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Investigating the influence of stress hormones on the partial migratory tendencies of the American goldfinch (*Spinus tristis*)

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Migrants may employ different strategies that best optimize energetic trade-offs/ costs associated with specific metabolic demands in preparation for and during their seasonal movements. One strategy is known as partial migration, where members of the same population exhibit either migratory or sedentary behavior patterns. The American goldfinch (*Spinus tristis*), a known partial migratory species, displays a latitudinal distribution of age-sex classes across the winter range during their migratory phase. Females and adult males migrate further south than juvenile males to overwinter. The purpose of this study was to determine whether corticosterone (CORT), a hormone related to high-energy costs or activity, might explain this migratory behavior. Increases in CORT have been found in several seasonal migrants and may support associated physiological and behavioral changes during this life-history stage. We measured basal and stress induced CORT levels among age and sex classes influencing migratory status. Samples were extracted during late-breeding and migratory phase. A general linear model determined predictor variables that induced basal and stress-induced CORT levels. Body condition was the only significant variable negatively correlated with stress-induced CORT levels ($p = 0.03$). In addition we observed a significant negative relationship between body mot intensity and stress responsiveness in Goldfinches ($p = 0.05$). Our findings add to our understanding of how glucocorticoids support seasonal life history stages comparing the different CORT levels across age and sex classes.

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INVESTIGATING THE INFLUENCE OF STRESS HORMONES ON THE PARTIAL
MIGRATORY TENDENCIES OF THE AMERICAN GOLDFINCH
(*SPINUS TRISTIS*)

By

Katherine E. Campbell

A Senior Thesis Submitted to the

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in Partial Fulfillment of the Requirements for Graduation

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Abstract

Migrants may employ different strategies that best optimize energetic trade-offs/ costs associated with specific metabolic demands in preparation for and during their seasonal movements. One strategy is known as partial migration, where members of the same population exhibit either migratory or sedentary behavior patterns. The American goldfinch (*Spinus tristis*), a known partial migratory species, displays a latitudinal distribution of age-sex classes across the winter range during their migratory phase. Females and adult males migrate further south than juvenile males to overwinter. The purpose of this study was to determine whether corticosterone (CORT), a hormone related to high-energy costs or activity, might explain this migratory behavior. Increases in CORT have been found in several seasonal migrants and may support associated physiological and behavioral changes during this life-history stage. We measured basal and stress induced CORT levels among age and sex classes influencing migratory status. Samples were extracted during late-breeding and migratory phase. A general linear model determined predictor variables that induced basal and stress-induced CORT levels. Body condition was the only significant variable negatively correlated with stress-induced CORT levels ($p = 0.03$). In addition we observed a significant negative relationship between body mot intensity and stress responsiveness in Goldfinches ($p = 0.05$). Our findings add to our understanding of how glucocorticoids support seasonal life history stages comparing the different CORT levels across age and sex classes.

Introduction

The burning of fossil fuels and land-use change, such as urbanization and deforestation, have contributed to rising global temperatures that negatively effect ecological systems, where migratory organisms are particularly impacted by these changes (Bruthe et al. 2014; Fenkes et al. 2015; Pautasso 2012; and Root et al. 2005). Shifts in climate alter seasonal climate patterns and phenological events such as migration (Guillemain et al. 2015; Van Buskirk et al 2009). For many avian species, their migratory period is intrinsically dependent on specific external cues from the environment that induce an appropriate physiological and/or behavioral response (Cornelius et al. 2013). A shift in these cues during their molting, breeding, and migratory phase leads to the disassociation of highly specific internal rhythms with the external stimuli that drive these seasonally specific life history stages (Hahn and MacDougall-Shackleton 2008).

Synchronization between physiological processes and environmental cues plays an important role in the execution of seasonally specific life history stages in migratory birds, such that these traits are selected via the evolutionary mechanism of natural selection (Bauer et al. 2008). Annual increases in photoperiod have been shown to trigger specific behavioral changes in avian species, conveying in depth information relevant to current and future seasonal events during the molting, migratory, and breeding stages (Cornelius et al., 2013; Moore et al. 1982; Ramenofsky and Wingfield 2007). Migration is a very costly life history stage that requires a significant amount of energy expenditure to maintain metabolic activity as birds cover long distances to specific breeding and overwintering grounds. For example, wheateaters (*Oenanthe onanthe*) spend at least

4,000kJ—if not more—during their 30,000km migration between Alaska and Africa (Schmaljohann et al. 2012). The migratory phase necessitates physiological changes within an individual in preparation for these energy intensive flights.

Specific physiological changes associated with migratory preparation and departure, such as increased metabolic activity and locomotion, may be coordinated by hormones. Glucocorticoid hormones (GCs) are chemical messages released by the hypothalamic-pituitary-adrenal (HPA) axis into the bloodstream that interact with target tissues and drive necessary physiological life cycle transitions (Piersma et al. 2000). Production of GCs facilitates the appropriate stress-response during different physiological states that encompass both life threatening and seasonal processes (e.g. feeding behavior, locomotor activity, and energy metabolism) (Landys et al. 2006). The dominant GC, corticosterone (CORT) increases in response to energy demanding processes, for example, during migration in at least one seasonal migratory species study (Cornelius et al. 2013; Landys-Ciannelli et al. 2002). Previous research suggests elevated CORT levels are an adaptive metabolic trait in birds because they transform stored energy into a usable form through protein catabolism (Asthemier et al. 1992; Le Ninan et al. 1988; and Davison et al. 1982).

Elevated CORT levels that reflect increased metabolic demands associated with flight activity may also act as a regulatory mechanism to induce migratory behavior (Landys-Ciannelli et al. 2002). Although much of the research that focuses on the role of GCs in avian species investigates whether seasonal elevations of CORT are linked with specific life history stages, there is no definitive indication that CORT is the underlying

mechanism (Landys-Ciannelli et al. 2006). Understanding what drives specific migratory activity in avian populations is needed to fill in this fundamental gap in our knowledge.

Up-regulation of CORT may be inducing specific migratory behavioral responses in avian migrants and therefore must be further researched to discern the significant role GC's play in affecting a bird's decision to migrate. Different species have adapted their migratory responses in such a way that within a population, certain individuals will display anywhere from rigid genetic control to more plasticity in behavioral responses. (Newton 2011). Partial migration is one such behavioral response that is a transitional adaptation between two dissimilar life history stages: migratory and sedentary tendencies (Fudickar et al.2013). Despite the different life history approaches, both seasonal migrants and year-round residents may be present in the same population, suggesting there is some fluidity between these two behavioral responses (Fudickar et al. 2013). The American Goldfinch (*Spinus tristis*), has been documented as a partial migratory species based on the behavioral pattern of juvenile males wintering in the north whereas adults and juvenile females, by comparison, migrated further south (Prescott and Middleston 1990).

The purpose of this study was to explore how CORT levels influence migratory behavior by studying the partial migrant, the American Gold Finch because this species displayed specific behavioral variation between individuals in the population. Prescott and Middleston's (1990) work inspired our primary research question, which addressed whether latitudinal variation in age-sex classes during fall migration correlated with CORT levels across age and sex classes of Goldfinches. We hypothesized that CORT is a hormonal mechanism underlying physiological and behavioral changes among migrants

and non-migrants. We predicted that differences in basal and stress-induced plasma CORT levels across age and sex classes would be a strong indicator of migratory status/activity. Our specific predictions included the following: 1) Elevated CORT levels (i.e., basal and stress-induced) would be highest among the adult class, as an indication of their migratory activity 2) Seasonal CORT levels would differ between two specific life history stages: late breeding and migration 3) Body molt and body condition would be heavily influenced by elevated CORT levels and therefore both variables would be strong predictors of stress responsiveness in Goldfinches. To address these predictions we extracted blood from Goldfinch specimen to determine if there were plasma CORT level distinctions that corresponded to specific migratory activity between age-sex classes.

Methods

Study Site:

Species

The American Goldfinch (*Spinus tristis*) is a member of the passerine bird family, fringillidae that are characterized by distinctive sexual dimorphic plumage among males and females (Middleton 1993). Males are most noticeably recognized by their bright orange bill and carotenoid-based yellow plumage where as females have more of a muted yellow pigmentation (Middleton 1993; Kelly et. al 2012). Goldfinches exhibit partial migratory behavior, which is documented as latitudinal distribution of specific age and sex classes during the overwintering migratory phase (Prescott and Middleton 1990). Distinctions between age-sex classes is reflected in the dissimilar migratory tendencies between adults that winter further south while juveniles remain in the northern range of their breeding grounds.

Foraging and Habitat Preference

Goldfinches are granivorous, relying almost exclusively on a seed based-diet (Furlonger et al. 2012). Thistle seeds (Cynareae) often times are a preferred food source for these birds in the wild. Seed production and abundance of thistle may also drive specific life history stages such as initiation and duration of their breeding season (Furlonger et. al 2012). Thistle seeds are also used commercially, attracting these birds to more urban and suburban sites. Due to a steady food supply, reduced predation, fewer nest-parasitism by other birds residential areas have become a productive habitat for goldfinches (Middleton 1979).

Sampling Locations

Field sites were chosen based on the frequency of goldfinch activity according to reports from residents within the contiguous communities of Ann Arbor, Ypsilanti and Canton located in the lower southern region of the state of Michigan. Locations were designated by the resident birding community and homeowners of those areas. We visited rural and residential areas throughout Southeastern Washtenaw and Western Wayne counties at least twice to determine their feasibility as field sites. Goldfinches visited at least one feeder at these designated areas in some capacity, which was a means to gauge capture success of a potential.

Those that had the frequent goldfinch activity were our primary captures sites. Of the four sites visited, three were located in rural areas and one in a suburban area. Rural areas tended to yield the most capture rates due to the high frequent goldfinch activity at feeders. In contrast, suburban areas yielded the least amount of captures. Data collection coincided with the late breeding period and into the migratory phase of our organismal

model, the American Goldfinch (*Spinus tristis*) during mid-June through early December. The breeding period was limited to August while molt and subsequent migratory movements occurred during the fall (Sept.-Nov). Sites were visited twice weekly for sample collection.

Capture Technique:

Apparatus

Mistnets and a feeder trap apparatus were used to capture specimen at field sites. Nets were set up on either side of the thistle feeder, which overlapped with peak bird activity during the morning and into the afternoon (Keyes and Grue 1982). Favorable weather conditions for mistnetting included little wind and some cloud cover in order to minimize visibility and maximize captures. When mistnets were not being used, we set up a feeder trap apparatus that was placed over the thistle feeder. Goldfinch playbacks (i.e., recordings of calls and songs) were used to attract birds to our feeders at each field site. Trapping times were standardized across sites during sample collection (08:00-12:00) to account for diurnal rhythms that might affect plasma CORT levels (Wingfield et al. 1992).

Recapture

Each specimen was banded and monitored for recapture at our four designated field sites. Specimens were only sampled for an additional blood sample if it had been at least two weeks since previous sampling to avoid negatively affecting specimen fitness.

Blood Sample Extraction:

Blood Extraction

To determine if seasonal shifts in plasma CORT corresponded with migratory patterns across different age and sex classes we extracted ~50ml of blood into a 75-microcapillary tube immediately after capture. The initial extraction established the specimen's basal plasma CORT level. Blood was taken from the alar vein located inside the left wing using a 25-gauge needle. The 75-microcapillary tubes were sealed with molding clay at one end, and kept on ice between 2-4 hours until plasma could be separated from red blood cell hematocrit using a clinic IEC microcapillary centrifuge later that same day. To account for capture stress due to the sensitivity of plasma CORT, the first blood sample was extracted within three minutes of the initial capture (Wingfield et al. 1992). Birds were then held inside a cloth bag for ~30minutes before a second blood sample was extracted in under five minutes.

Plasma Extraction

In order to assess stress responses in target specimen plasma was separated from whole blood samples to determine baseline CORT (0 minute post-capture) and stress-induced CORT levels (30 minutes post-capture) (Wingfield et al. 1992). The centrifuge was spun at 13,000g for eight minutes at room temperature (21°C). Hematocrit values were recorded for 0' and 30' samples using a microhematocrit reader card. After plasma extraction, samples were stored in -80°C freezer until assay analysis. By measuring stress-induced elevations of CORT concentrations at specific time points, we were able to directly assess stress responses in captured specimen that was comparable across seasons and age-sex classes.

Physical Fitness:

Body Measurements

Age and sex of specimen were designated based on plumage characteristics (Pyle 1997). Measurement of the wing (W), tarsus (T) and keel (K) lengths were taken to the nearest 0.1 mm with calipers and mass was measured using a 30g Pesola spring scale (to the nearest 0.5g). Specimens were fat scored according to furculum and abdominal fat deposits on a scale from 1 (hardly any fat) to 5 (excess fat) (Helms and Drury 1960; Cornelius and Hahn 2012). Cloacal protuberance (CP) length (to the nearest 0.1mm) was measured in males, while brood patch (BP) stage was recorded for females: absence (0) or development (1-5) of a BP (Ralph et al. 1993; Cornelius et al. 2011a).

Body Molt

To determine if changes in CORT levels were a determinant of molt intensity we recorded body molt status. Molt intensity was scored based on a four-point scale :0 – no feathers growing; 1 – few feathers growing in one feather tract; 2 – few feathers growing in multiple feather tracts; 3 – many feathers growing in multiple feather tracts. We also recorded status (percent of molt complete) of the right and left primary and secondary wing feathers (Cornelius et al. 2011b).

Body Condition

We also determined if reduction in physical fitness corresponded with elevations in CORT levels. For each bird, a first principal component (PC1) was calculated as a single measure of body size (this is method), which included tarsus and keel length measurements. Because of molting phase, wing measurements were not as reliable and were excluded from the single measure of body size.

Hormone Assay:

Enzo-Life Sciences EIA kits were used to determine CORT levels from extracted 0' and 30' plasma samples (Enzo Life Sciences 2015). Competitive binding occurred between CORT from the sample/standard and an alkaline phosphatase molecule to the polyclonal antibody. After a period of incubation at room temperature the excess reagents were washed away and then a substrate was added. CORT present in the sample was inversely proportional to the yellow color generated by the enzyme reaction (Appendix 1). Based on the measured optical density, concentrations of CORT were calculated and a standard curve was generated for each plate. Samples were randomized across plates and run in triplicate at once throughout December. Intra-assay variation (CV) was 11.8% and our minimum detectability values ranged from 25ng/ml to 40ng/ml. Samples that fell below levels detectable by the assay were assigned the minimum value for that plate.

Data Analysis:

To determine significant predictors based on the CORT assay results, a general linear model was used to analyze samples collected across the migratory phase (Sept-Dec) (i.e., samples collected in Aug. were initially excluded). Predictors for baseline and stress-induced (30-min) CORT included the following variables: 1) age and sex class, 2) body condition, 3) body molt, and 4) flight feather molt for fall baseline and 30-min CORT samples (n = 44, Table 1).

All samples and all months were included in the data set (0 minute n = 59; 30 minute n = 65) to determine differences in seasonal CORT. Months were designated as

either summer (June-Aug) or fall (Sept-Dec) to test for differences between late summer breeding phase and fall migratory phase. Categories agreed with seasonal changes in reproductive fitness for males and females based on CP (cloacal protuberance) and BP (brood patch) measurements. Suggesting breeding occurred in the summer and non-breeding in the fall. We then ran post-hoc ANOVA or correlation analyses for significant predictors to compare means and assess changes across the late breeding transition to autumn migration. Outliers (defined as being greater than 2 standard deviations from the mean) were excluded from this data set when analyzing general patterns in baseline and 30-min plasma CORT. In addition, we ran a Cook's test to assess whether specific data points were driving the statistical significance that negatively correlated to status of body condition (Figure 1).

Results

<u>Data Analysis:</u>

CORT across Age and Sex Classes

Predictor variables had no significant effect on baseline CORT (Table 1, $p >$ 0.05), although averages did trend in the predicted direction. Juvenile males had the lowest baseline CORT across age and sex classes, however when compared to adult male basal levels this trend was rather weak (figure 4, $p = 0.31$). There was even less variation among the 30-min CORT when comparing levels between age and sex classes (Figure 5 and 4).

Seasonal CORT

A comparison between summer and fall showed a highly significant decline in baseline CORT (0' $t_{1,59} = 10.7$; $p = 0.0018$) and more modest reductions in 30-min CORT

$(30'\ t_{1,65} = 4.4;\ p = 0.04)$ during the fall. Post-hoc Tukey tests revealed baseline CORT levels were highest, on average, in Aug compared to levels in Sept-Nov (Figure 3). 30-min CORT between Aug and Nov showed a similar trend, however, the results were not significant $(p > 0.05)$.

Physical Fitness:

Body Molt

Body molt negatively predicted 30-min CORT stress responsiveness in Goldfinches (Table 1, $p = 0.05$). Specimen that exhibited heavy body molt had significantly lower stress-induced CORT levels compared to non-molting individuals (Figure 6)

Body Condition

PC1 was comprised of the tarsus and keel length measurements and was used to positively predict mass based on linear fit to estimate body condition based on the age-sex class residuals (Figure 1). Body condition was the only significant variable negatively correlated with 30-min CORT stress responsiveness (Figure 1, $p < 0.05$, $R^2 = 0.07$). Further statistical analysis revealed a specific data point—previously designated as an outlier—was not driving this particular trend according to Cook's test, which was based off a threshold of 1.0 (Figure 1, D-value = 0.2). Body condition varied by age and sex class indicating that juveniles had lower body condition when compared to adults (Figure 2). Adult females were only marginally higher in body condition than juveniles; however, adult males had significantly higher body condition than juveniles (Figure 2). Body condition was negatively correlated with 30-min CORT (p= 0.03) but not with baseline CORT (Table 1, $p > 0.05$).

Table 1: The dependent variables used to generate a linear model in order to compare relationships between age-sex class, body condition, body molt, and flight feather molt to basal and stress-induced CORT concentration

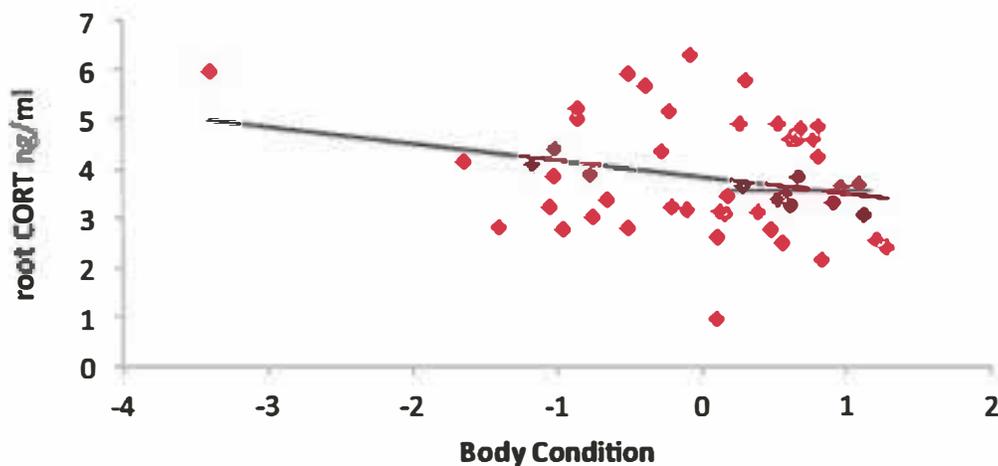|	0 minute CORT, n=44			30 minute CORT n=44		
Dependent variables	df	F	P	df	F	P
Age-Sex Class	3	1.22	0.31	3	0.02	0.99
Body Condition	1	0.47	0.49	1	2.23	0.03
Body Molt	3	1.09	0.3	3	2.8	0.05
Flight Feather Molt	1	1.42	0.24	1	0.005	0.94

Fig. 1. The influence of body condition on stress-induced plasma Corticosterone (CORT) levels regardless of Goldfinch age-sex classes ($p = 0.03$, $R^2 = 0.07$). The outlier does not drive statistical significance of this negative correlation. Cook's test confirms general pattern is not influenced by single data point that reflects significantly poor body condition as a determinant of exceptionally high stress responsiveness.
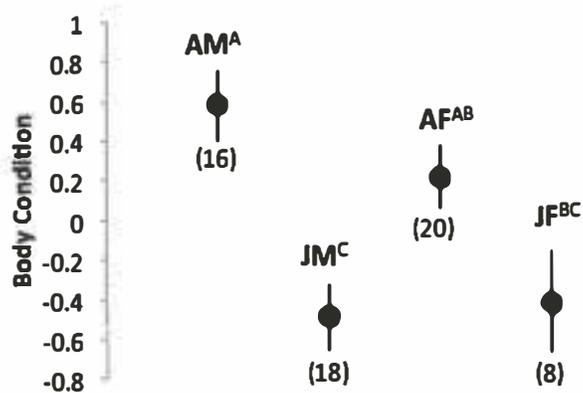
Fig. 2. The residuals from the PC1 to mass linear fit estimate body condition across the four age and sex classes (AM= Adult Male, mean = 0.58 ± 0.18 SE; AF= Adult Female, mean = 0.22 ± 0.16 SE; JF= Juvenile Female, mean = -0.41±0.26 and JM=Juvenile Male, mean = -0.49±0.17). Numbers inside parentheses represent individuals from the designated age and sex class categories. Single letters (A, B, C) identify which statistically significant differences in body condition among the different age-sex classes while double letters represent a lack of statistical significance ($p > 0.05$).
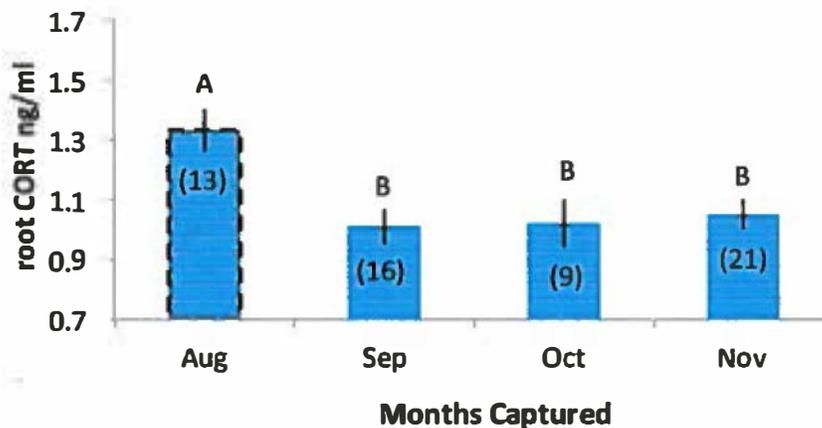

Fig. 3. Seasonal changes in baseline CORT from August (late-breeding phase, Aug mean = 1.33 ± 0.07 SE) to September, October, and November (molting/migratory phase, Sept mean = 1.01 ± 0.06 SE; Oct mean = 1.02 ±0.08 SE; Nov mean = 1.05 ± 0.05 SE; 0' $t_{1,59}$ = 10.7; p = 0.0018). Numbers in parentheses designate basal plasma CORT samples from each month. Dashed line represents statistical significance trend in summer samples. Letters designate the statistically significant difference between summer (A) and fall (B) basal CORT levels.
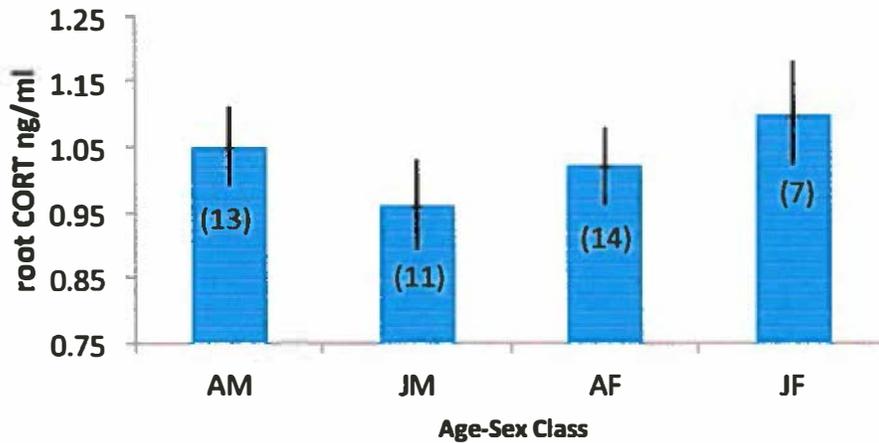
Fig. 4. Changes in baseline plasma CORT categorized according to age and sex classes (AM= Adult Male, mean = 1.05 ± 0.06 SE; AF= Adult Female, mean = 1.02 ± 0.06; JF= Juvenile Female, mean = 1.10 ± 0.08; and JM=Juvenile Male mean = 0.96 ± 0.07). Basal plasma samples were from individuals captured during their migratory phase (Sept-Dec). Numbers in parentheses designate samples from the designated age-sex class categories.

Fig. 5. Changes in stress-induced plasma CORT according to age and sex classes. (AM= Adult Male, mean = 3.69 ± 0.35; AF= Adult Female, mean= 3.96±0.29; JF= Juvenile Female, mean= 4.2 ± 0.5; and JM=Juvenile Male, mean = 3.82 ± 0.32). 30-min CORT samples were from individuals captured during their migratory phase (Sept-Dec). Numbers in parentheses designate 30-min CORT samples from the designated age-sex class categories.
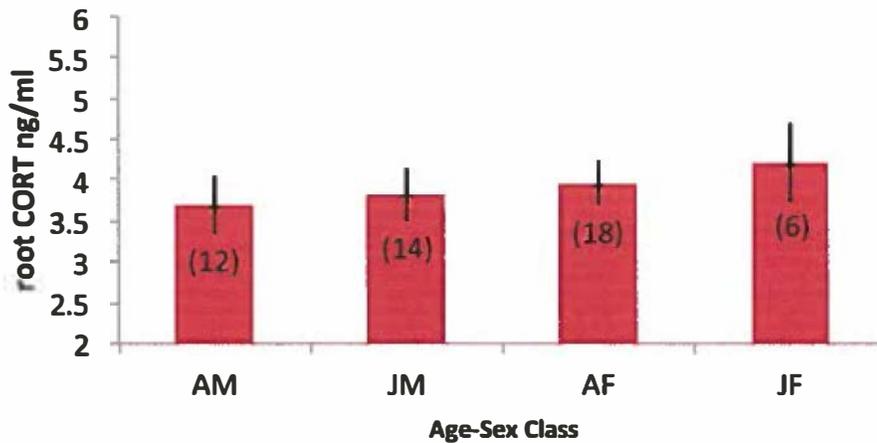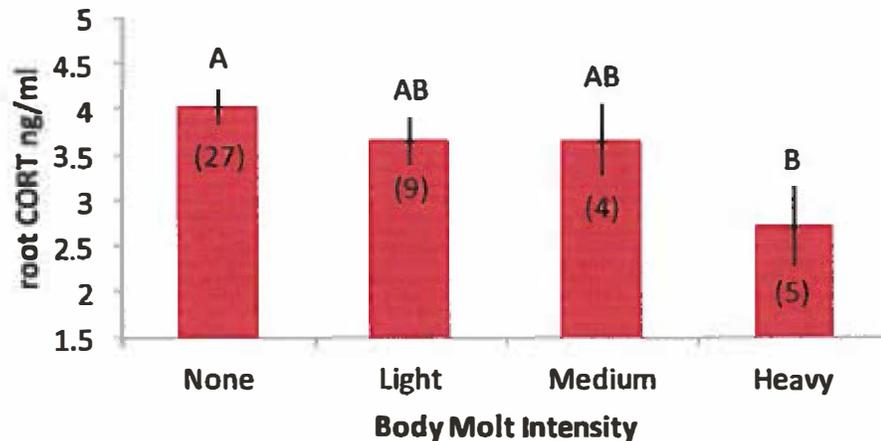
Fig. 6. The influence of molt intensity on stress-induced CORT levels regardless of Goldfinch age-sex classes ($p = 0.05$). Body molt classifications include the following: None (mean $= 4.02 \pm 0.20$ SE, 1.14 SD), Light (mean $= 3.63 \pm 0.26$, 0.87 SD), Medium (mean $= 3.64 \pm 0.38$, 0.76 SD), and Heavy (mean $= 2.69 \pm 0.43$, 1.07 SD). Numbers in parentheses designate 30-min plasma CORT samples from individuals that displayed body molt sufficient to fall under one of the four categories. Letters designate the statistically significant difference between None (A) and Heavy (B) molt intensity as it differences to stress-induced responsiveness.

Discussion

Age-Sex Class Hypothesis:

Our findings did not support the hypothesis that differences in basal and stress-induced plasma CORT levels may be the underlying mechanism of partial migration in Goldfinches. Although there was a statistical trend showing on average adult males had higher basal CORT levels than juvenile males, the results do not indicate that CORT levels in age-sex class alone are the primary cause for the latitudinal distribution reported in Prescott and Middleston's (1990) study. It is possible, however, that these weak differences between adult and juvenile male CORT levels are physiologically significant, and may become statistically significant if a larger sample size were analyzed to determine specific CORT profiles among age and sex classes of Goldfinches.

We observed little variation between average basal CORT and average stress-induced CORT levels across age and sex classes. While our residuals (Figure 2.) confirmed that juvenile males were the age-sex class that had the lowest body condition, it is interesting that this group's stress-induced CORT profiles did not differ significantly from the adults, given that the two measurements are often correlated in wild birds (Breuner and Hahn 2003). Our inability to determine migratory and sedentary status of captured individuals may also have contributed to our lack of a statistically significance difference between average basal and stress-induced CORT profiles for each age-sex class. Furthermore, baseline CORT was often extremely low and near the minimum detectability value of the assay. It may be that the lack of sensitivity to detect extremely low basal concentrations obscured important differences. If this is the case, age-sex class may be a more important predictor of stress-responsiveness than what our current findings indicated. More description of individual and age-sex class movements in years of variable environmental conditions may reveal distinctions in CORT levels previously overlooked between adult males and juvenile males.

Seasonal Effects on CORT:

Despite the lack of significance in differences in baseline CORT across age and sex classes, basal samples during the late breeding (Aug) and migratory phases (Sept-Nov) did in fact show clear differences that coincided with two distinct life history stages. Higher basal CORT concentrations during the breeding season and lower CORT concentrations during the autumn feather molt is a common seasonal pattern observed in other birds, not just Goldfinches, suggesting that individuals modulate their stress response seasonally (Romero 2002; Astheimer et al.1994). One explanation for these

seasonal patterns may be the active suppression of CORT release during the feather

molting stage to protect developing feathers from the protein catabolizing effects of

CORT (Cornelius et al 2011). Our data showed individuals in heavy body molt had lower

CORT levels compared to individuals in lighter stages of molt or non-molters.

These findings suggest that Goldfinches have evolved a mechanism that allows

them to down regulate plasma CORT during the molting phase (Romero et al. 2005;

DesRochers et al. 2009). In comparison, Red Crossbills and Zebra Finches rely on a

different physiological trade-offs (i.e. slower feather growth, production of higher quality

feathers) that better suits their nomadic tendencies, foraging for an unpredictable food

source, and opportunistic breeding cycles (Cornelius et al. 2011). Despite being close

relatives to Goldfinches, Crossbills do not suppress CORT response during molt, which

suggests the metabolic demands on a nomadic facultative migrant that breeds

opportunistically, are unlikely to impose similar constraints on a partial migratory species

(Cornelius and Hahn 2012). The suppression of CORT while molting may be an

adaptation by partial migrants to optimize energy expenditure specific to a particular life

history strategy. Crossbills fitness may be adversely affected by a similar metabolic

tradeoff. Indeed, American Goldfinches grow feathers more rapidly than crossbills and

may therefore need to protect stores from CORT to a greater degree than do Crossbills

(Cornelius et al. 2011; Middleton 1990).

Physical Fitness:

Because of the role CORT plays in mobilizing energy stores and physiological

responses, it may also function as a mediator of energetic tradeoffs associated with life

history stages and the various strategies implemented to reduce metabolic costs (French

et al. 2007). Therefore, differences in CORT levels may reflect variations in physical condition that could affect a bird's decision to migrate (Cornelius et al. 2013; Long and Holberton 2004). The correlation we observed between poor body condition and elevated 30-min CORT levels may indicate that physical fitness is a determinant of stress responsiveness in Goldfinches. Although baseline CORT did not show a similar trend, our findings for stress-induced CORT are consistent with another study that investigated the relationship between 30-min CORT levels and body condition across finch species. Body condition for Goldfinches and Pine Siskins (*Carduelis pinus*) correlated with magnitude of stress response, which was indicative of elevated plasma CORT levels with the exception of the Purple Finch (*Haemorhous purpureus*) (Knutie and Pereyra 2012).

It is likely that body condition is driving CORT variations. Testing seasonal body condition of Black-legged Kittiwakes, (*Rissa tridactyla)* demonstrated how declines in physical condition during breeding caused elevated basal CORT, and consequently was the reason these birds were more susceptible to acute stress (Kitaysky et al. 1991). However it may be just as likely that elevated CORT induces detrimental effects on body condition through influence on protein stores (Asthiemer et al. 1992). One of the specimen we encountered in our study had very poor body condition and very high baseline CORT levels. It is possible that chronically elevated CORT levels induced a maladaptive response that severely impacted the individual's fitness (Blas et al. 2005; Wingfield and Romero 2011). Initially we suspected this outlier in our data set was driving the significant trend between body condition and induced CORT (Figure 1). However, Cook's test confirmed that this data point was not disproportionately influencing the statistical significance of the relationship between body condition and

induced CORT levels. Goldfinches with this biological imperative high stress-induced CORT and low body condition should take precedence in future research exploring how CORT as a mediator of energetic tradeoffs between molt physiology and physical fitness.

Our data demonstrate that Goldfinches respond to stressors with strong HPA activity. However, the theoretical stressors that induce migration through HPA activity are yet to be determined. Daily energy demands, degradation of muscle tissue, chronic stress, or general life history stages such as breeding and molting are potential physiological and phonological stressors that could simultaneously alter sensitive HPA activity and induce basal CORT levels in an individual. We therefore consider three possible alternative hypotheses that may better explain what mechanism is driving the underlying partial migratory patterns of the American Goldfinch.

Alternative Hypotheses:

Condition Dependent-Hypothesis:

The premise of the Condition-Dependent Hypothesis is that condition-dependent variables may alter behavioral patterns and therefore influence migrant status/activity (Boyle 2008). For example, partial migratory tendencies may evolve if the breeding grounds do not have the capacity to support the full population during the non-breeding season. Certain individuals within the population may have adapted a specific strategy to avoid competition for limited resources such as food and habitat/territory (Fudickar et al. 2013). This hypothesis is one possible explanation for the latitudinal variation in winter range among Goldfinches. However, it does not explain why juvenile males might remain in northern breeding grounds while adults and juveniles females tend to migrate further south (Prescott and Middleston 1990).

To satisfy the conditions of this hypothesis, we would expect juveniles to display migratory tendencies more readily than the adults because of high HPA activity that would induce a migratory response through elevated CORT levels. Based on this alternative hypothesis, birds with high body condition and lower HPA activity would not be as inclined to migrate because their low CORT profiles would not induce the physiological and behavioral drive underlying the migratory response. This hypothesis this does not support with Prescott's and Middleston's (1990) findings, which determined the adult males (i.e., those in better body condition in our study migrate further south as opposed to the juvenile males. That is if this migratory pattern is still pertinent to its scientific niche, which has not since been verified.

Dominance Hypothesis

A second alternative hypothesis is that one group, usually older individuals, vies for food resources that are scarce and eventually out-competes subordinate groups (the Dominance Hypothesis). Dominant individuals would thus remain in the vicinity of their breeding grounds year round while the other groups are forced to migrate to other areas in search of food. Again, this hypothesis is not likely to support the migratory pattern of juvenile males wintering north of adults, since this age-sex class would be considered the subordinate group based on a vast literature in birds demonstrating juvenile subordinate behavior (Chappman et al. 2011; Catry et al. 2004; Pérez-Tris and Tellería 2002). We would not expect juveniles to be capable of out-competing adult males for limited resources, and therefore, the Dominance Hypothesis is likely to be supported (Fudickar et al. 2013; Ketterson and Nolan 1976). While juveniles generally have lower body condition than adult males, it does not explain why the subordinate group remains in the

northern breeding grounds while the dominant group is displaced further south to winter.

Arrival-Time Hypothesis

Lastly, the Arrival-Time Hypothesis suggests that the sex experiencing more intense intraspecific completion for territories is more likely to benefit from arriving earlier to breeding grounds (Bai et al. 2011). One explanation for why juveniles winter closer to their breeding grounds, may be to maximize reproductive success for the upcoming year by arriving early to these sites before adult males return form their over-winter destination (Ketterson and Nolan 1976). This hypothesis makes sense in light of the fact that juvenile males, being subordinate to adult males, may be at a disadvantage in locating and defending a high- quality breeding territory. Conversely, this hypothesis also predicts that adult males are less likely to migrate otherwise they run the risk of losing holds on breeding territories to other competitors (i.e., other adult males and juvenile males) (Boyle et al. 2008). Studies on Goldfinch territoriality do not definitively refute or support findings of extensive agonistic behavior within intraspecific and interspecific interactions (Coutlee 1967). In order to directly test this hypothesis more data concerning juvenile male migratory and reproductive strategies is required to analyze the extent of Goldfinch territoriality and the distinct strategies implemented by different age-sex classes to optimize breeding season.

Conclusions

We found no definitive evidence demonstrating CORT is driving the previously reported age-sex class differences in winter range observed in the partial migrant the American Goldfinch. Important differences among age-sex class CORT levels, however, may have been obscured in our experimental protocol given our inability to accurately

determine which individuals were seasonal migrants compared to those that were year round residents. Further research investigating potential underlying CORT driven mechanisms that influence migratory physiology and behavior is crucial to determine hormonal responsiveness during distinct life history stages. The decision to migrate essentially dictates where migratory populations will exist; how disturbances in seasonal patterns and disassociations with important environmental cues negatively impact the distribution as well as movement strategies/pathways of migratory birds. These earlier shifts uncouple migration phenology: altering ecological, agricultural, and even pathological processes, which are a consequence of anthropogenic stressors exacerbating the effects of global climate change.

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Appendix 1

The following protocol was taken directly from the Corticosterone EIA Kit Catalog No.

ADI-900-097 96 Well Kit Assay Design Booklet.

Sampling Handling

Materials:

1. CORT Standard to allow extraction efficiency to be accurately determined
2. ACS Grade Ethyl Acetate
3. Glass test tubes

Procedure:

1. Add sufficient Corticosterone to a typical sample for determination of extraction efficiency.
2. In a fume hood, add 1 mL of Ethyl Acetate for every mL of sample. Stopper and shake sample.
3. Allow layers to separate. Carefully pipet off the top organic layer and place in a clean test tube.
4. Repeat steps 1 and 2 twice more, combining the organic layers.
5. Evaporate the Ethyl Acetate to dryness under nitrogen.
6. Dissolve the extracted Corticosterone with at least 250 µL of Assay Buffer 15. Vortex well then allow to sit for five minutes at room temperature. Repeat twice more.
7. Run the reconstituted samples in the assay immediately or keep the dried samples frozen below -20°C in desiccation.

Reagent Preparation

1. Just before use, prepare the Assay Buffer 15 by diluting 10 mL of the supplied concentrate with 90 mL of deionized water. Discard unused buffer or add up to 0.09% sodium azide (w/v) for storage.

2. Corticosterone Standard Allow the 200,000 pg/mL Corticosterone standard solution to warm to room temperature. Label five 12 x 75 mm glass tubes #1 through #5. Pipet 1,000 µL of standard diluent (Assay Buffer 15 or Tissue Culture Media) into tube #1. Pipet 800 µL of standard diluent (Assay Buffer 15 or Tissue Culture Media) into tubes #2 through #5. Remove 100 µL of diluent from tube #1. Add 100 µL of the 200,000 pg/mL standard to tube #1. Vortex thoroughly. Add 200 µL of tube #1 to tube #2 and vortex thoroughly. Add 200 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 and #5. The concentration of Corticosterone in tubes #1 through #5 will be 20,000, 4,000, 800, 160 and 32 pg/mL respectively. See the Corticosterone Assay

Layout Sheet for dilution details. Diluted standards should be used within 60 minutes of preparation.

3. Wash Buffer Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening. All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4°C.
2. Pipet 100 µL of standard diluent (Assay Buffer 15 or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 µL of Standards #1 through #5 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Pipet 50 µL of Assay Buffer 15 into the NSB wells.
6. Pipet 50 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of yellow Antibody into each well, except the Blank, TA and NSB wells.
NOTE: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.
8. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 washes.
10. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 µL of the blue Conjugate to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.
13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, prefer- ably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.