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# Hypothalamic–pituitary–adrenal axis activity is not elevated in a songbird (*Junco hyemalis*) preparing for migration

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ABSTRACT

During spring, increasing daylengths stimulate gonadal development in migratory birds. However, late-stage reproductive development is typically postponed until migration has been completed. The hypothalamic–pituitary–adrenal (HPA) axis regulates the secretion of glucocorticoids, which have been associated with pre-migratory hyperphagia and fattening. The HPA-axis is also known to suppress the hypothalamic–pituitary–gonadal (HPG) axis, suggesting the possibility that final transition into the breeding life history stage may be slowed by glucocorticoids. We hypothesized that greater HPA-axis activity in individuals preparing for migration may foster preparation for migration while simultaneously acting as a "brake" on the development of the HPG-axis. To test this hypothesis, we sampled baseline corticosterone (CORT), stress-induced CORT, and negative feedback efficacy of Dark-eyed Juncos (*Junco hyemalis*) in an overwintering population that included both migratory (*J.h. hyemalis*) and resident (*J.h. carolinensis*) individuals. We predicted that compared to residents, migrants would have higher baseline CORT, higher stress-induced CORT, and weaker negative feedback. Juncos were sampled in western Virginia in early March, which was about 2–4 wk before migratory departure for migrants and 4–5 wk before first clutch initiation for residents. Contrary to our predictions, we found that migrants had lower baseline and stress-induced CORT and similar negative feedback efficacy compared with residents, which suggests that delayed breeding in migrants is influenced by other physiological mechanisms. Our findings also suggest that baseline CORT is not elevated during pre-migratory fattening, as migrants had lower baseline CORT and were fatter than residents.



## 1. Introduction

By migrating, temperate-zone birds take advantage of seasonally abundant food resources during the summer months while improving their likelihood of survival by departing from locales that are harsh and resource-poor during winter (Lack, 1968). In preparation for mid- and long-distance migration, birds undergo a migratory developmental phase that includes several physiological changes such as flight muscle hypertrophy, red blood cell proliferation (erythropoiesis), and fattening (Cornelius et al., 2013; Piersma et al., 1999; Ramenofsky, 2011). Fattening, driven by hyperphagia, is especially important for migrants because fat is the main fuel utilized during extended periods of flight (Ramenofsky, 1990). Initiation of the migratory developmental phase in spring is regulated by both endogenous circannual rhythms and increasing photoperiod or daylength (Gwinner, 1996a; Ramenofsky and Wingfield, 2007). Exposure to lengthening days causes increased secretion of gonadotropin-releasing hormone (GnRH), which plays a critical role in male spring migratory development through its stimulatory effects on androgen production and release (Deviche, 1995; Owen et al., 2014; Ramenofsky and Nemeth, 2014). Increasing daylengths also stimulate secretion of other neuropeptides, whose specific roles in migratory preparation are still largely unknown (Cornelius et al., 2013; Ramenofsky, 2011).

In addition to stimulating migratory development, increasing daylengths also activate gonadal development and other physiological and behavioral changes essential for breeding (Gwinner, 1996b; Perrins, 1970; Rowan, 1926). Androgens in particular are known to have a permissive effect on pre-migratory fattening in male birds (Mattocks, 1976; Ramenofsky and Nemeth, 2014; Tonra et al., 2011). Partial reproductive development before migratory departure allows migrants to commence breeding shortly

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after arrival to the breeding grounds. Nevertheless, migrants typically delay complete reproductive maturity until migration has been completed, likely because full gonadal development could interfere with migratory performance owing to increased body mass, metabolic pathway constraints, increased lipoprotein use, and decreased hematocrit (Williams, 2012).

Final transition into the breeding life history stage could be mediated by glucocorticoid production and release, which is controlled by the hypothalamic–pituitary–adrenal (HPA) axis. Corticosterone (CORT), the main glucocorticoid in birds, has numerous physiological effects and is generally considered important for migratory development due to its permissive effects on hyperphagia and fattening (Landys et al., 2004a,b). Additionally, CORT has well-known suppressive effects on the hypothalamic–pituitary–gonadal (HPG) axis (Wingfield and Sapolsky, 2003). Therefore, CORT could be an important regulator for promoting migratory preparation and delaying full reproductive maturity until arrival at the breeding grounds. Indeed, in most bird species CORT levels increase during spring migratory preparation (Holberton et al., 2008; Romero, 2002). However, we also know that in many bird species gonadal recrudescence increases during migration (Bauchinger et al., 2007), apparently despite high CORT levels. One possible way to reconcile these observations is to suggest that gonadal recrudescence occurs during stopovers when CORT decreases (Eikenaar et al., 2014) from elevated levels during migratory flight (Falsone et al., 2009).

To determine whether increased CORT exposure during migratory preparation might act as a "brake" on the HPG-axis, we compared CORT profiles of migrant and resident Dark-eyed Juncos (*Junco hyemalis hyemalis* and *J.h. carolinensis*, respectively) that share the same overwintering site. Juncos were sampled for baseline CORT, stress-induced CORT, and negative feedback efficacy. Baseline CORT concentrations represent levels needed for everyday, predictable activities, and generally reflect current energy use (Sapolsky et al., 2000). Because baseline CORT concentrations represent CORT levels only when birds are undisturbed, we also measured stress-induced CORT to determine relative sensitivity of migrants and residents to stressors (Sapolsky et al., 2000). Negative feedback is another important component of the endocrine stress response, as strong negative feedback can quickly reduce CORT secretion, whereas weak negative feedback prolongs CORT exposure. Compared to other components of the HPA-axis, weak negative feedback is the strongest indicator of chronic stress (Dickens and Romero, 2013). By measuring baseline CORT, stress-induced CORT, and negative feedback strength, we estimated each individual's potential *total exposure* to CORT. We predicted that if CORT plays a stimulatory role in migratory preparation, then prior to migratory departure migrant birds would have higher baseline CORT, higher stress-induced CORT, weaker negative feedback, and thus higher total CORT exposure as compared to resident birds.

## 2. Methods

### 2.1. Study animals

We studied an overwintering population of Dark-eyed Juncos (*J. hyemalis*) at Mountain Lake Biological Station (37°22′N, 80°32′W), located in Giles County, western Virginia and managed by University of Virginia. We used *J. hyemalis* in this study because both resident (*J.h. carolinensis*: hereafter called "residents") and migrant (*J. h. hyemalis*: hereafter called "migrants") subspecies overwinter at Mountain Lake. On average, residents weigh more and are slightly larger than migrants, and males weigh more and are slightly larger than females (Nolan et al., 2002). Migrant males, migrant females, resident males, and resident females can be identified based on bill color, wing chord length, and plumage characteristics (Ketterson and Nolan, 1976; Nolan et al., 2002). Plumage characteristics can also be used to age juncos as young (less than 1 year old) or old (greater than 1 year old) (Ketterson, 1979). This study was carried out March 12–19, 2015, which is about 4–5 wk before first clutch initiation for residents (April 16) and 2–4 wk before migratory departure for migrants. We estimated migrant departure date based on evidence that there were very few migrants (<3% of junco population) when we returned to Mountain Lake in mid-April, and because migrants from this population showed very low levels of Zugunruhe throughout the month of March in a captive study (A. Fudickar, personal communication). During this study, both resident and migrant juncos were observed foraging in winter flocks and showed little evidence of territorial behavior.

### 2.2. Trapping and blood sampling

Birds were captured in baited Potter traps and mist-nets. Traps and nets were opened 1 h after sunrise and closed at least 2.5 h before sunset (0830–1700 h). While many vertebrates possess diel CORT rhythms (Rich and Romero, 2001; Romero and Remage-Healey, 2000), we found no relationships between baseline CORT concentrations and time of capture in our study (regression analysis, data not shown). While traps were open, 2–4 observers would scan with binoculars in order to determine time of capture. Birds were bled within 3 min of capture to obtain a baseline CORT sample (~60 μL) as CORT levels typically increase within 3 min of disturbance (Romero and Reed, 2005). Birds for which capture time could not be assigned were excluded from all CORT analyses. For a sub-set of male resident and migrant juncos, we took an additional 60 μL of blood within 5 min of capture so we could measure baseline testosterone, which would give us an estimate of relative reproductive readiness in resident and migrant males (Fudickar et al., in press). All blood samples were taken from the alar vein and were collected with heparinized, microhematocrit capillary tubes. Birds were kept in opaque, cloth bags between blood sampling time-points.

After baseline blood sample collection, birds were identified as residents or migrants, sexed, aged, banded if needed, and weighed to the nearest 0.25 g. Birds were given both abdominal and furcular subcutaneous fat scores, with a score of 0 meaning no visible fat and 5 meaning visible fat bulging (Nolan and Ketterson, 1983; O'Neal et al., 2011). Stress-induced blood samples (~30 μL) were then taken 30 min after capture, as CORT peaks around this time-point in Dark-eyed Juncos (Schoech et al., 1999). Birds were then immediately given an intramuscular injection of dexamethasone (DEX) into the pectoral muscle. DEX is a synthetic glucocorticoid and is commonly used for determining negative feedback efficacy (Dickens et al., 2009; Romero, 2004; Sapolsky and Altmann, 1991). We used a 1 mg/kg of body weight dose, as previous studies have shown this dosage to effectively suppress endogenous CORT production in songbirds (Rich and Romero, 2001). Through a validation study, we determined that juncos maximally decrease CORT production 90 min after DEX injection. Therefore, we assessed negative feedback 90 min after DEX injection in our study (Fig. 1). After this last blood sample (~60 μL), birds were then released at their site of capture.

Blood samples were kept in a cooler with icepacks for no more than 9 h before centrifugation. After centrifugation, plasma was drawn off and stored at −80° C until further analysis. Sample sizes for full stress series were: migrant males ($n = 17$), migrant females ($n = 3$), resident males ($n = 24$), and resident females ($n = 6$). Sample sizes for testosterone were: migrant males ($n = 11$) and resident males ($n = 11$). Sample sizes for body weight and fat were: migrant males ($n = 29$), migrant females ($n = 12$), resident males ($n = 32$), and resident females ($n = 9$). All procedures were

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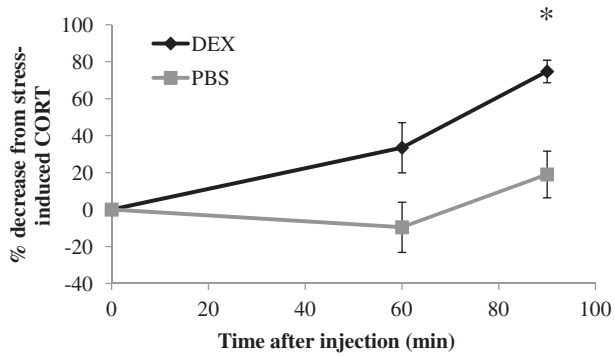


**Fig. 1.** Mean ± SE negative feedback efficacy 60 and 90 min after dexamethasone (DEX) ($n = 8$) or PBS ($n = 8$) injection in migratory Dark-eyed Juncos (*Junco hyemalis hyemalis*) captured during autumn in North Dakota, USA (45°53′N, 96°47′W). Negative feedback efficacy was calculated as the percent decrease from CORT levels 30 min after capture (stress-induced levels) to those 60 and 90 min after injection with DEX or PBS. Asterisks indicate significant differences ($p < 0.05$) between DEX and PBS-injected birds.

approved by the North Dakota State University Institutional Animal Care and Use Committee.

### 2.3. DEX validation study

Briefly, *J.h. hyemalis* individuals were trapped in Fargo, ND, USA during migration in November 2014. Stress-induced samples were taken 30 min after capture, and birds were then injected with DEX ($n = 8$) or a control phosphate buffer solution (PBS) ($n = 8$). Subsequent blood samples (~60 μL) were taken 60 and 90 min after injection. While CORT levels did not significantly change over time after injection with PBS (Fig. 1; one-way repeated measures ANOVA, $F_{2,7} = 2.65$, $p = 0.11$); CORT levels significantly decreased both 60 and 90 min after injection with DEX (Fig. 1; one-way repeated measures ANOVA $F_{2,7} = 12.16$, $p < 0.01$, LSM post-hoc tests all ps < 0.05). However, compared to PBS-injected birds, DEX-injected birds had significantly stronger negative feedback only at the 90 min time-point (Fig. 1; *t*-tests with Bonferroni corrections, 60 min $t_{(1,14)} = 2.24$, $p = 0.084$; 90 min $t_{(1,15)} = 2.80$, $p = 0.03$).

### 2.4. CORT enzyme immunoassay

We measured CORT concentrations with an enzyme immunoassay kit (#KO14, Arbor Assays, Ann Arbor, MI, USA) that had low, reported cross-reactivity with DEX (0.12%). While previous studies have successfully used this kit in songbirds (DeVries and Jawor, 2013; Pryor and Casto, 2015), we further validated the kit for use in the Dark-eyed Junco. To test for parallelism, we compared slopes between our standard curve (created from kit-provided reference standards) and serially diluted pooled junco plasma. Parallelism assumptions were met ($F_{1,10} = 5.5$, $p = 0.96$) as residual variance did not significantly increase when the two lines shared a common slope (Grotjan and Keel, 1996). Briefly, we added 20 μL plasma to 200 μL ultra-pure water. Samples were then extracted two times with diethyl ether in a methanol/dry ice bath, dried with $N_2$, and reconstituted with 200 μL assay buffer. Samples were then plated in duplicate and processed according to the manufacturer's instructions. Samples were spread across plates to balance migrant/resident status, sex, and age. Reported assay sensitivity was 18.6 pg/mL and intra- and inter-assay variation were 4.5% and 9.3%, respectively. For samples with undetectable CORT levels, we assigned them a value of 62 pg/mL, as this was the lowest level of CORT we detected among our samples.

### 2.5. Testosterone enzyme immunoassay

We measured testosterone concentrations with an enzyme immunoassay kit (#901-065, Enzo Life Sciences Inc., Farmingdale, NY, USA) that had previously been validated for use in the Dark-eyed Junco (Clotfelter et al., 2004; Rosvall et al., 2012). Briefly, we added 30 μL plasma to 200 μL ultra-pure water. Samples were then extracted two times with diethyl ether in a methanol/dry ice bath, dried with $N_2$, and reconstituted with 300 μL assay buffer. Samples were then plated in duplicate and processed according to the manufacturer's instructions. All samples were run on a single plate. Reported assay sensitivity was 5.67 pg/mL and intra-assay variation was 2.3%. For samples with undetectable testosterone levels, we assigned them a value of 10.8 pg/mL, as this was the lowest level of testosterone we detected among our samples.

### 2.6. Statistical analyses

Negative feedback efficacy was measured as the percent decrease in CORT from stress-induced levels (collected 30 min after capture) to those 90 min after DEX injection: (stress-induced CORT – post-DEX CORT)/(stress-induced CORT) * 100 (Lattin et al., 2012). Since baseline CORT, stress-induced CORT, and negative feedback have been shown to be regulated independently, we analyzed each CORT variable separately (Landys et al., 2006; Romero, 2006). Baseline CORT, stress-induced CORT, negative feedback efficacy, baseline testosterone, body weight, abdominal fat, and furcular fat were analyzed using mixed model ANOVAs. For all mixed models except testosterone, fixed effects included migrant/resident status, sex, and age (first-year/adult young or older than first-year/adult old). For testosterone, fixed effects include migrant/resident status and age. For baseline CORT, stress-induced CORT, and negative feedback analyses, EIA plate number was included as a random effect. Cohen's $f^2$, a commonly used effect size measure for mixed models (Cohen, 1988; Selya et al., 2012), was reported for all significant main effects. Cohen's $f^2$ values of 0.02, 0.15, and 0.35 are considered to represent small, medium, and large magnitudes of effect, respectively (Cohen, 1988). All mixed models were performed using the PROC MIXED procedure in SAS (Version 9.4).

## 3. Results

### 3.1. CORT and testosterone

Migrants had significantly lower baseline CORT levels compared with residents (Fig. 2a; mean ± SE, 0.2 ± 0.1 vs. 0.7 ± 0.2 ng/mL, $F_{1,42} = 4.87$, $p = 0.03$, Cohen's $f^2 = 0.09$). Baseline CORT levels were undetectable for 60% of migrants ($n = 30$) and 27% of residents ($n = 20$). However, baseline CORT did not significantly differ with sex ($F_{1,42} = 1.33$, $p = 0.25$) or age ($F_{1,42} = 2.15$, $p = 0.15$). Migrants also had significantly lower stress-induced CORT levels compared with residents (Fig. 2b; mean ± SE, 7.7 ± 1.0 vs. 20.6 ± 1.6 ng/mL, $F_{1,41} = 40.07$, $p < 0.01$, Cohen's $f^2 = 0.91$), and stress-induced CORT also did not significantly differ with sex ($F_{1,41} = 0.02$, $p = 0.88$) or age ($F_{1,41} = 3.39$, $p = 0.07$). Negative feedback efficacy did not significantly differ between migrant and resident (Fig. 2c; $F_{1,41} = 0.62$, $p = 0.44$), male and female ($F_{1,41} = 0.06$, $p = 0.44$), and adult young and adult old ($F_{1,41} = 0.00$, $p = 0.97$) birds. Resident males had significantly higher baseline testosterone levels than migrant males (Fig. 3; mean ± SE, 1.2 ± 0.4 vs. 0.1 ± 0.03 ng/mL, $F_{1,19} = 9.25$, $p < 0.01$, Cohen's $f^2 = 0.41$), although testosterone did not significantly differ with age ($F_{1,19} = 1.58$, $p = 0.22$). Baseline

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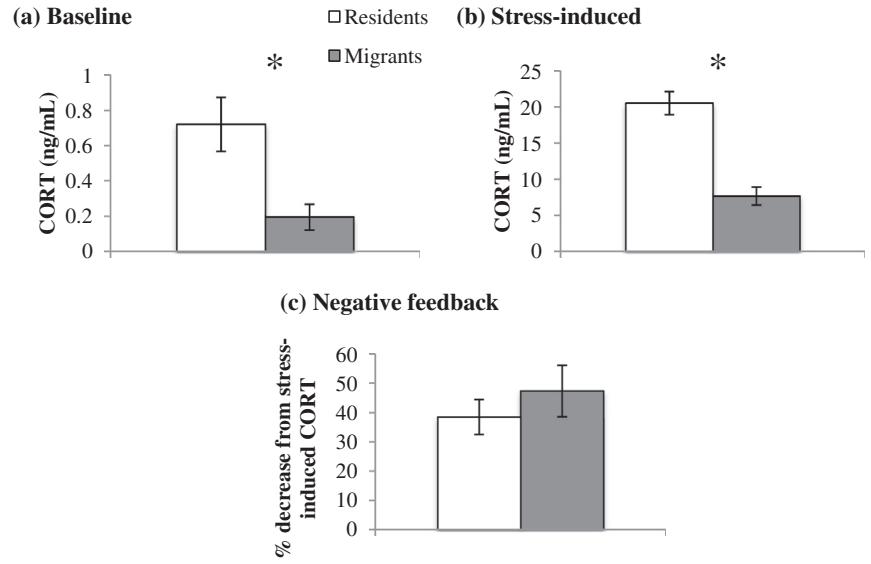


**Fig. 2.** Mean ± SE (a) baseline corticosterone (CORT) ($n$ = 20 and 30), (b) stress-induced CORT ($n$ = 18 and 31), and (c) negative feedback efficacy levels ($n$ = 18 and 31) measured in Dark-eyed Junco residents (*Junco hyemalis carolinensis*) and migrants (*J.h. hyemalis*). Birds were captured during spring in VA, USA (37°22′N, 80°31′W). Negative feedback efficacy was calculated as the percent decrease from stress-induced CORT levels to those 90 min after dexamethasone (DEX) injection. Asterisks indicate significant differences ($p$ < 0.05) between residents and migrants.

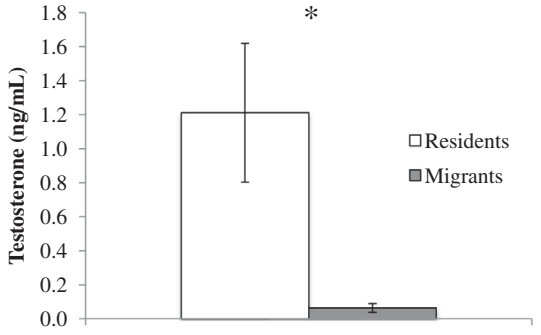


**Fig. 3.** Mean ± SE baseline plasma testosterone concentrations of male Dark-eyed Junco residents (*Junco hyemalis carolinensis*) ($n$ = 11) and migrants (*J.h. hyemalis*) ($n$ = 11), respectively. Birds were captured during spring in VA, USA (37°22′N, 80°31′W). Asterisks indicate significant differences ($p$ < 0.05) between residents and migrants.

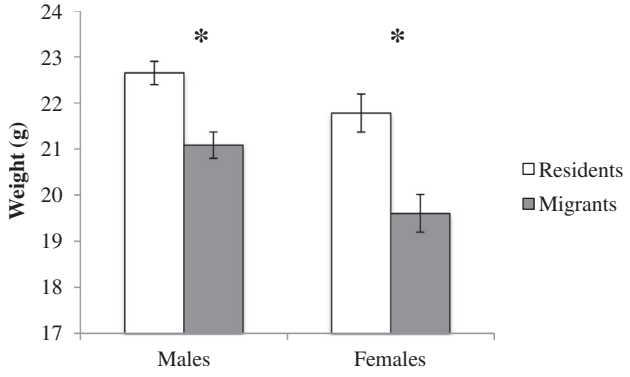


**Fig. 4.** Mean ± SE body weight of male and female Dark-eyed Junco residents (*Junco hyemalis carolinensis*) ($n$ = 32 and 9) and migrants (*J.h. hyemalis*) ($n$ = 29 and 12), respectively. Birds were captured during spring in VA, USA (37°22′N, 80°31′W). Males weighed significantly more than females. Migrants are known to be smaller-bodied than residents (Nolan et al., 2002). Asterisks indicate significant differences ($p$ < 0.05) between residents and migrants.

testosterone levels were undetectable for 18% of migrant males ($n$ = 11) and 0% of resident males ($n$ = 11).

### 3.2. Body condition

As expected based on established sub-species and sex differences in size, residents weighed significantly more than migrants (Fig. 4; $F_{1,78}$ = 27.07, $p$ < 0.01, Cohen's $f^2$ = 0.33), and males weighed significantly more than females (Fig. 4; mean ± SE, resident males: 22.7 ± 0.3 g, resident females: 22.3 ± 0.4 g, migrant males: 21.7 ± 0.5 g, migrant females: 20.3 ± 0.5 g, $F_{1,78}$ = 8.43, $p$ < 0.01, Cohen's $f^2$ = 0.09). Adult young and old birds did not significantly differ in weight ($F_{1,78}$ = 0.22, $p$ = 0.64). Compared with residents, migrants had significantly more abdominal and furcular fat (Fig. 5a; mean ± SE, 2.8 ± 0.4 vs. 1.2 ± 0.3, abdominal fat: $F_{1,78}$ = 11.52, $p$ < 0.01, Cohen's $f^2$ = 0.13; Fig. 5b; mean ± SE, 2.9 ± 0.3 vs. 1.3 ± 0.3, furcular fat: $F_{1,78}$ = 15.56, $p$ < 0.01, Cohen's $f^2$ = 0.18). Neither abdominal nor furcular fat differed significantly with sex (abdominal fat: $F_{1,78}$ = 0.45, $p$ = 0.51; furcular fat: $F_{1,78}$ = 1.84, $p$ = 0.18) or age (abdominal fat: $F_{1,78}$ = 0.03, $p$ = 0.86; furcular fat: ($F_{1,78}$ = 0.00, $p$ = 0.96).

## 4. Discussion

The purpose of this comparative study was to determine whether heightened HPA-axis activity during spring migratory development acts as a "brake" on the HPG-axis in birds preparing to migrate. Counter to expectation, we found that within an over-wintering population of *J. hyemalis*, migrant individuals had less-reactive HPA-axes than resident individuals prior to migratory departure. Therefore, our results suggest that the HPA-axis is not responsible for delayed reproductive maturation during spring migratory development.

Migrants were observed to have significantly greater fat stores compared with residents, thus suggesting that migratory development was well under way. Despite apparent evidence that these individuals were undergoing migratory preparation, their baseline and stress-induced CORT levels were lower than resident individuals. One possibility may be that we sampled migrant juncos too early during migratory development (2–4 wk before departure) to see heightened HPA-axis activity, although Holberton et al.

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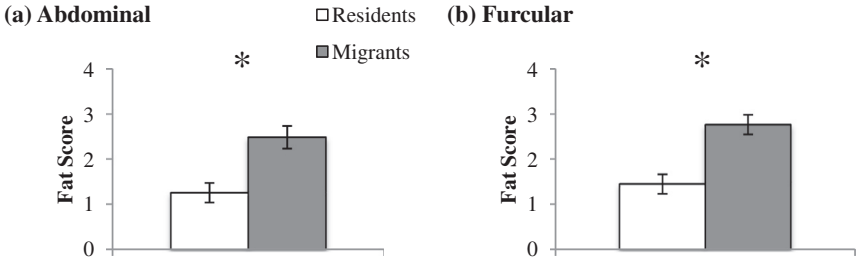


**Fig. 5.** Mean ± SE (a) abdominal and (b) furcular fat scores of Dark-eyed Junco residents (*Junco hyemalis carolinensis*) ($n$ = 41) and migrants (*J.h. hyemalis*) ($n$ = 41). Fats scores ranged from 0 (no visible fat) to 5 (visible fat bulging). Birds were captured during spring in VA, USA (37°22′N, 80°31′W). Asterisks indicate significant differences ($p < 0.05$) between residents and migrants.

(2008) found that captive Dark-eyed Juncos increased baseline CORT in concert with fat scores. Furthermore, a similar migrant vs. resident junco study in captivity found that prior to anticipated migratory departure, migrants had higher fat scores but lower baseline CORT concentrations compared to residents (Fudickar et al., in press). It is also possible that migrant and resident juncos have inherently different HPA-axes; future studies should measure winter CORT profiles of resident and migrant juncos to assess the relative increase in CORT from winter to pre-migration, which may be greater in migrants than residents. While this alternative is possible, it seems unlikely given how low baseline CORT levels already were in migrants.

Lower baseline and stress-induced CORT levels in migrants versus residents could also be caused by up-regulation of the HPA-axis prior to breeding (Eikenaar et al., 2015), as residents were at least a few weeks closer to breeding than migrants, and previous studies have found baseline CORT levels significantly increase from winter to pre-breeding (Hegner and Wingfield, 1990 but see Lattin et al., 2012). However, past studies have also shown that testosterone levels and testes size start to increase 1–2 mo before CORT levels start to increase from winter levels (Hegner and Wingfield, 1990). Given that all juncos were still in their winter flocks and showed little signs of territorial behavior, we doubt that pre-breeding up-regulation of baseline and stress-induced CORT levels explain higher CORT levels in residents vs. migrants. Future studies should also better determine pre-migratory CORT profiles of migrant and resident female juncos. While our study found no significant CORT or negative feedback differences between sexes, our female sample size was very small ($n$ = 3 and 6 for migrant and resident females, respectively).

Additionally, while migrant juncos did not have higher circulating levels of CORT or weaker negative feedback compared to resident juncos, downstream components of the migrant HPA-axis could have caused migrants to be more sensitive to stress. For example, altered concentrations of glucocorticoid receptors, corticosteroid binding globulin (CBG), and 11ß-hydroxysteroid dehydrogenase (11ß-HSD) could all affect stress reactivity (Sapolsky et al., 2000). There is increasing evidence that seasonal changes in downstream components of the HPA-axis may contribute to increased stress sensitivity before breeding (Lattin et al., 2012, 2015), thus helping birds optimally time breeding initiation.

Several other physiological mechanisms may mediate delayed reproductive development in migrants. One possibility is that migrants may require longer daylengths than residents to initiate reproductive development, as other studies have found population differences in photoperiodic thresholds (Lambrechts et al., 1997; Lambrechts et al., 1996; Silverin et al., 1993). An increased photoperiodic threshold in migrants could cause them to start gonadal recrudescence and reproductive development later than residents, which is supported by our finding that resident males had significantly higher testosterone levels than migrant males during this study. Alternatively, migrants and residents may share the same photoperiodic threshold but differ in the rate of later-stage gonadal recrudescence and reproductive maturation (Caro et al., 2005, 2006). Later-stage reproductive development could also be contingent on the relative rate of increasing photoperiod, as migrants will experience rapidly increasing daylengths as they migrate toward higher latitudes. However, evidence suggests that gonadal recrudescence rates in birds are driven by current photoperiod, rather than the rate or change in direction of photoperiod (Dawson, 2015). Delayed reproductive development in migrants could also be mediated by gonadotropin-inhibiting hormone (GnIH) either through increased GnIH secretion or GnIH receptor concentrations (Kriegsfeld et al., 2015). Thyroid hormones could also play a role in delayed reproductive development, as thyroid hormone levels have been positively associated with fattening, flight muscle hypertrophy, and migratory restlessness (Bishop et al., 2000; Pant and Chandolasaklani, 1993). However, studies have reported both suppressive and permissive effects of the thyroid hormones on reproductive development in seasonally breeding birds (Dawson et al., 2001).

Reported baseline CORT levels in this study were surprisingly low, as many sample concentrations were less than 1 ng/mL. Only one other avian study has measured baseline CORT using this same kit (DetectX Corticosterone EIA Kit, Arbor Assays, Ann Arbor, MI, USA), but their lowest reported CORT concentration was 3.08 ng/mL (Pryor and Casto, 2015), which is more typical of other studies. However, our results may differ from Pryor and Casto (2015) because they used the kit-supplied dissociation reagent whereas we did a double-extraction with diethyl ether. Therefore, lower-calculated CORT concentrations may be specific to the EIA kit we used, as antibody and protocol differences can significantly alter measured hormone concentrations (Brown et al., 2003; Lattin et al., 2011), which is why different studies should not directly compare hormone concentrations (Brown et al., 2003). Lower measures of CORT caused by kit differences are also supported by the fact that our stress-induced CORT measurements are in the lower range of other studies in wild birds. Regardless, these "lower" CORT measurements should not matter since migrant/resident status, sex, and age were evenly distributed among plates.

Contrary to previous studies, our results found that baseline CORT is not elevated during migratory preparation since migrants had lower baseline CORT and higher fat scores compared with residents. However, studies that have reported positive relationships between baseline CORT and pre-migratory hyperphagia and fattening have predominately been correlative (Fudickar et al., 2013; Holberton, 1999; Holberton et al., 1996, 2008; Long and Holberton, 2004), while the majority of manipulative studies have found that neither CORT nor CORT-agonist administration increases hyperphagia in birds (Astheimer et al., 1992; Gray et al., 1990; Holberton et al., 2007; Saldanha et al., 2000; Simon, 1989 but see Landys et al., 2004b; Lohmus et al., 2006). Further-

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more, studies in Northern wheatears (*Oenanthe oenanthe*) during migratory stopovers found negative correlations between baseline CORT levels and refueling rates (Eikenaar et al., 2013, 2014). Eikenaar et al. (2013) suggested that since CORT levels rapidly decrease after arrival to stopover sites and then steadily increase as birds put on more fat, CORT acts more as a departure signal. This hypothesis fits well with similar studies in Bar-tailed Godwits (*Limosa lapponica*) (Landys-Ciannelli et al., 2002; Ramenofsky et al., 1995), Red Knots (*Calidris canutus*) (Piersma et al., 2000), and Gray Catbirds (*Dummetella carolinensis*) (Holberton et al., 1996), which also found that fatter birds had higher baseline CORT levels. Therefore, low baseline CORT levels may be necessary during hyperphagia and fuel deposition, while high baseline CORT levels may signal sufficient fat stores for departure. Future studies should determine whether *J.h. hyemalis* baseline CORT levels continue to rise during fattening and peak immediately before departure.

## 5. Conclusions

By comparing resident and migrant Dark-eyed Juncos that share the same overwintering site, this study examined whether up-regulated HPA-axis activity in migrants could potentially suppress later-stage reproductive development during spring migratory preparation. Contrary to our predictions, we found that migrants actually had lower circulating CORT levels and similar negative feedback efficacy compared with residents, thus suggesting that systemic glucocorticoids do not act as a brake on reproductive maturation prior to migratory departure. Future studies should examine the relationship between individual CORT levels and fueling rates during pre-migratory development, as our study design did not allow us to test how CORT changed over the migratory preparation stage. Future studies should also examine when residents start to increase CORT from winter to breeding levels, as it's possible we sampled residents too close to breeding. These studies would also verify whether migrants and residents have inherently different HPA-axes. Regardless, our results strongly indicate that delayed reproductive development in migrants is not being driven by increased HPA-axis activity, therefore future studies should examine whether higher photoperiodic thresholds or other, physiological differences mediate delayed reproductive development in migrants. Finally, our data lead us to propose that high CORT levels may not be required for pre-migratory hyperphagia and fattening.

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