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Corticosterone negative feedback is weaker during spring vs. autumn migration in a songbird (*Junco hyemalis*)

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ABSTRACT

Birds face many challenges during seasonal migrations and must make important decisions about whether to accelerate, maintain, or delay travel to their final destinations. Spring migration is likely more challenging than autumn migration as spring journeys are completed more quickly and weather conditions are harsher during this time. These differential challenges may be reflected in the endocrine stress response, as the hypothalamic–pituitary-adrenal (HPA) axis is important for both daily energetic needs and coping with stressors. Indeed, most avian studies have found that both baseline and stress-induced corticosterone (CORT) levels tend to be higher in spring migrants than in autumn migrants. We hypothesized that CORT negative feedback efficacy also differs across the season, and is likely weaker during times of year when birds must be most sensitive to stressors. We therefore predicted that CORT negative feedback efficacy would be weaker during spring vs. autumn migration as spring migrants are more likely to encounter situations where they must decide whether to turn back or delay their travel. We examined male dark-eyed juncos (*Junco hyemalis*) during their spring and autumn stopovers in Fargo, ND, USA. Our prediction was met as we did find that negative feedback efficacy was weaker during spring vs. autumn, although we notably did not find any seasonal differences in baseline and stress-induced CORT. We also found that spring migrants were heavier, had greater subcutaneous fat stores, and had slightly higher hematocrit compared to autumn migrants. These findings suggest that CORT negative feedback sensitivity may help migrating birds effectively cope with the differential challenges of autumn and spring migration.

1. Introduction

To cope with seasonal fluctuations of key resources and environmental conditions, a large portion of avian species undergo biannual migrations (Lack, 1968). These migratory journeys can range up to several thousand kilometers, and thus provide substantial energetic challenges (Wikelski et al., 2003). Migration can be separated into several stages including preparation, flight, stopovers, and arrival. During migratory preparation, birds undergo several physiological and behavioral changes to facilitate flight ability (e.g. flight muscle hypertrophy), energy storage (e.g. hyperphagia, increased subcutaneous fat stores), and departure (e.g. nocturnal migratory restlessness). Similar processes occur during stopovers, as migrants use these time periods to rest and replenish their energy stores before continuing their migratory journeys (Ramenofsky and Wingfield, 2007). At stopover sites, birds also must assess weather conditions to decide whether to proceed, stay, or reverse direction. As inclement weather may cause migrants to move towards lower latitudes, this would delay and accelerate journeys for spring and autumn migrants, respectively.

Spring migrations likely are more challenging than autumn migrations due to a number of reasons. First, migrants have been observed to complete their journeys in a shorter period of time in the spring than in the autumn, mostly by decreasing time spent at stopovers (Nilsson et al., 2013; Schmaljohann, 2018), but also by increasing flight speed (Horton et al., 2016; Karlsson et al., 2012; Nilsson et al., 2013). This suggests that spring migrants use energy at higher rates than autumn migrants. A more rapid transit during spring is likely related with reproductive success, as early arriving migrants can increase their reproductive success by obtaining choice territories and breeding earlier (Smith and Moore, 2005; Takaki et al., 2001). Long-distance migrants that arrive too early to the breeding grounds, however, face decreased survival rates (Lerche-Jørgensen et al., 2018), likely due to the unpredictable weather conditions of early spring. This tradeoff between reproductive success and survival can be more effectively balanced if

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spring migrants are sensitive to environmental conditions (Lattin et al., 2015). Challenging environmental conditions are encountered during migratory journeys as well, and are likely more harsh in the spring as migrants move towards their breeding grounds at higher latitudes, rather than in the autumn when migrants move toward their overwintering grounds at lower latitudes (King et al., 1963; Lack, 1960; Newton, 2007; Sandberg and Moore, 1996). This is evidenced by the fact that mass mortality events have been reported more frequently during spring migration than autumn migration (27 vs. 8 reported events, respectively) (Newton, 2007). In general, these findings strongly indicate that avian migrants face greater daily energetic demands and unpredictable conditions during their journeys in the spring than in the autumn.

The differential challenges between spring and autumn migration may be reflected in the endocrine stress response, as this system facilitates effective physiological processes and behavioral decisions in response to perturbations (Romero et al., 2017; Sapolsky et al., 2000). The endocrine stress response is regulated by the hypothalamic–pituitary-adrenal (HPA) axis, which in birds ultimately results in secretion of corticosterone (CORT). CORT interacts with several different physiological systems and plays important roles in energy use, metabolism, and stress reactivity. At baseline levels, CORT mostly has permissive effects on metabolism and is generally linked with current energy use (Sapolsky et al., 2000). After confronted with a stressor, CORT concentrations elevate to “stress-induced” levels which help mobilize energy stores, suppress unnecessary functions (e.g. reproduction), and increase sensitivity to catecholamines (Sapolsky et al., 2000). Several studies have found both higher baseline and stress-induced CORT levels in spring vs. autumn migrants (Liu and Swanson, 2015; Loshchagina et al., 2018; Piersma and Ramenofsky, 1998; Raja-aho et al., 2013; Romero et al., 1997), which suggests that higher CORT levels may facilitate increased energy utilization and greater responsivity to stressors.

Negative feedback is another important component of the stress response, as it causes the decrease of CORT after stressful encounters. In general, weak negative feedback has been thought to be maladaptive as it increases an animal’s total CORT exposure, which over time can result in stress-related pathologies (Romero and Wikelski, 2010). However, weaker negative feedback may play an adaptive role during times of the year when animals need to make critical decisions. For example, during an early spring snowstorm a weak negative feedback response will result in increased exposure to CORT, which may cause breeding birds to abandon their clutches and thus forgo their current reproductive effort in favor of maximizing personal survival and future reproduction (Breuner and Hahn, 2003; Lattin et al., 2015; Wingfield et al., 2017). To our knowledge, however, there are no studies examining whether or how negative feedback differs between autumn and spring migration. We hypothesized that negative feedback is weaker during the migratory period with more inclement and unpredictable weather, as enhanced exposure to glucocorticoids may help individuals adaptively respond to the more unpredictable conditions.

We measured baseline CORT, stress-induced CORT, and negative feedback efficacy of male dark-eyed juncos (*Junco hyemalis*) during their autumn and spring migratory stopovers in Fargo, ND, USA. Based on prior studies that have compared CORT levels between autumn and spring migration, we predicted that dark-eyed junco migrants would have higher baseline and stress-induced CORT in spring than in autumn. Further, based on our hypothesis that weaker negative feedback efficacy would enable migrants to more effectively respond to inclement weather events, we predicted that CORT negative feedback efficacy would be weaker during spring vs. autumn migration.

2. Material and methods

2.1. Study animals

Slate-colored dark-eyed juncos (*Junco hyemalis hyemalis*) were captured during their autumn and spring migratory stopovers in Fargo, ND (46°52′N, 96°47′). As juncos do not breed in Fargo, ND (Nolan et al. 2002; T. Greives, personal observation), we are confident that all captured individuals were migrants. Autumn migrants were captured from October 13, 2015 through November 1, 2015, and spring migrants were captured in the following spring from March 22, 2016 through April 12, 2016. By examining relative junco abundance reported to eBird (Sullivan et al. 2009), we confirmed these dates corresponded with peak migratory activity in Fargo. We also verified that temperatures were colder and wind gust speeds were faster during spring vs. autumnal stopovers in Fargo, ND (Table S1).

We identified males and females based on their plumage characteristics (Ketterson and Nolan, 1976; Nolan et al., 2002). Because we e juncos (autumn: $N = 5$, spring: $N = 7$) we restricted our analysis to males. As female juncos winter further south than male juncos (Ketterson and Nolan, 1982), females may have migrated through Fargo earlier than males during the autumn, but later than males during the spring. If this was the case, our sampling periods could have missed peak female activity, and hence resulted in a low female sample size.

Young males (< 1 year old) and old males (1 + years old) were identified via plumage characteristics and iris color (Ketterson, 1979). While young males made up 74% of autumn migrants but only 58% of spring migrants, the proportion of younger and older males did not significantly differ between autumn and spring migration ($x^2_{(1)} = 1.27$, $p = 0.26$). As stress response and morphological variables did not significantly differ with age (Table S2–S3), we therefore examined all males together in our endocrine and morphological analyses.

2.2. Trapping and sampling

Juncos were trapped with baited Potter traps starting ~1 h after sunrise and ending ~5 h later. Although baseline CORT levels do show diel rhythms in wild birds (Rich and Romero, 2001; Romero and Remage-Healey, 2000), we verified that capture time did not significantly correlate with baseline CORT concentrations in this study (Spearman’s rank order correlation, $r_s(43) = -0.24$, $p = 0.11$; data not shown).

Traps were continually monitored and as soon as the trap door shut, a baseline blood sample (~60 μL) was taken within 3 min of capture (Romero and Reed, 2005). Juncos were then weighed to the nearest 0.1 g, placed in opaque cloth bags, and held for 30 min after which a stress-induced sample was taken (~30 μL) to assess how much CORT birds secreted in response to the stress of capture and restraint (Schoech et al., 1999). Immediately after collecting the stress-induced blood sample, we then injected juncos intramuscularly in the right pectoralis with a 1 mg/kg of body weight dose of dexamethasone (DEX), a synthetic glucocorticoid used to stimulate negative feedback. A final blood sample (~60 μL) was then taken 90 min later (Bauer et al., 2016). Heparinized microhematocrit capillary tubes were used to collect blood samples from the alar vein.

Blood was stored on ice for no more than 9 h before centrifugation at 10,600 g for 10 min. Plasma was then drawn off and stored at −80 °C until further analysis. Hematocrit was measured as the ratio of packed red blood cells to total blood volume. Birds were transported to the lab and euthanized via isoflurane overdose. Furcular fat mass, the left pectoralis (not injected with DEX), and the heart were dissected out and weighed to the nearest 0.0001 g within 15 min of euthanasia. While juncos had significant fat masses in the abdominal region and elsewhere, we chose to solely examine furcular fat due to time pressure. We do note, however, that visual furcular and abdominal fat scores were similar, and that furcular fat subjective scores correlated well with the actual, dissected mass (data not shown). While testes were too small to measure in autumn migrants, spring migrants did have distinguishable testes (5.3 ± 0.4 mg, mean ± SEM). Additional organs were collected for other studies. For all variables except baseline CORT and hematocrit (due to sample loss during centrifugation), sample sizes were $N = 23$ and $N = 24$ for autumn and spring males, respectively. For baseline CORT and hematocrit, autumn and spring sample sizes were $N = 21$ and $N = 24$, respectively. Samples were collected under the appropriate permits from the U.S. Fish and Wildlife Service, and the North Dakota Game and Fish Department. All protocols were approved and carried out according to the North Dakota State University Institutional Animal Care and Use Committee guidelines.

2.3. Enzyme immunoassays

CORT concentrations were measured with an enzyme immunoassay kit (#K014, Arbor Assays, Ann Arbor, MI, USA) that we had previously validated for use in dark-eyed juncos (Bauer et al., 2016). Briefly, a measured volume of plasma (~30 µL for baseline and post-DEX samples and ~20 µL for stress-induced samples) was added to 200 µL of ultra-pure water. We extracted samples two times via diethyl ether in a methanol/dry ice bath, dried extracts with N_2, and then reconstituted samples with 200 µL of assay buffer. Samples were plated in duplicate according to the kit instructions. All three of an individual's samples (baseline, stress-induced, and post-DEX CORT) were included on the same plate, and each assay plate contained an equal number of autumn and spring individuals. Reported assay sensitivity was 18.6 pg/mL. Intra- and inter-assay sensitivity were 4.8% and 14.9%, respectively.

2.4. Statistical analyses

We analyzed baseline CORT, stress-induced CORT, and negative feedback efficacy separately as these different levels of the endocrine stress response are regulated independently (Landys et al., 2006; Romero, 2006). We calculated negative feedback efficacy as the percent decrease in CORT from stress-induced levels to those 90 min after DEX injection: 100 * [(stress-induced CORT − post-DEX CORT)/stress-induced CORT] (Lattin et al., 2012). If post-DEX CORT levels were higher than stress-induced CORT levels (2 out of 47 birds), a negative feedback level of zero was assigned, as a negative feedback level of less than zero is not biologically relevant. We recognize, however, that individual variation in the timing of maximum CORT response may impact our negative feedback calculations, as some individuals may have already started declining their circulating CORT levels while others may have still been increasing at the time of DEX injection.

We used two-tailed independent-samples t-tests to analyze whether body mass, pectoralis mass, heart mass, wing chord, and hematocrit differed between autumn and spring migrants. Normality and homogeneity of variance assumptions were tested via Shapiro-Wilk and Levene's tests, respectively. Cohen's d test was used to calculate effect sizes, where 0.1 = small, 0.5 = medium, and 0.8 = large magnitudes of effect, respectively (Cohen, 1988). Mann-Whitney U tests (non-parametric equivalent of independent-samples t-tests) were used to determine whether baseline CORT, stress-induced CORT, negative feedback efficacy, furcular fat mass, and tarsus length differed between autumn and spring migrants, as transformation failed to satisfy both normality and homogeneity of variance assumptions for these data. The equation $r = Z/\sqrt{N}$ was used to calculate effect sizes, where 0.1 = small, 0.3 = medium, and 0.5 = large magnitudes of effect, respectively (Cohen, 1988). All analyses were performed in SPSS (Version 24).

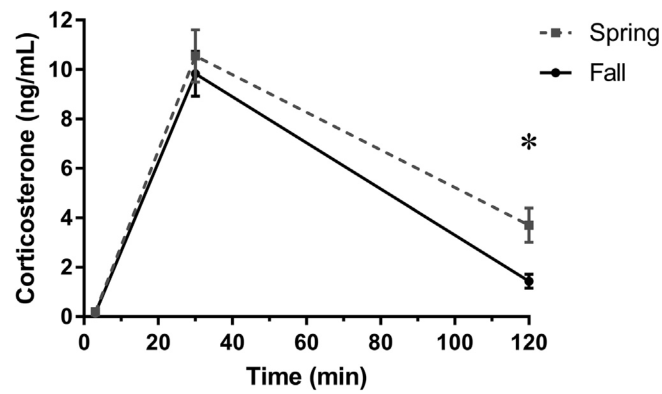

Fig. 1. Mean (±SE) plasma corticosterone levels in male dark-eyed juncos (*Junco hyemalis*) at autumn and spring migratory stopovers in Fargo, ND. Baseline levels were measured within 3 min of capture (autumn: $N = 21$, spring: $N = 24$). Stress-induced levels were measured 30 min after capture (autumn: $N = 23$, spring: $N = 24$), after which birds were injected with dexamethasone and a final sample was taken 90 min later (autumn: $N = 23$, spring: $N = 24$) to assess negative feedback efficacy. Asterisks indicate differences between autumn and spring where $p < 0.05$.

3. Results

3.1. Endocrine stress response

While neither baseline nor stress-induced CORT levels significantly differed between spring and autumn migrants (Fig. 1: $U = 184$, $p = 0.11$, $r = 0.24$; $U = 260$, $p = 0.73$, $r = 0.05$, respectively), spring migrants did have significantly weaker negative feedback compared to autumn migrants (Fig. 1: $U = 162$, $p < 0.02$, $r = 0.35$). From stress-induced levels, migrants reduced their CORT concentrations on average by 62.7 ± 6.4% in the spring vs. 82.6 ± 3.8% in the autumn. Spring and fall stress-induced CORT concentrations were 10.6 ± 1.1 ng/mL and 9.8 ± 0.9 ng/mL, respectively, and spring and fall baseline CORT concentrations were 0.19 ± 0.1 ng/mL and 0.15 ± 0.1 ng/mL, respectively. Descriptive data are means ± SEM.

3.2. Morphological measurements

Spring migrants weighed significantly more than autumn migrants (Fig. 2**a**: $t_{45} = -5.28$, $p < 0.001$, Cohen's $d = 1.54$). Spring migrants also had significantly more furcular fat (Fig. 2**b**: $U = 71$, $p < 0.0001$, $r = 0.64$) than autumn migrants but did not differ in pectoralis or heart mass (Fig. 2c-d: $t_{45} = 0.209$, $p = 0.84$, Cohen's $d = 0.06$; $t_{45} = -0.61$, $p = 0.54$, Cohen's $d = 0.18$, respectively). Spring and fall migrants also did not significantly differ in tarsus length or wing chord length (Fig. 2e-f: $U = 256$, $p = 0.67$, $r = 0.06$; $t_{45} = -0.23$, $p = 0.82$, Cohen's $d = 0.07$, respectively).

3.3. Hematocrit

Compared to autumn migrants, spring migrants showed a trend toward higher hematocrit levels (Fig. 3: $t_{43} = -2.01$, $p = 0.051$, Cohen's $d = 0.60$).

4. Discussion

We examined whether CORT negative feedback efficacy differs between spring and autumn migration in male dark-eyed juncos, as we had hypothesized that negative feedback efficacy is weaker during time periods with more challenging conditions. Our predictions were met, as we found that males had weaker negative feedback at migratory stopovers in spring than in autumn and that spring conditions were cooler and windier compared with autumn. We did not find any seasonal

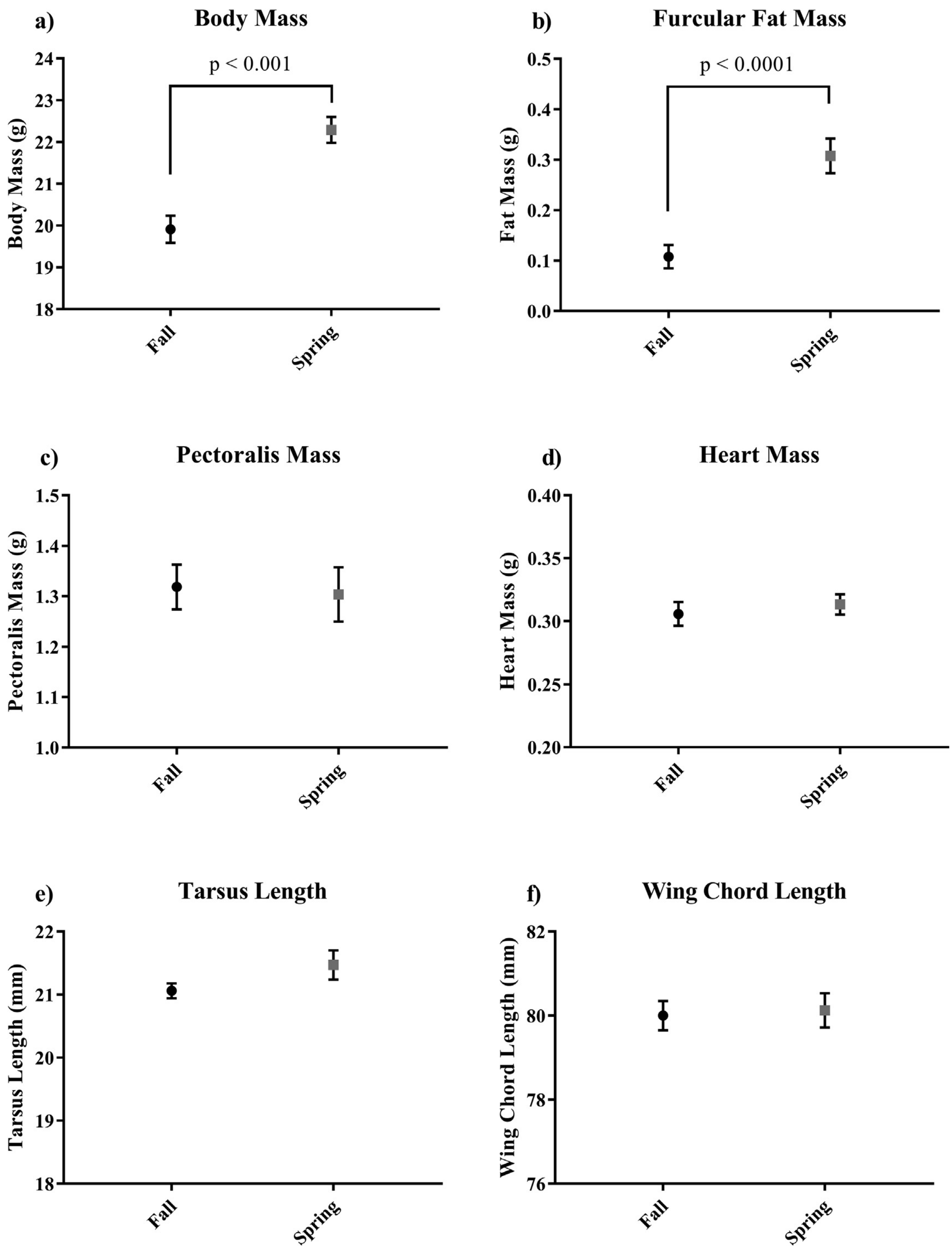

Fig. 2. Mean (±SE) morphological measurements of male dark-eyed juncos (*Junco hyemalis*) at autumn and spring migratory stopovers in Fargo, ND, including **a)** body mass, **b)** wet furcular fat mass, **c)** wet pectoralis mass, **d)** wet heart mass, **e)** tarsus length, and **f)** wing chord length (autumn: $N = 23$, spring: $N = 24$ for all measurements).

differences in baseline and stress-induced CORT, however, and therefore did not meet our predictions that baseline and stress-induced CORT would be higher during spring compared to autumn. These results suggest that the regulation of the HPA-axis varies with the differential challenges of spring and autumn migration.

While weak negative feedback efficacy increases the total amount of CORT an animal is exposed to after a stressful encounter, this may actually be a useful tactic during times of year when animals would benefit from being sensitive to stressors (Lattin et al., 2015). We predicted that migrating birds would be more sensitive to stressors during spring vs. autumn migratory stopovers, as environmental conditions are often more challenging and unpredictable during the spring and birds must make critical decisions about whether to proceed, stay, or turn back in the face of inclement weather or other challenging conditions. We also recognize that decreased negative feedback efficacy in the spring could be related to the CORT-Flexibility Hypothesis (Lattin et al. 2015). This hypothesis posits that negative feedback should be weak during early breeding as this is another critical time period when

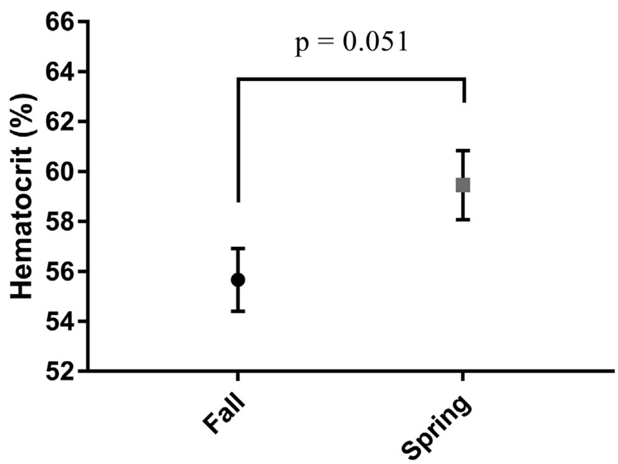

Fig. 3. Mean (±SE) hematocrit of male dark-eyed juncos (*Junco hyemalis*) at autumn and spring migratory stopovers in Fargo, ND (autumn: $N = 21$, spring: $N = 24$).

birds must make crucial decisions about whether to abandon a current breeding attempt in favor of a future one. This hypothesis was supported by Lattin et al. (2012), which demonstrated that negative feedback efficacy is weakest during the pre-breeding stage in resident house sparrows (*Passer domesticus*) compared to the rest of the year.

Our findings, however, do not indicate whether these seasonal migratory differences in negative feedback efficacy have been shaped by natural selection or are instead responses to current environmental conditions. To differentiate between these two possibilities, future studies could compare CORT negative feedback efficacy in captive birds held under the same conditions with autumn vs. spring photoperiod schedules. We also recognize that negative feedback strength is just one of many methods that may increase vertebrate's exposure to glucocorticoids. Increased glucocorticoid release, altered corticosteroid binding globulin levels, increased corticosteroid receptor abundance and distribution, and altered levels of enzymes that convert glucocorticoids to active or inactive forms (11β hydroxysteroid dehydrogenase Type I and Type II, respectively) may all increase exposure to glucocorticoids (Lattin et al., 2015). Finally, we also recognize that our method of assessing negative feedback could not differentiate between the sensitivity of the HPA-axis to high CORT levels vs. inherent CORT clearance and deactivation rates.

While we did find significant differences in negative feedback efficacy between autumn and spring migration, we did not see differences in baseline and stress-induced CORT between these time periods. Based on previous studies (Liu and Swanson, 2015; Loshchagina et al., 2018; Piersma and Ramenofsky, 1998; Raja-aho et al., 2013; Romero et al., 1997), we had originally expected that both baseline and stress-induced CORT levels would be higher during spring vs. autumn. Our results may not be in line with these studies for a variety of reasons. First, autumn vs. spring differences in baseline and stress-induced CORT may be less distinct during the stopover period compared to other stages of migration. If we confine our comparisons to studies that took place during stopovers, only three studies found significantly higher baseline and stress-induced CORT during spring vs. autumn (Liu and Swanson, 2015; Loshchagina et al., 2018; Romero et al., 1997) while two did not (Eikenaar et al., 2015; Tsipoura et al., 1999). Second, our method of trapping may have biased our sampling towards birds in the early stages of refueling, as we trapped birds via baited Potter traps while Liu and Swanson (2015), Loshchagina et al. (2018), and Romero et al. (1997) predominately used mist nets. Birds in the early stages of refueling may have been more likely to enter baited Potter traps, and some studies have found that baseline CORT levels positively relate with body fat (Eikenaar et al., 2013) and negatively relate with time until departure (Eikenaar et al., 2017, 2014). Seasonal differences in our study may have been obscured, then, if we were predominately trapping juncos during the stopover period when their CORT levels were at their nadir. Finally, the lack of a seasonal difference in baseline and stress-induced CORT levels may have been caused by the location of our stopover site along the dark-eyed junco migration route. As we may have we caught juncos during the late stages of autumn migration and the early stages of spring migration (as evidenced by low female capture rates), it is therefore possible that our autumn and spring juncos were at their highest and lowest CORT levels of their migration periods, respectively, as some studies have found that CORT levels increase over the course of both autumn (Eikenaar et al., 2017, 2018; Falsone et al., 2009; Loshchagina et al., 2018) and spring migration (O'Reilly and Wingfield, 2003; Wingfield and Farner, 1978).

Future studies should examine whether female dark-eyed juncos have different CORT responses between autumn and spring migration. While we did attempt to measure females in this study, our low sample size caused us to restrict our analysis to males. As mentioned earlier, our low female capture rate may have been related with the timing of our trapping periods, as females may predominantly migrate during the early portion of the autumn season but during the later portion of the spring season (Ketterson and Nolan, 1982). We note that other studies examining both males and females found no sex differences in baseline or stress-induced CORT (O'Reilly and Wingfield, 2003; Raja-aho et al., 2013; Romero et al., 1997), although it is unknown whether sex differences may be present in CORT negative feedback efficacy. It is difficult to predict whether the sexes would differ in CORT negative feedback strength during spring migration, as increased sensitivity to stressors may be beneficial for males as they arrive earlier to the breeding grounds when weather conditions are more unpredictable (Ketterson and Nolan, 1982), whereas females may also benefit from increased stress sensitivity as they are the ultimate decision makers of breeding initiation (Ball and Ketterson, 2008).

While our male dark-eyed juncos weighed more and had higher furcular fat stores during spring migration vs. autumn migration, we did not find any seasonal differences in pectoralis mass, heart mass, tarsus length, and wing chord length. These results suggest that body weight differences were driven more by fat deposits, rather than muscle mass or structural differences. This is not surprising, as fat may compose 7–60% of total body mass in migrant birds (Guglielmo, 2018). While fat may be stored in up to 16 different location in the avian body, we considered furcular fat deposits indicative of total body fat, as we found that furcular and abdominal visual fat scores were similar within individuals (data not shown). Greater fat deposits during spring migration is well supported in the literature, as Holzschuh and Deutschlander (2016) found that 11 out of 12 warbler species had higher fat scores during spring vs. autumn migratory stopovers. If greater fat deposits serve as an energetic buffer during unpredictable and inclement weather, then this may aid migrants during the more challenging environmental conditions of spring migration. Alternatively, seasonal differences in fat deposits may be related with seasonal differences in average flight distance between stopovers.

We also found that male dark-eyed juncos trended towards higher hematocrit during spring stopovers compared to autumn stopovers. As hematocrit increases oxygen delivery capabilities (Piersma et al., 1996), this supports our assumption that spring migration is more challenging than autumn migration. Our findings are mirrored by Krause et al. (2016), as they found male Gambel's white-crowned sparrows (*Zonotrichia leucophyrs gambelli*) had higher hematocrit levels during migratory departure in the spring compared to the autumn. Data from Morton (1994) also shows a trend towards higher hematocrit levels in male mountain white-crowned sparrows (*Zonotrichia leucophyrs oriantha*) during their spring arrival to the breeding grounds compared to their autumn migration preparation phase. However, Owen and Moore (2006) found no differences in hematocrit between spring and autumn migration in two different thrush species. Increased hematocrit levels during spring versus autumn migration, however, could be related with the upcoming challenges of breeding, but we find this unlikely as most studies see an immediate decline in hematocrit after arrival to the breeding grounds (Hatch and Smith, 2010; Krause et al., 2016; Morton, 1994; Saino et al., 1997). Future studies should further examine seasonal hematocrit differences during the stopover stage in a wide variety of avian species.

5. Conclusions

This study supports the hypothesis that migrating songbird HPA-axes are more sensitive to stressors during spring migration compared to autumn migration, as we found that male dark-eyed juncos had weaker negative feedback efficacy during spring vs. autumn stopovers. High stress-induced CORT levels can also increase sensitivity to stressors, yet we did not find increased stress-induced CORT during spring vs. autumn migration as we had originally predicted. This may suggest that birds vary their stress sensitivity via negative feedback rather than glucocorticoid release. Future studies should examine whether other components of the endocrine stress response related to glucocorticoid regulation, such as receptor abundance and 11β hydroxysteroid dehydrogenase concentrations, consistently vary between spring and autumn migration. While we had also expected to find higher baseline CORT during spring vs. autumn stopovers, we did not find any significant seasonal differences. As baseline CORT is important for metabolic activities, this may suggest that spring and autumn stopovers have similar energetic challenges. Therefore, the increased challenges of spring migration may be due to higher unpredictability of weather conditions, rather than greater energetic costs. Our study also found that migrants weighed more, had more subcutaneous fat, and slightly higher hematocrit during spring vs. autumn migration, which is mostly in line with previous studies. In conclusion, we found that male juncos display weakened CORT negative feedback during spring vs. autumn stopovers, which may suggest that spring migration is more unpredictable and challenging. Future work should further examine the seasonal profiles of CORT negative feedback efficacy in juncos and other migratory bird species, and whether these trends differ between the sexes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.04.008.

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