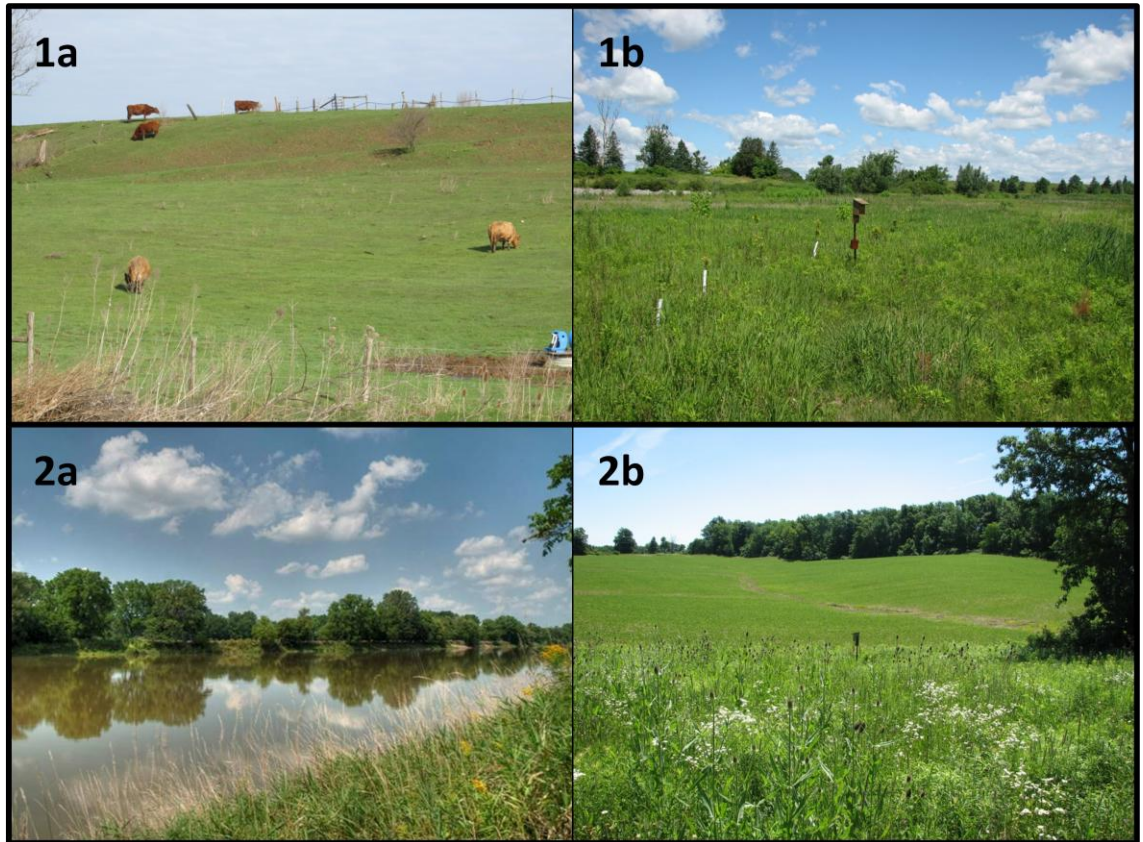
Figure 5.1 - Key landscape features (a) and representative nest box placement (b) in each

of two habitat types used in this study: Inland-Pasture (1) and Riparian-Cropland (2).
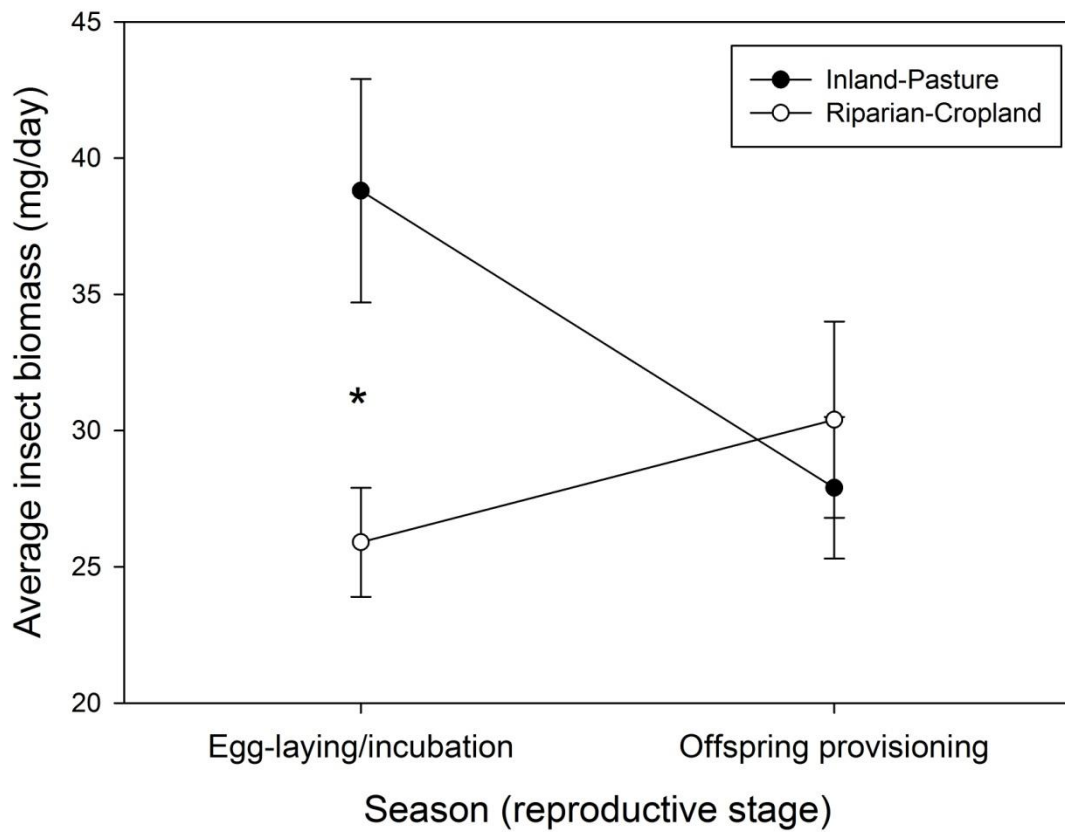
Figure 5.2 - Average daily insect biomass in two tree swallow habitat types (Inland-Pasture and Riparian-Cropland). Insects were sampled using passive traps at three locations within Riparian-Cropland and in two locations within Inland-Pasture (see Methods for details).

181
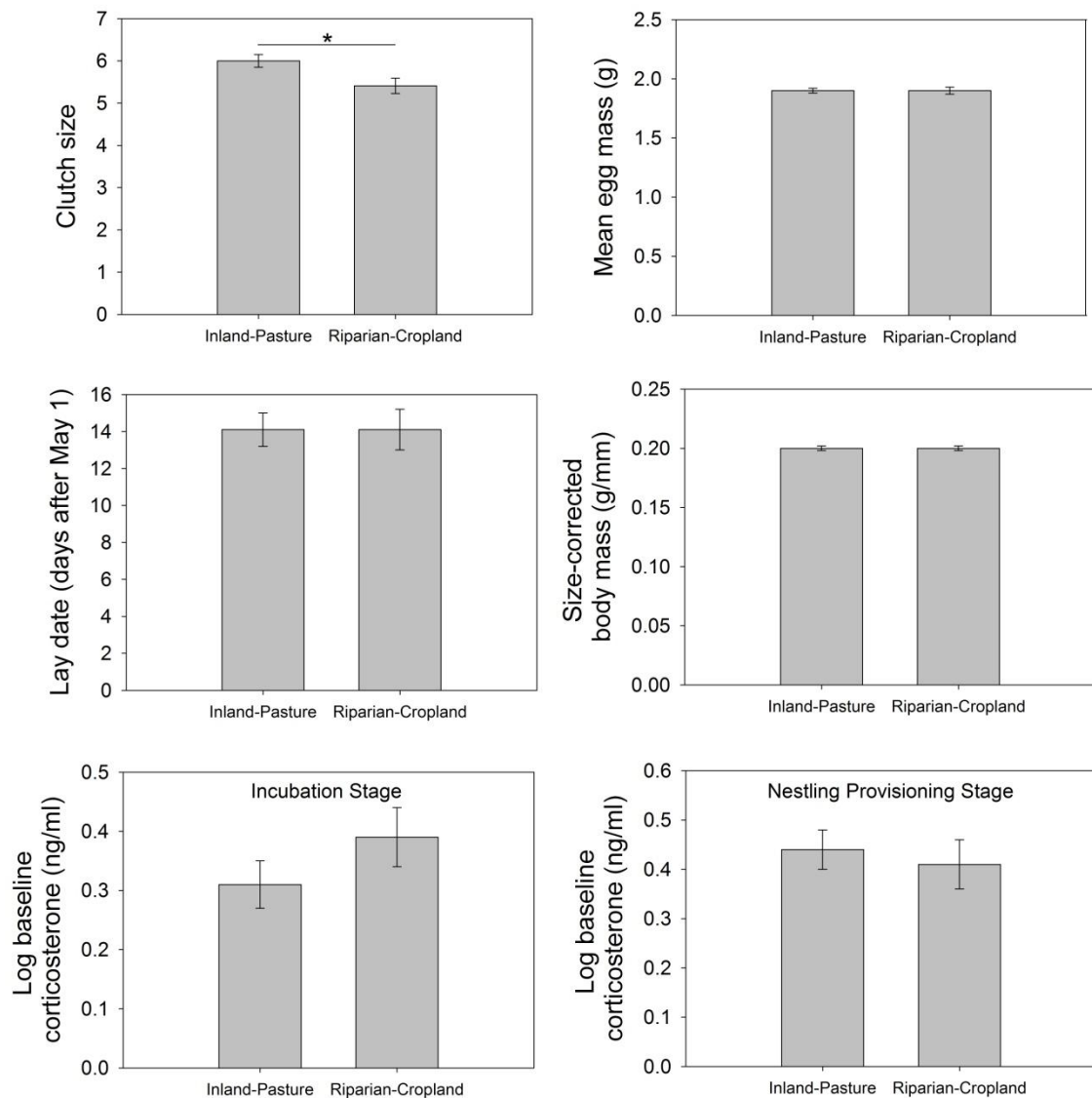
Figure 5.3 - Female tree swallow investment (clutch size, egg mass, lay date) and condition (body mass, baseline corticosterone) between two breeding habitat types (Inland-Pasture and Riparian-Cropland; see Methods for description of habitats).
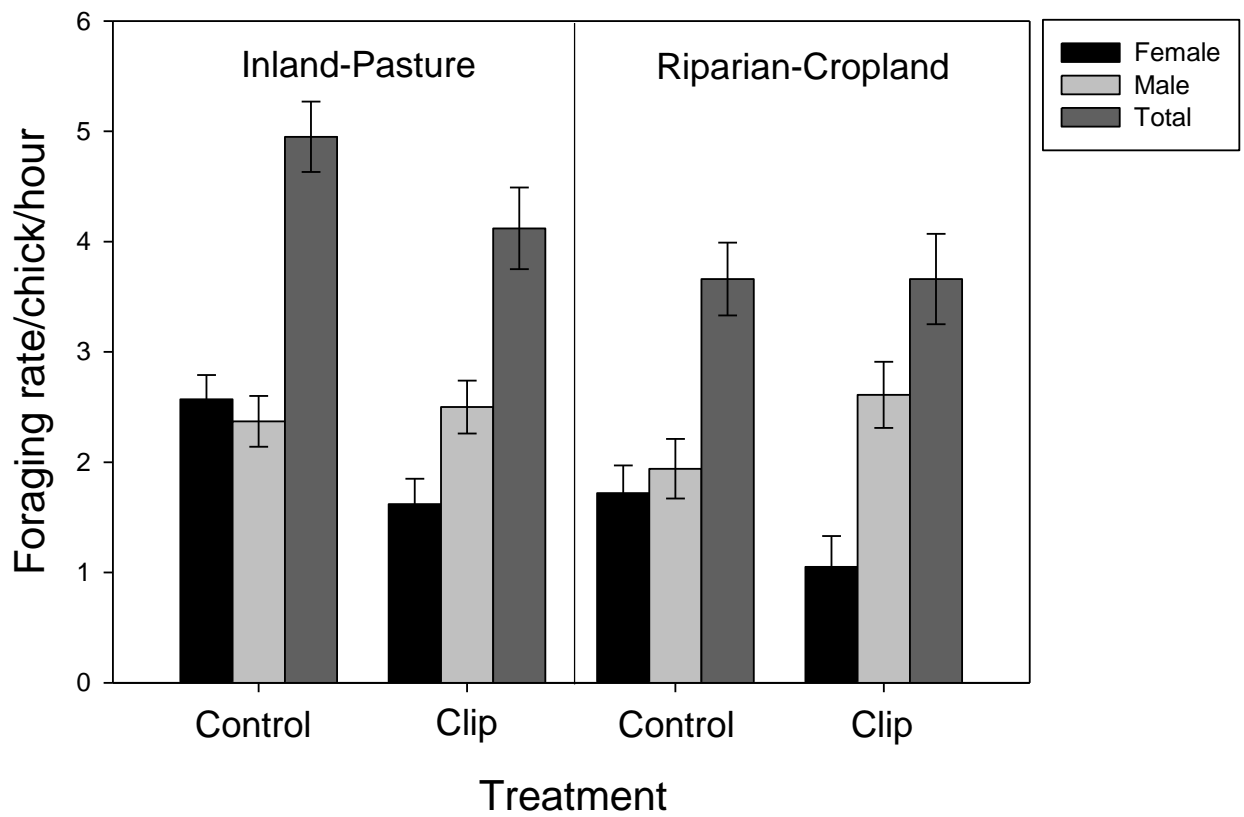
Figure 5.4 - Female, male, and total tree swallow foraging (nestling provisioning) rates at control and manipulation (female feather-clipped) nests in two habitat types, Inland-Pasture and Riparian-Cropland (see Methods for details regarding habitat types).
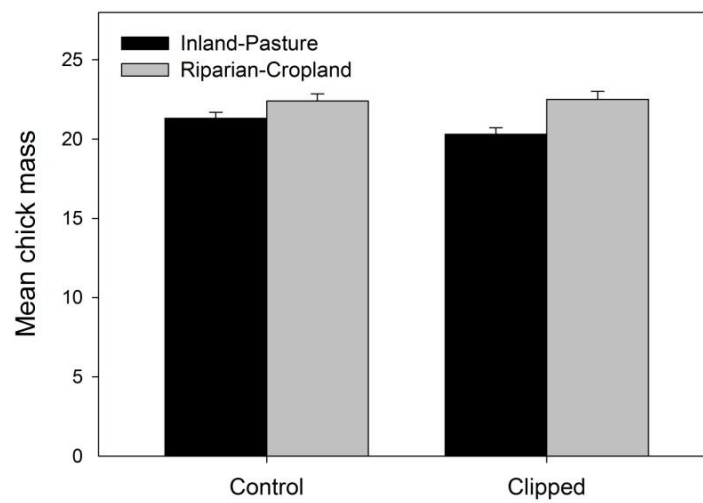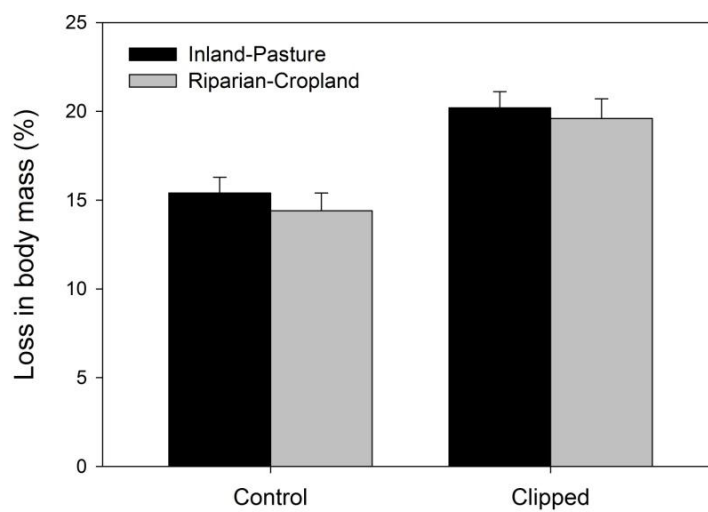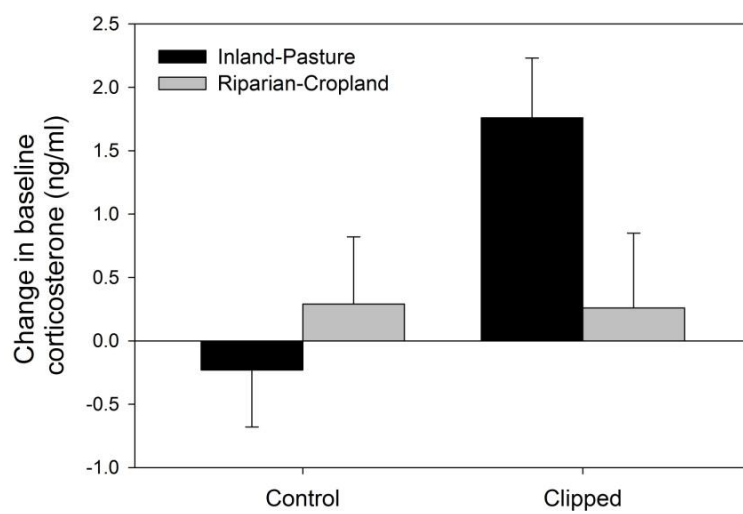
Figure 5.5 - Change in baseline corticosterone over the breeding season, loss in body mass over the breeding season, and chick quality of control and manipulated (feather-clipped) female tree swallows in two habitat types (Inland-Pasture and Riparian-Cropland; see Methods for details of habitat types).
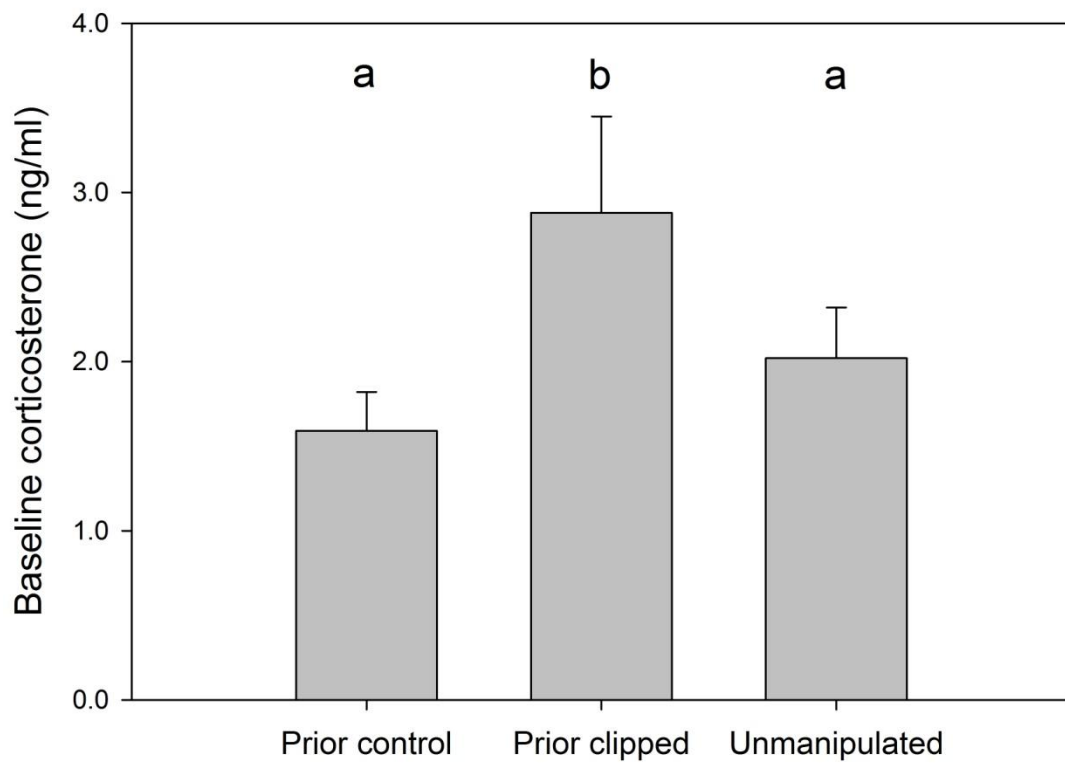
Figure 5.6 - Baseline corticosterone levels of control and manipulated (feather-clipped) female tree swallows at the incubation stage in the year following the manipulation.

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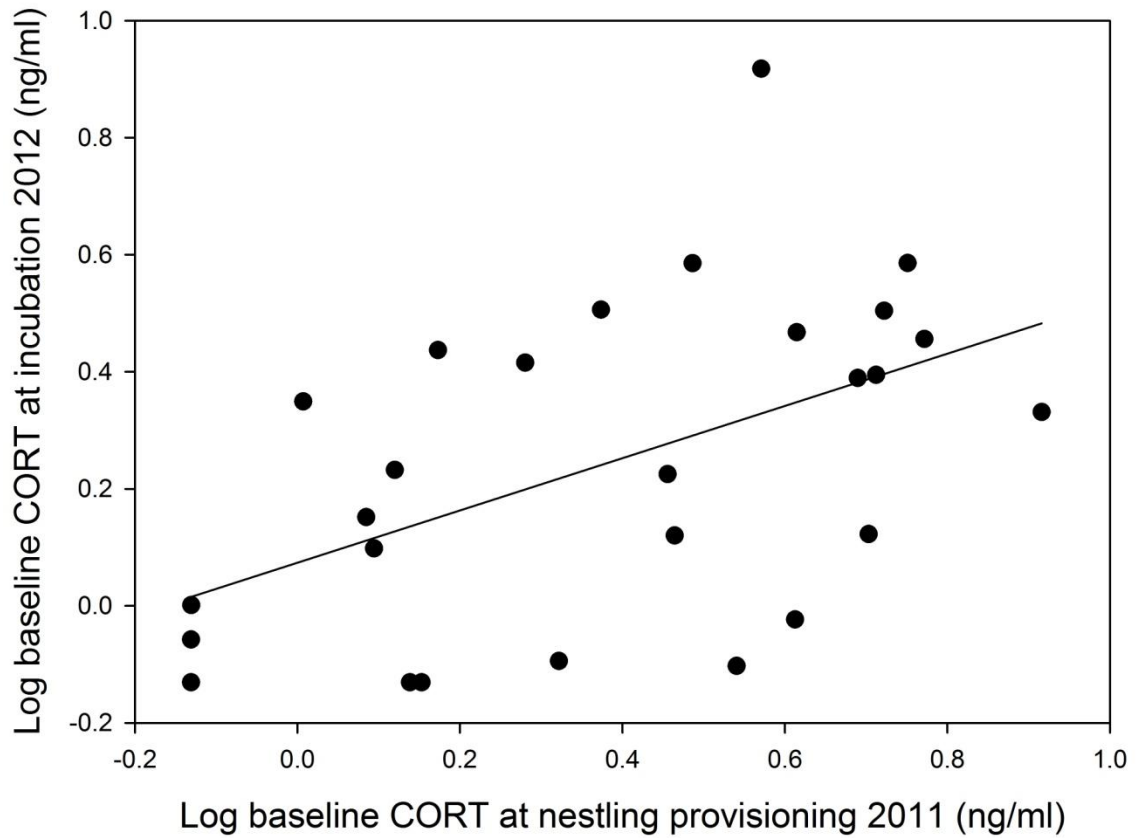
Figure 5.7 - Relationship between baseline corticosterone (CORT) levels in 2011 (at the nesting provisioning stage) and baseline CORT levels in 2012 (at the incubation stage) (n=27; overall model R^2=0.47).

CHAPTER 6 - CONCLUSIONS AND FUTURE DIRECTIONS FOR THE USE OF BASELINE GLUCOCORTICOIDS IN CONSERVATION*

Introduction: Linking multiple validations of baseline glucocorticoids

With the advent of non-lethal field endocrinology (Wingfield and Farner, 1975) came vast opportunities for studying the hormonal regulation of behaviour, development, phenology, and life-history trade-offs in free-ranging wildlife. The physiological metrics available to ecological and evolutionary ecologists today are myriad, spanning the sub-disciplines of health, metabolism, nutrition, growth and development, oxidative status, and reproduction. More recently, conservation biologists have begun to add these physiological measures to their toolbox, paying particular attention to glucocorticoids (GCs) as potential sensitive metrics to monitor population disturbance or health (Cooke et al., 2013; Wikelski and Cooke, 2006). The "stress hormone" moniker undoubtedly set the initial trajectory for the expectation that GCs could be interpreted as straightforward indicators of habitat quality and disturbance level (i.e., individuals or populations with higher GCs are "stressed") (Baker et al., 2013; Bonier et al., 2009; Busch and Hayward, 2009; Reeder and Kramer, 2005). In some cases, this expectation is wholly plausible; however, only after considering GCs in light of their underlying (and arguably complex and context-dependent) physiological role can we delineate when this assumption holds true. Of paramount importance are explicit validations that ask whether baseline glucocorticoids simultaneously integrate environmental variability, change predictably in response to habitat change, and predict fitness metrics. A diversity of anthropogenic pressures are on the rise including pollution, exploitation, invasive alien species, and

resource consumption, and, as a result, rates of biodiversity loss have not been slowing

(Butchart et al., 2010). Consequently, conservation biologists can only benefit from

having an assortment of validated, evidence-based approaches to monitor populations and

demarcate and proactively manage threats.

Taken together, the results of this thesis serve as a caution to the interpretation of

baseline GCs as biomarkers of habitat quality or disturbance prior to validation. Most

importantly, the results draw attention to multiple ways that GCs may fail to fulfil this

role. Specifically, in tree swallows, baseline GC levels were not reflective of

environmental variation across a gradient or discrete habitat types (Chapter 2 and 5),

showed a moderate level of intra-individual variability across reproduction and in

response to a decline in environmental quality (Chapter 3), and did not reflect any within-

or across-season components of fitness (Chapter 4 and 5). Importantly, these findings

occurred despite the consideration of a large number of contexts that could influence

variation in baseline GCs including reproductive stage, age, body condition, reproductive

investment, and underlying habitat type. In addition, an experimental manipulation of

foraging efficiency generally failed to bring anticipated relationships to the surface or

strengthen existing relationships that are necessary for baseline GCs to act as reliable and

predictable biomarkers of environmental change. While these results may seem

discouraging to the application of baseline plasma GCs in conservation monitoring, it

remains unclear how pervasive they may be across species. Given that I have provided

detailed implications of each result from the perspective of conservation applications

within each data chapter, here I will provide some insight regarding how the results

interact with one another with the goal of paving the way for future work.

Why might baseline glucocorticoids fail and when might they be more straightforward to interpret?

It is becoming increasingly clear that the timing of GC measurements across daily, seasonal, and lifetime scales will be integral to their interpretation in natural systems. We completed our validation studies during the breeding season and, across taxa, the vast majority of studies attempting to link GCs to fitness or environmental quality have been completed during reproduction. This is likely a reflection of the greater ease with which individuals can be captured in the breeding season and, for many migratory species, represents the only time during the lifecycle that individuals are accessible. Our results, along with the previously variable GC-fitness and GC-environment relationships across species, may be reflecting that the breeding season is a particularly difficult time period to interpret changing GC levels. This variability likely stems from two main factors. First, GCs play a complex and likely preparative role during the breeding season, a role that is already well-documented during migration (Holberton, 1999; Holberton et al., 2007; Lõhmus et al., 2003; Long and Holberton, 2004; Munakata et al., 2007; Piersma et al., 2000), dispersal (Belthoff and Dufty, 1998; Cease et al., 2007; Dufty and Belthoff, 2001; Silverin, 1997), and hibernation (Reeder et al., 2004; Sheriff et al., 2011). For example, GCs may preparatively mediate changes in body mass, foraging, or investment for different components of the breeding cycle (Crossin et al., 2012; Love et al., 2014). Importantly, this could lead to variable GC levels across individuals due to small differences in sampling time or intrinsic differences between individuals in when they initiate these changes. Overall, especially in species or sexes which invest heavily in offspring care, the non-breeding and non-migratory seasons may provide the clearest links between GC activity and disturbance. However, this suggestion holds important

limitations for many species (e.g., if the breeding season is the only accessible period or represents the time period when disturbance is most expected). If this is the case, the pre-breeding or post-breeding portion of the season where individuals are still on the breeding grounds, but not actively caring for offspring, may be best for relating GCs and environmental quality to subsequent reproductive success or survival. Future studies of species where taking a full life cycle approach is conceivable will be helpful in validating this proposition.

Second, from a life history perspective (sensu Stearns, 1992), the breeding season may be a complex period in which to interpret GC levels because certain species display down-regulated HPA/HPI activity to allow for the continuation of reproduction despite environmental perturbation (Wingfield and Sapolsky, 2003). More specifically, species that are short-lived, semelparous, or have seasonally or socially constrained breeding opportunities are more likely to maintain a reproductive attempt in the face of an unexpected environmental change (Crossin et al., 2015; Wingfield and Sapolsky, 2003). Such "resistance to stress" could mask relationships between GCs and fitness and thus potentially suggests that longer-lived, non-constrained species may be better candidates for conservation monitoring using baseline GC levels during these time periods. Although validating GC-fitness relationships in long-lived, iteroparous species is particularly difficult because it necessitates detailed longitudinal datasets to adequately quantify fitness, these types of studies have been accomplished at established study sites (e.g., Angelier et al., 2006; Satterthwaite et al., 2010).

As a whole, our results also draw attention to the presence of environmental and behavioural compensatory mechanisms that can potentially "buffer" individuals to the influences of habitat change, leading to a potential disconnect between GC levels and

fitness. For example, buffers could take the form of alternative foraging tactics or shelter, flexibility in mate performance, or advantages of social structure (e.g., helpers). Beyond transitory adjustments in behaviour, different environments may select for alternative coping strategies or behavioural types (e.g., reactive versus proactive individuals) and these strategies have been characterized by alternative HPA/HPI profiles (Atwell et al., 2012; Cockrem, 2007; Cristóbal-azkarate et al., 2007; Koolhaas et al., 1999; Partecke et al., 2006), potentially leading to GC level differences between populations that are the result of alternative tactics, rather than disturbance levels (i.e., alternative tactics may not result in differences in reproductive potential or survival) (Dantzer et al., 2014). For example, in species undergoing range expansion, leading edge populations are often characterized by reactive individuals with greater exploratory behaviour and heightened stress responses compared to populations at range interiors (Addis et al., 2011; Jessop et al., 2013; Krause et al., 2015; Liebl and Martin, 2012; Walker et al., 2015). Such changes in HPA/HPI activity at the population level have been observed in as few as 12 generations (Atwell et al., 2012). These patterns and possibilities reinforce the importance of linking environment-GC investigations to fitness outcomes prior to interpretation; an anthropogenic perspective of what constitutes a disturbance may not actually provide a sufficient enough challenge to wildlife to alter success.

Lastly, GCs may be more readily interpretable when measured in conjunction with other physiological or biochemical traits. For example, blood or fecal panels (i.e., full suites of physiological traits comparable to veterinary blood panels) can provide simultaneous information on sex, reproductive status, HPA activity, immune function, and nutrition. Recent work in the endangered Northern spotted owl (*Strix occidentalis caurina*) found that only through the consideration of thyroid hormone metabolites (an

192

indicator of nutrition) was it possible to establish a link between acute vehicle exposure, fecal GCs, and reproductive success (Hayward et al., 2011). Combinations of physiological traits related to stress, reproductive status, and nutritional state have also helped delineate the various threats faced by caribou (*Rangifer tarandus caribou*) living in proximity to oil sands operations in Alberta (Joly et al., 2015; Wasser et al., 2011) and killer whales (*Orcinus orca*) exposed to boat traffic and nutritional limitation in Puget Sound (Ayres et al., 2012). By combining GC measures with metrics of nutritional state or metabolic rate (e.g., thyroid hormone, beta-hydroxy-butyrate, or triglyceride levels) and reproductive state, it may be possible to tease apart underlying variability in GC levels and more easily interpret whole-organism response to environmental change. Nonetheless, from a practical perspective, the larger size of the plasma or fecal samples required, the greater laboratory time, and the higher costs associated with assays needed to assess multiple physiological traits simultaneously will (at least currently) make this approach much more applicable to large wildlife species and projects with greater scope.

General considerations and recommendations for future work

As the previous section contains some suggestions for future work, here I will provide other considerations not encompassed by the discussion therein. For any future study or meta-analysis designed to assess the relevance of GCs to conservation or citing potential for this application, it is paramount to consider on-the-ground implementation (i.e., logistical feasibility, cost, and ease of use). For example, it is possible that certain thresholds of environmental degradation will result concurrently in changes in GC levels and fitness (Dantzer et al., 2014). However, the magnitude of this threshold is integral; the suggested power of GCs in conservation lies in their ability to impart sensitivity and

predictive capacity. Therefore, if it is not possible to establish an underlying relationship between GCs and fitness that will be applicable across gradual (attritional) changes in environmental quality, the tool forfeits considerable appeal. More severe changes in environmental quality can often be observed and assessed visually, and therefore GCs are not necessarily needed in these situations as wildlife responses to these changes can be confirmed through observational studies of behaviour, or indeed the repercussions (i.e., fitness losses) are so severe or immediate that they cannot be reversed. The necessity that GCs must be indicators of gradual, sub-lethal effects must be kept in mind when defining what constitutes a "success" story for GCs in conservation physiology.

Similarly, if the collection of certain contextual variables is necessary to interpret GC levels, the feasibility of their measurement to different organisms becomes potentially problematic. For example, if only a short temporal window during the breeding or other season can provide insight into fitness in the context of environmental quality, it may limit this approach in many species where reproductive status is difficult to ascertain, or where breeding attempts are not easily monitored. Furthermore, if detailed reproductive monitoring is possible, the underlying value of GCs is limited since demographic information may be more easily obtained and can provide direct estimates of viability. On a finer scale, it has been suggested that the measurement of cellular GC receptor densities may provide insight into variability in GC levels (Crespi et al., 2013; Dantzer et al., 2014; Lattin et al., 2012; Romero et al., 2015); however, this type of investigation necessitates invasive tissue or lethal sampling. While these types of studies can undoubtedly help to illuminate the mechanisms underlying variation in GC levels, if receptor density characterization is a prerequisite for interpreting GC levels, their utility as a conservation biomarker essentially vanishes.

I propose that a profitable future endeavour will involve the explicit comparison of GCs with other metrics available for conservation monitoring. Tarlow and Blumstein (2007) performed a non-quantitative investigation nearly 10 years ago by comparing seven metrics of potential anthropogenic stress in animals (GCs, cardiac response, mate choice, flight initiation distance, immunocompetence, fluctuating asymmetry, and breeding success) and assigned each metric a relative rating of high, medium, or low in terms of ease of use, ability to quantify impact, reflection of population viability, and repeatability. GCs were given a ranking of medium in all categories. However, given the torrent of investigations involving GCs since the formal description of conservation physiology and the even greater literature base that has been accumulating in eco- and evolutionary physiology since the formalization of the Cort-Fitness Hypothesis, there now exists the possibility to compare metrics using a quantitative, meta-analytic framework. In particular, estimates of cost, time investment, sample storage requirements, and invasiveness are warranted, as well as the inclusion of other physiological and behavioural metrics. For example, a recent analysis investigating experimentally-induced chronic stress across laboratory and field studies identified body mass as a more consistent consequence than altered GC levels (Dickens and Romero, 2013), indicating the importance of considering traditional (and often simpler) measures of organismal state as viable metrics of disturbance. Additionally, behavioural assessments in coho salmon (*Oncorhynchus kisutch*) have been shown to correlate well with many physiological variables known to relate to post-capture health and survival, illustrating the potential for low-cost methods of detecting disturbance (Davis, 2010; Raby et al., 2012). Overall, comparisons among metrics can help practitioners weigh the costs and benefits of alternative techniques for their wildlife systems.

Our understanding of the additive effect of multiple environmental stressors on GCs in wild populations is still limited. Experimentally applying combinations of environmental changes (e.g., predation pressure and food limitation: Clinchy et al., 2004) will offer additional insight in this regard, and dose-response relationships where the intensity or duration of different anthropogenic stressors are varied will help to identify whether physiological thresholds exist. It is clear from the mixed results of the small contingent of studies in which environmental quality, GCs, and fitness have been measured simultaneously that our understanding of the predictive value of GCs is currently limited. As such, moving forward, the value of such investigations cannot be overstated. Because such relationships can display alternative patterns at the individual and population-level (Riechert et al., 2014), examining linkages at multiple scales is necessary to fully appreciate how physiological measurements taken at the individual scale spur population patterns (Cooke, 2014; Cooke and O'Connor, 2010). Beyond investigations that identify correlations between GCs and fitness, explicit demographic models (e.g., matrix models), incorporation of parameter elasticities, survival analysis, and path analysis can estimate population dynamics, selection differentials, and help to identify the fitness metrics that will most likely be linked to GC levels (Crespi et al., 2013). Similarly, remaining aware of well-appreciated concepts in population ecology, such as negative density-dependence, will also help to more accurately interpret individual-level changes in GCs in the light of population-level change.

Finally, as a broad recommendation for expediting the validation of GCs (or any physiological metric) as conservation biomarkers, I suggest the relatively novel flow of information from traditional ecological and evolutionary physiologists explicitly to conservation biologists. Indeed, there is enormous capacity for growth in this regard;

since 2006, less than 1% of articles that were published in major plant and animal physiological journals contain conservation-specific keywords (Lennox and Cooke, 2014). This pattern may partly stem from the discipline of conservation physiology being viewed primarily as an opportunity for conservation biologists to assimilate techniques and tools in physiology. Such a scenario is characterized by the flow of information beginning with conservation biologists so that case-directed endeavours in conservation can be addressed. However, traditional ecological or evolutionary physiologists can specifically generate or re-purpose information with the targeted goal of progressing conservation physiology. As individuals whose research is dependent on wildlife, many physiologists have a vested practical interest in the natural world and addressing conservation issues can provide a way to invest in the perpetuity of the systems we rely so heavily upon (Caro and Sherman, 2013).

Relevance of results for tree swallows as an aerial insectivore

I conducted the validations comprising this thesis in a declining member of an avian aerial insectivore guild that is gaining conservation concern in North America, rather than in a surrogate species. I sought to provide insight into how changes in environmental quality during the breeding season may be monitored through physiological biomarkers, and how they may influence individual performance and fitness. My results indicate that there may be a disconnect between early- and late-season breeding habitat quality from the perspective of food availability that could cause a disparity between expected and realized breeding success in certain habitats. Over a larger gradient of agricultural intensifications, Ghilain and Bélisle (2008) found that the availability of *Diptera* prey is correlated with tree swallow breeding success, but it is unclear if this is altering

population dynamics, and more recent work is drawing attention to carryover effects from the breeding grounds (Paquette et al., 2014). Indeed, the fact that baseline GC levels of individuals breeding in my study site remained elevated even subsequent to the entire migratory and wintering season may signal that individuals are not able to compensate in overwintering areas, potentially due to increased pressures there. While my sites may allow individuals to successfully breed despite these effects, other areas with greater intensive agriculture or predation risk may not provide the same opportunity. I parallel other investigators in calling for a full life cycle approach to gain insight into mechanisms of decline, likely necessitating international collaborative effort (McCracken, 2008). While aerial insectivores share a common food resource, they are a diverse grouping of avian species in terms of migratory distance, foraging tactic (sallying versus coursing), foraging altitude, nesting habitat, overwintering range, etc. With many of these species we still lack basic information on ecology, migration routes, overwintering sites, and changing insect resources (quantity and quality) (Nebel et al., 2010; Nocera et al., 2012). Adaptive management techniques for conservation that operate under a scarcity of information, but that can be actively altered as evidence accumulates, should be designed to simultaneously generate scientific knowledge and monitor populations in cost-effective ways (Nichols and Williams, 2006; Rioux et al., 2010).

Conclusion

Despite extensive interest and investigation of GCs in the context of conservation, their interpretation, even in many species-specific applications, has been premature due to the systemic omission of fitness metrics, comparatively few validation studies, and highly variable results. Regardless of which metric of GCs (plasma, salivary, fecal, hair, or

feather) is under consideration, there still remains considerable validation prior to feasible implementation (Dantzer et al., 2014; Goymann, 2012) and none will constitute an unequivocal indicator of "stress level". However, this should not be viewed as a fatal flaw; implements in any discipline's toolbox will only be suitable for certain tasks. Through the perspective of my introductory chapter and the investigations comprising my subsequent data chapters, I have drawn attention to a number of validations of relevance to both eco- and evolutionary physiologists and conservation biologists. These investigations are transferable across species and GC metrics, and even further, to any physiological trait that is being considered for conservation monitoring (e.g., oxidative stress, telomere length, immunocompetance). By appreciating the functional role of physiology and the goals and limitations of working within conservation-focused systems, the field of conservation physiology can yield truly integrative approaches for addressing and preventing further loss of biodiversity worldwide.

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