1 Patterns of testosterone in three Nearctic-Neotropical migratory songbirds during spring
2 passage

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15 Abstract

16 Preparation for breeding may overlap extensively with vernal migration in long-distance
17 migratory songbirds. Testosterone plays a central role in mediating this transition into breeding
18 condition by facilitating changes to physiology and behavior. While changes in testosterone
19 levels are well studied in captive migrants, these changes are less well known in free-living
20 birds. We examined testosterone levels in free-living Nearctic-Neotropical migrants of three
21 species during their vernal migration. Testosterone levels increased during the migratory period
22 in males of all three species but significantly so in only two. Testosterone levels in females
23 remained the same throughout their migration. Our results support the extensive overlap
24 between vernal migration and breeding preparation in male songbirds. The pattern of
25 testosterone changes during vernal migration is far from clear in females.

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27 Keywords: testosterone, migration, birds, breeding preparation, annual cycle

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30 1. Introduction

31 Animals express different phenotypes at different times of the year as they transition
32 through their annual cycle. Piersma and van Gils (2011) purport that life-history stages are the
33 specific phenotypic periods that exist within a single individual at different points during the
34 annual cycle. Finite State Machine Theory posits that there is a limit to the number of
35 behavioral, physiological, and morphological traits that can be expressed within an individual at
36 any time (Jacobs and Wingfield, 2000). Accordingly physiological tradeoffs must occur as an
37 organism transitions between states, i.e. stages in the annual cycle (Jacobs and Wingfield,
38 2000; Ramenofsky, 2011). Migratory songbirds typically exhibit the following series of annual
39 life-history stages: breeding, pre-basic molt, autumn migration, overwintering, and vernal

migration; and some species also complete a pre-alternate molt prior to vernal migration (sensu Jacobs and Wingfield, 2000). The expression of traits associated with these different life-history stages of migratory species must coincide due to temporal constraints, i.e. the addition of the two lengthy migratory periods necessitates overlap between the migratory stage and the previous and subsequent stages. If we are to understand the biology of migratory species we must understand how these life-history stages interact with one another (Greenberg and Marra, 2005).

Hormones mediate many of the physiological and behavioral changes as individuals transition between life-history stages (Wingfield, 2008). For example, the termination of breeding and the onset of pre-basic molt is promoted by prolactin (Dawson and Sharp, 1998; Dawson, 2006). Numerous hormones regulate a variety of physical and behavioral traits during breeding, so it is critical to gain a complete understanding of the hormonal changes that take place as seasonally-breeding birds prepare for reproduction. Preparation for breeding in migratory songbirds may be particularly intricate because these birds are balancing the energetic and physiological constraints they experience in two successive and particularly demanding phases of the annual cycle, migration and breeding. While it is clear that events occurring in one life history stage influence survival and reproductive success in subsequent stages (Paxton and Moore, 2015; Studds et al., 2008; Tonra et al., 2011a), studies focusing on the physiological overlap between stages in free-living migrants are limited and their results are inconsistent. For example, while Tonra et al. (2013) detected increases in androgens in American Redstarts (*Setophaga ruticilla*) on the wintering grounds prior to vernal migration and Wingfield and Farner (1978a, 1978b) showed that testosterone increased during vernal migration in White-crowned Sparrows (*Zonotrichia leucophrys*), Bauchinger et al. (2007) did not detect any increase in migrating Garden Warblers (*Sylvia borin*).

Testosterone (T) is a steroid hormone that mediates physiology and behavior throughout the annual cycle. T plays a central role during reproduction and may be important in facilitating

the transition between breeding and the stages preceding it (Ramenofsky and Wingfield, 2006). In seasonally-reproducing birds, breeding preparation involves photostimulation and subsequent recrudescence of the hypothalamic-pituitary-gonadal (HPG) axis (Hahn et al., 2009). T is present in both males and females at varying levels throughout the annual cycle (Ketterson et al., 2005) and plays a primary role in the expression of breeding behaviors in both sexes (Balthazart, 1983; Wolfgang Goymann and Wingfield, 2014; Rosvall, 2013; Staub and De Beer, 1997; Wingfield et al., 2001). Testosterone is also a precursor to the production of estradiol in females, which is required for ova development (Norris, 1997). Since physiological breeding preparation takes approximately one month to complete (Ramenofsky, 2011), this process necessarily overlaps with the previous life-history stage and testosterone levels may increase well before the breeding season begins (Tonra et al., 2013; Wingfield and Farner, 1978a, 1978b; see Wingfield et al., 1990).

In addition to its influence during the breeding season, T plays a role during vernal migration. Almost a century ago, Rowan (1925) showed that T is required for birds to develop normal migratory behaviors. Many subsequent studies have also revealed that T and/or other gonadal hormones influences the expression of various migratory traits including migratory restlessness, hyperphagia, fat deposition, and accompanying mass gains (Deviche, 1995; King and Farner, 1962; Morton and Mewaldt, 1962; Schwabl and Farner, 1989a, 1989b; Schwabl et al., 1988; Stetson and Erickson, 1972; Weise, 1967). Further, the schedule for the expression of these migratory traits is advanced when T levels are experimentally elevated (Owen et al., 2014; Tonra et al., 2013, 2011b). These studies indicate that T may influence physiological aspects of migration related to energetic condition (fattening, mass gains) and given T's influence on aggression and territoriality during the breeding season (Balthazart, 1983; Goymann and Wingfield, 2014) we might expect a link between T and resource competition during migration. While elevated T leads to increases in activity and food intake in captive birds (Wikelski et al., 1999) and to faster movement rates and more time spent foraging in free-living

breeding birds (Lynn et al., 2000), T's influence on competition during migration has yet to be explored.

This study investigates variation in T for long-distance migrants as they progress towards their breeding grounds. To do so, we measured T levels in three Nearctic-Neotropical passage migrants at a southern and a northern site during vernal migration thus representing “far” and “near” relative distances to the breeding grounds. Our major hypothesis was that T increases as birds move closer to their breeding grounds during vernal migration. We tested the following specific predictions: (1) birds sampled at our northern site would have higher circulating T when compared to conspecifics sampled at our southern site; (2) males will have higher circulating T than females; (3) T and energetic condition would be positively correlated; and (4) T and the potential for competitive interactions would be correlated. In addition, we investigated the potential for an interaction between corticosterone (CORT) and T since some studies have indicated that CORT may suppress T (Deviche et al., 2001; Swett and Breuner, 2008).

2. Materials and Methods

2.1. Study Species

The focal species of this study were three boreal-breeding Nearctic-Neotropical passerines: Swainson's Thrush (SWTH; *Catharus ustulatus*), Northern Waterthrush (NOWA; *Parkesia noveboracensis*), and Magnolia Warbler (MAWA; *Setophaga magnolia*). All three winter in Central and/or South America and breed primarily in boreal regions of North America, with the southern-most breeding in Eastern North America around 39 degrees North latitude (Poole, 2005; Fig. 1). These species do not winter or breed at either of our study locations and thus are transient migrants at both.

2.2. Capture and Sampling

We passively captured migrants at two study locations that represent “far” and “near” relative distances to their breeding ranges (Fig. 1). Our southern, “far”, location was Johnson’s Bayou in Cameron Parish, Louisiana (29° 45’ N 93° 30’ W; hereafter “Louisiana”) where we operated up to 29 mist nets during April and May 2011-2014. This site is approximately two hectares in size but is located within a larger chenier forest extending approximately 20 miles along the northern coast of the Gulf of Mexico in southwest Louisiana. Our northern, “near”, location was Appledore Island in York County, Maine (42°58’N, 70°36’W; hereafter “Maine”) where we operated up to 10 mist nets during May and June 2011-2014. This site is a 33-hectare island located in the Isles of Shoals archipelago and is approximately 9.5 km from the nearest point on the mainland. We checked nets at both locations at least every 20-30 minutes.

We obtained a blood sample via brachial puncture from each individual within 10 minutes of extraction from a net (mean $\pm$ SD; 4.5 ± 2.0 min). We used either a 26- or 27-gauge needle, depending on the species, and collected blood into heparinized capillary tubes. Samples were placed on ice or in a refrigerator until centrifuged later that same day at 14,000 rpm for 10 minutes. Plasma was extracted and stored at -20°C until analyzed. Red blood cells were placed in approximately 500 μ l of lysis buffer (50mM TRIS, 10mM EDTA, 1% SDS, 0.1 M NaCl) and then stored at either -20°C or 4°C. These red blood cells were used to determine the sex of individuals genetically because neither NOWA nor SWTH exhibit extensive sexual dimorphism.

Each bird was banded with a USGS aluminum leg band and measurements of wing length (unflattened wing chord; nearest 0.5 mm), cloacal diameter (nearest 0.1 mm), and body mass (nearest 0.01g) were taken. Age was determined according to Pyle (1997) as either second-year (SY) or after-second-year (ASY). Occasionally we recorded a bird as the less specific age class of after-hatching-year (AHY) if we were unable to differentiate further. For MAWA, sex was determined based on plumage characteristics (Pyle, 1997). Subcutaneous fat

deposits were assessed to quantify energetic condition, according to Helms and Drury (1960).

Sample sizes by species, location, age, and sex are provide in Table 1.

2.3. Plasma Testosterone Assays

Plasma T was determined with an enzyme immunoassay (EIA; Assay Designs, Inc. #901-065; Jawor, 2007; Jawor et al., 2007). Depending on sample volume, 20-40 μ l of plasma was used and 2000 cpm of H3 labeled T (PerkinElmer) was added to each plasma sample to allow calculation of recoveries after three extractions with diethyl ether. Extracts were re-suspended in 50 μ l of ethanol and 300 μ l of the assay buffer provided in the EIA kit. Recoveries were determined with 100 μ l from each reconstituted sample. Samples were run in duplicate with 100 μ l of each reconstituted sample in the EIA. Testosterone concentrations were determined using a logistic curve (Microplate Manager; Bio-Rad Laboratories, Inc., Hercules, California) and corrected for incomplete recoveries and initial plasma volume. Intra- and inter-assay variations were determined based on three standard samples of known T concentration placed in each assay plate. Intra-assay variation ranged from 2% to 13% and inter-assay variation was 13.8%.

2.4. Plasma Corticosterone Assays

Given the possibility that CORT may suppress T (Deviche et al., 2001; Swett and Breuner, 2008), we determined CORT levels in a subset of our samples, dependent on adequate plasma volume. Plasma CORT was determined with an enzyme immunoassay (EIA; Arbor Assays Inc. #K014; DeVries and Jawor, 2013). Ten μ l of plasma were used with 2000 cpm of H3 labeled CORT (PerkinElmer) to allow calculation of recoveries after three extractions with diethyl ether. Extracts were re-suspended in 400 μ l of the assay buffer provided in the EIA kit. Recoveries were determined with 100 μ l from each reconstituted sample. Samples were run in duplicate with 50 μ l of each reconstituted sample on the EIA. CORT concentrations were determined using a logistic curve (Microplate Manager; Bio-Rad Laboratories, Inc., Hercules, California) and

corrected for incomplete recoveries and initial plasma volume. Intra- and inter-assay variations were determined based on four samples from a plasma pool placed in each assay plate. Intra-assay variation ranged from 6% to 9% and inter-array variation was 6%.

2.5. Genetic Sex Determination

We extracted DNA using a DNeasy Tissue Kit, #69506, Qiagen, Valencia, CA, following standard protocol from Qiagen for nucleated erythrocytes. Polymerase chain reaction was used to amplify the Chromobox-Helicase-DNA binding gene, different versions of which are found on the Z and W chromosomes of birds, using the P2 and P8 primers (Griffiths et al., 1996, 1998). PCR products were run on an agarose gel where a single band at 325 bp indicated male and two bands (one each at 325 and 375 bp) indicated female.

2.6. Statistical Analyses

Prior to analyses, T and CORT data were corrected for inter-assay variation based on the standards within each assay. Further, both T and CORT failed to meet normality assumptions. Natural log (NOWA and MAWA T), square root (SWTH and NOWA CORT), or sixth root (SWTH T) transformations corrected the disparity, and thus transformed data were used in all analyses. Also, birds aged as unknown/AHY were excluded from all analyses that included age.

We calculated energetic condition for each bird by subtracting the species- and size-specific fat-free mass from the individual's body mass, thus larger values of energetic condition are indicative of birds with larger fat stores, after correcting for size (Ellegren, 1992; Owen and Moore, 2006). For this calculation we used the combined long-term data sets from both study sites, Louisiana (1993-2010) and Maine (1990-2009), for each of the three species (SWTH: N = 1,877; NOWA: N = 2,265; MAWA: N = 6,011). For each species and wing chord, we regressed body mass on fat score and then used the resulting intercept in a final regression of fat-free

mass on wing chord. The resulting equations solve for fat-free mass (y) where ' x ' represents wing chord (SWTH: $y = 0.20x + 5.8$; NOWA: $y = 0.09x + 7.9$; MAWA: $y = 0.09x + 2.6$).

All analyses were performed in Program R (version 3.1.2). We used linear mixed effects models to allow the inclusion of random effects (packages lme4 and lmerTest; Bates, 2010; Bates et al., 2014). We selected the best model for each set of analyses using likelihood ratio tests by comparing full models (with all possible interactions) to simpler models, which allowed detection of significant interactions and first-order effects. We evaluated significance for main effects using likelihood ratio tests that compared a model with the variable in question to the null model. If necessary, we evaluated pairwise effects using least squares means tests with Tukey correction (package lsmeans in R).

We examined T in each study species in a series of analyses. First, we investigated whether circulating T was influenced by a variety of factors measured in this study that should be considered when testing our other predictions. These factors included date (day of year), the latency between extraction from nets and blood sampling (handling time), energetic condition, and time of day (calculated as sampling time less the time of local sunrise). Analyzing these factors first allowed us to identify which variables should be included as random variables in subsequent analyses and thus in this first set analyses, age, sex, year, and location were set as random effects. Second, we analyzed whether variation in circulating T levels can be explained by age, sex, or location of sampling (far or near the breeding grounds). In this second set of analyses, year and any effects of date, handling time, time of day, and energetic condition that were supported by model selection from the first analysis were kept as separate random effects. Third, we examined whether T was correlated with two measures of competition a migrant would experience on the day of sampling. For this analysis we compared circulating T to both the total number of other migrants captured and the number of migrants captured within each species' foraging group. MAWA, NOWA and SWTH were considered to be upper canopy, ground, and ground/midlevel foragers respectively. All species captured at our two study

locations (# species = 98, # migrants = 21,050) were broadly designated as ground/understory, midlevel/lower-canopy, and/or upper canopy foraging guilds (loosely based on De Graaf et al., 1985). Since it is likely that site-specific factors influence these measures of competition, this third set of analyses were run for each site separately and all aforementioned variables that were supported by the model selection in our first and second sets of analyses were included as random effects.

In a separate analysis, we examined the potential influence of CORT on circulating T on a subset of sampled birds. Because of their small body size we were unable to obtain enough blood from MAWA for this additional hormone analysis (mean mass $\pm$ SD; 8.2 ± 1.0 g); therefore we only investigated the potential influence of CORT in NOWA and SWTH. For these analyses, we used ANOVAs to compare CORT and T levels in both males and females sampled within 3 minutes of capture in a net (baseline CORT samples) to individuals for whom precise capture time was unknown. This design allowed us to determine whether CORT levels were higher in birds with unknown capture times and whether this influenced circulating T levels as has been indicated in some studies (Deviche et al., 2001; Swett and Breuner, 2008). Finally, we also analyzed the relationship between T and diameter of the cloaca using ANOVA.

3. Results

3.1. Testosterone patterns related to sex, location, and age

There were no significant interactions between location, age and sex on T levels of SWTH and none of these factors had a significant effect on T in SWTH (Table 2; Fig. 2). T levels of NOWA showed no significant interactions between location, age, or sex. There was a significant effect of location on T levels in NOWA but the effects sex and age were not significant (Table 2). Post-hoc pairwise analyses revealed that male NOWA have higher T in Maine ($t = 2.6$, $p = 0.048$; Fig. 2) while T in female NOWA was not different between sites ($t = 0.49$, $p = 0.96$). Further, T in male NOWA in Maine was higher than in female NOWA from both locations but only

significantly so for females in Louisiana (Female Maine-Male Maine: $t = 2.5$, $p = 0.07$; Female Louisiana-Male Maine: $t = 2.7$, $p = 0.04$). We found no significant interactions between sampling location, age, or sex on T of MAWA thus first-order effects were investigated. There were significant effects of sex and sampling location on T, but not age (Table 2). While both male and female MAWA had higher T in Maine, as shown by pairwise analyses, the difference is significant in males only (Males: $t = 3.7$, $p < 0.01$; Females: $t = 1.6$, $p = 0.4$; Fig. 2). Additionally female MAWA in Maine had significantly higher T than males in Louisiana ($t = 4.0$, $p < 0.01$; Fig. 2).

3.2. Other factors in relation to testosterone

For SWTH, none of the other variables examined (date, handling time, energetic condition, and time of day) had a significant effect on T (Table 3). There was no effect of energetic condition or time of day on T in NOWA (Table 3). In NOWA, date had a significant and positive effect on T (Table 3). Handling time also had a significant effect on T in NOWA however the 95% confidence interval for the parameter estimate overlapped zero indicating that there is no net effect of handling time. In MAWA, date had a significant positive relationship with T while the other variables tested were not related to T levels (Table 3).

There was no effect of sex on either CORT or T levels between birds sampled within 3 minutes of capture compared to those with unknown capture times (which could have been up to 30 minutes) in either species tested (T, SWTH: $F_{1,22} = 0.37$, $p = 0.55$; T, NOWA: $F_{1,16} = 0.03$, $p = 0.86$; CORT, SWTH: $F_{1,22} = 0.34$, $p = 0.57$; CORT, NOWA: $F_{1,16} = 0.16$, $p = 0.70$). CORT was significantly higher in birds with unknown capture times compared to those sampled within 3 minutes (SWTH: $F_{1,22} = 4.6$, $p = 0.04$; NOWA: $F_{1,16} = 24.6$, $p < 0.001$; Fig. 3). However, there was no significant difference in T between birds sampled within 3 minutes of capture and those with an unknown capture time (SWTH: $F_{1,22} = 0.001$, $p = 0.97$; NOWA: $F_{1,16} = 2.70$, $p = 0.12$; Fig. 3).

Neither the total number of migrants nor the number of migrants in each species' foraging group was related to T at either location for any of the three species studied (Table 4). Diameter of the cloaca was not different between males and females in any of the three species studied (SWTH: $F_{1,151} = 0.39$, $p = 0.54$; NOWA: $F_{1,59} = 0.01$, $p = 0.92$; MAWA: $F_{1,60} = 0.19$, $p = 0.67$). Additionally, cloacal diameter was not related to circulating T levels in any three species (SWTH: $F_{1,151} = 0.55$, $p = 0.46$; NOWA: $F_{1,58} = 1.34$, $p = 0.25$; MAWA: $F_{1,60} = 0.05$, $p = 0.83$).

4. Discussion

4.1. Geographic patterns of testosterone

In all three species studied, males had higher T in Maine, where they are closer to their breeding grounds, compared to Louisiana, although this pattern was not statistically significant in SWTH. This increase in T likely coincides with testis recrudescence which has been shown to occur throughout vernal migration in other passerine species (Bauchinger et al., 2008, 2007; Wingfield and Farner, 1978a, 1978b). Whereas studies of captive birds also indicate that T increases as males progress through simulated vernal migration (Bauchinger et al., 2008; Bluhm et al., 1991; Ramenofsky et al., 1999; Schwabl and Farner, 1989a, 1989b), it should be noted that hormone levels between captive and free-living birds may not be comparable (see Wingfield et al., 1990).

Within songbirds, the extent of overlap between migration and breeding preparation is likely to be driven by the length of the migratory journey such that long-distance migrants, including Nearctic-Neotropical migrants, are expected to have substantial temporal overlap between breeding preparation and vernal migration (Ramenofsky and Wingfield, 2006; Ramenofsky, 2011). Field studies investigating T levels in relation to breeding ground proximity in male migrants have yielded conflicting results. Wingfield and Farner (1978a, 1978b) found that T increases during the migratory period as males approach the breeding grounds in both the long-distance subspecies (*Z.l. gambelii*) and the medium-distance subspecies (*Z. l.*

298 *pugetensis*) of the White-crowned Sparrow. Bauchinger and colleagues (2007) did not find an
299 increase in T until Garden Warblers arrived on the breeding grounds. It should be noted that
300 many of their samples were below their assay's detection limit, which may have impeded the
301 ability to detect changes in the T levels of migrating Garden Warblers. Tonra et al. (2013) also
302 failed to find a relationship between androgen levels and distance to the breeding grounds in
303 American Redstarts as they prepared for their vernal migration.

304 While androgens, including T, are generally considered to be "male" hormones, female
305 vertebrates also have meaningful levels of circulating androgens and possess androgen
306 receptors (Staub and De Beer, 1997). In female birds, T is produced largely by the ovaries but
307 also may be secreted by the adrenal glands, and T levels vary seasonally (Ketterson et al.,
308 2005; Staub and De Beer, 1997). In this study, we did not detect any changes in T levels in
309 female songbirds during spring migration. Wingfield and Farner (1978a) found T to increase
310 during migration in the long-distance migrant subspecies of White-crowned Sparrow but not in
311 their medium distance conspecifics (Wingfield and Farner, 1978a). However in both of these
312 studies by Wingfield and Farner (1978a, 1978b), estradiol was higher in late migrants and upon
313 arrival at the breeding grounds compared to earlier time periods. Once produced, T may be
314 immediately converted into estradiol or may be released into general circulation for direct use or
315 later conversion to estradiol by target tissues (Adkins-Regan, 2005). While we did not measure
316 estradiol in this study because of sampling limitations, one might predict estradiol to increase
317 throughout the migratory period in female songbirds.

318 In this study we found female MAWA to have relatively high T levels at both locations. In
319 fact, T levels in female MAWA were higher than in male MAWA at our Louisiana sampling
320 location. Outside of the breeding season T levels in songbirds remain fairly low in both sexes,
321 and it is apparently rare for females to have higher T than males in any season (see Ketterson
322 et al., 2005). However, circulating levels of T in male and female Northern Cardinals (*Cardinalis*
323 *cardinalis*) were similar during the non-breeding season (Devries et al., 2011; Jawor, 2007).

Similarly, T levels in Downy Woodpecker (*Picoides pubescens*) females were as high as or higher than in males during the non-breeding season (Kellam et al., 2004). Both of these species are non-migratory and both sexes may defend territories outside of the breeding season. In contrast, MAWA is a long-distance Nearctic-Neotropical migrant in which males are responsible for most territory defense in the breeding season (Dunn and Hall, 2010). However, other species of wood warblers are known to aggressively defend territories during the non-breeding season (Greenberg et al., 1996; Marra, 2000) and MAWA have been shown to segregate by sex on their wintering grounds (Ornat and Greenberg, 1990). While it is possible that female MAWA sustain high T throughout the year to promote aggressive behaviors related to winter territory defense, given the relatively low T levels seen in male MAWA early in migration and since males likely show aggressive behaviors as well, this potential explanation for female T levels in MAWA warrants more attention.

4.2. Temporal patterns of testosterone

In MAWA and NOWA, T increased with date, which may indicate that the pattern detected between sampling locations is a function of the time since breeding preparation began, rather than the geographic proximity to the breeding grounds. This observed pattern makes sense because after being photostimulated, the HPG axis increases production of hormones including T (Deviche and Small, 2001; Hahn et al., 2009). Vernal migration takes approximately 20 days for long-distance migrants complete (Ewert et al., 2012; Stutchbury et al., 2009; Wikelski et al., 2003). Based on our mixed-effect models, we estimate that circulating T would increase by 1.3 ng/ml in both NOWA and MAWA during that time. Testosterone levels in breeding male songbirds are generally within the 1 – 5 ng/ml range (Ketterson et al., 2005), so this observed within-season increase represents a meaningful change in hormone levels.

4.3. Other factors considered in relation to testosterone

T was not related to time of day, energetic condition, cloacal protuberance, or handling time. Previous studies have found that T follows a diel rhythm with highest levels during the overnight or early morning periods (Goymann and Trappschuh, 2011; Hau et al., 2002; Kempenaers et al., 2008). Given that we only sampled T during daytime hours, it is not surprising that we did not find T to correlate with time since sunrise. Removal of T has been shown to suppress the development of migratory traits including fattening and increases in mass (Deviche, 1995; Schwabl and Farner, 1989b; Stetson and Erickson, 1972) and experimental increases in T have been shown to promote the development of such traits (Owen et al. 2014; Tonra et al. 2011b; 2013). Although understudied, T may also play a role in the regulation of muscle anabolism (see Ramenofsky, 2011) and these seemingly opposing roles of T with regard to condition may explain the lack of relationship in this study. While some studies have indicated T levels may be related to condition changes in preparation for migration (Tonra et al. 2011b; 2013) it is likely that T's role in fattening and other migratory traits is complicated and requires further study (Ramenofsky and Németh, 2014).

The cloacal region is used for sperm storage in male birds and studies have shown that T plays a role in the development of the cloacal protuberance (Ramenofsky and Németh, 2014; Tonra et al., 2011b). Given that the proportions of migrating songbirds that have been found to produce measurable amounts of sperm is low (Quay, 1986, 1985a, 1985b) and that T levels we report in this study are lower than what is typical of breeding songbirds (Ketterson et al., 2005), the lack of relationship between cloacal diameter and T is not unexpected. That said, Tonra et al. (2011a) found an inverse relationship between cloacal diameter and arrival date on the breeding grounds with early arriving males having larger cloacal diameters, higher androgen levels, and better breeding success. In our study, cloacal diameter was not different between males and females indicating that that development of the cloacal protuberance in males had yet to commence.

Time spent in captivity has the potential to influence hormone levels and studies often exercise caution by including handling time in statistical analyses of T and other hormones. However, the effect of relatively short handling times (< 30 min), as in this study, are not likely to significantly influence circulating T (Peters et al., 2001; but see Devries et al., 2011). While we define handling time as the latency between extraction from a net and sampling, precise capture time in a net is unknown for most of the birds in this study. Even though previous studies have suggested that high levels of CORT caused by capture and handling may suppress T and other hormones of the HPG axis (Deviche et al., 2001; Swett and Breuner, 2008), we saw no indication of this in our study. Testosterone levels in birds with elevated “stress” levels or CORT were no different from birds with lower, baseline CORT levels. Our results are not novel, however, since other studies have found similar results (e.g. Devries et al., 2011; Wikelski et al., 1999), but this study does add to a growing body of evidence of the complex relationship between these two hormones. For example, higher CORT levels may increase free (unbound) T (Deviche et al., 2001) and variation among levels of T, CORT, and their shared binding globulin do not seem to influence T’s availability to target tissues (Swett and Breuner, 2008).

Testosterone is known to mediate inter- and intra-specific aggression and territorial defense in songbirds during the breeding season (Balthazart, 1983; Wingfield et al., 2001). In this study, however, we found no evidence that T was related to or influenced by competition during migratory stopover as assessed by both the total number of other migrants present and the number of migrants within a species’ foraging group. That said, T may influence the rate of and time spent foraging during the breeding season (Lynn et al., 2000) and thus the relationship between T, competition, and foraging activities during migration warrants further study during the migratory periods.

5. Conclusions

The growing consensus is that male migrants increase their T during vernal migration, likely as a result of overlapping life-history stages as breeding preparation begins. While female migrants did not vary during migration in this study, most birds of both sexes had slightly higher T levels than what is found in other Nearctic-Neotropical migrants during the wintering period (e.g. Tonra et al., 2013; Wingfield and Farner, 1978a, 1978b). Additionally, our observed temporal increase in T during migration may reflect the time that has elapsed since photostimulation rather than simply a geographic proximity to the breeding grounds. The overlapping life history stages of breeding and migration seen in this and other studies likely facilitate a smooth transition to commence breeding activities (e.g. territory establishment) immediately upon arrival on the breeding grounds.

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426 This is contribution 22 of the Appledore Island Migration Station and 182 of the Shoals Marine
427 Laboratory.

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429 References

430 Adkins-Regan, E., 2005. Hormones and animal social behavior. Princeton University Press,
431 Princeton, NJ.

432 Balthazart, J., 1983. Hormonal correlates of behavior, in: Farner, D., King, J., Parkes, K. (Eds.),
433 Avian Biology. Academic Press, London, pp. 221–365.

434 Bates, D., 2010. lmer4: mixed effects modeling with R. Springer, New York.

435 Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear mixed-effects models using
436 Eigen and S4. R package version 1.1-7.

437 Bauchinger, U., Hof, T., Biebach, H., 2007. Testicular development during long-distance spring
438 migration. Horm. Behav. 51, 295–305. doi:10.1016/j.yhbeh.2006.10.010

439 Bauchinger, U., Van't Hof, T., Biebach, H., 2008. Migratory stopover conditions affect the
440 developmental state of male gonads in garden warblers (*Sylvia borin*). Horm. Behav. 54,
441 312–318. doi:10.1016/j.yhbeh.2008.03.007

442 BirdLife-International, NatureServe, 2014. Bird species distribution maps of the world.

443 Bluhm, C., Schwabl, H., Schwabl, I., Perera, A., Follett, B., Goldsmith, A., Gwinner, E., 1991.
444 Variation in hypothalamic gonadotrophin-releasing hormone content, plasma and pituitary
445 LH, and in-vitro testosterone release in a long-distance migratory bird, the garden warbler
446 (*Sylvia borin*), under constant photoperiods. J. Endocrinol. 128, 339–345.

447 Dawson, A., 2006. Control of molt in birds: Association with prolactin and gonadal regression in
448 starlings. Gen. Comp. Endocrinol. 147, 314–322. doi:10.1016/j.ygcen.2006.02.001

449 Dawson, A., Sharp, P.J., 1998. The role of prolactin in the development of reproductive
450 photorefractoriness and postnuptial molt in the European starling (*Sturnus vulgaris*).
451 Endocrinology 139, 485–490. doi:10.1210/en.139.2.485

452 De Graaf, R.M., Tilghman, N.G., Anderson, S.H., 1985. Foraging guilds of North American
453 Birds.

454 Deviche, P., 1995. Androgen Regulation of Avian Premigratory Hyperphagia and Fattening :
455 From Eco- Physiology to Neuroendocrinology. Am. Zool. 35, 234–245.

456 Deviche, P., Breuner, C., Orchinik, M., 2001. Testosterone , Corticosterone , and Photoperiod
457 Interact to Regulate Plasma Levels of Binding Globulin and Free Steroid Hormone in Dark-
458 Eyed Juncos , *Junco hyemalis* 77, 67–77. doi:10.1006/gcen.2001.7613

459 Deviche, P., Small, T., 2001. Photoperiodic control of seasonal reproduction: neuroendocrine
460 mechanisms and adaptations, in: Dawson, A., Chaturvedi, C.M. (Eds.), Avian
461 Endocrinology. Narosa Publishing House, New Delhi, pp. 113–128.

462 DeVries, M.S., Holbrook, A.L., Winters, C.P., Jawor, J.M., 2011. Non-breeding gonadal
463 testosterone production of male and female Northern Cardinals (*Cardinalis cardinalis*)
464 following GnRH challenge. *Gen. Comp. Endocrinol.* 174, 370–378.
465 doi:10.1016/j.ygcen.2011.09.016

466 DeVries, M.S., Jawor, J.M., 2013. Natural variation in circulating testosterone does not predict
467 nestling provisioning rates in the northern cardinal, *Cardinalis cardinalis*. *Anim. Behav.* 85,
468 957–965. doi:10.1016/j.anbehav.2013.02.019

469 Dunn, E., Hall, G.A., 2010. Magnolia Warbler (*Dendroica magnolia*), in: Poole, A. (Ed.), The
470 Birds of North America Online. Cornell Lab of Ornithology, Ithaca. doi:10.2173/bna.136

471 Ellegren, H., 1992. Fat-free body mass of autumn migrating Bluethroats *Luscinia s. svecica*.
472 *Ardea* 80, 255–259.

473 Ewert, D.N., Hall, K.R., Wunderle, J.M., Currie, D., Rockwell, S.M., Johnson, S.B., White, J.D.,
474 2012. Duration and Rate of Spring Migration of Kirtland's Warblers. *Wilson J. Ornithol.* 124,
475 9–14. doi:10.1676/11-073.1

476 Goymann, W., Trappschuh, M., 2011. Seasonal and diel variation of hormone metabolites in
477 European stonechats: on the importance of high signal-to-noise ratios in noninvasive
478 hormone studies. *J. Biol. Rhythms* 26, 44–54. doi:10.1177/0748730410388394

479 Goymann, W., Wingfield, J.C., 2014. Male-to-female testosterone ratios, dimorphism, and life
480 history - What does it really tell us? *Behav. Ecol.* 25, 685–699. doi:10.1093/beheco/aru019

481 Goymann, W., Wingfield, J.C., 2014. Male-to-female testosterone ratios, dimorphism, and life
482 history--what does it really tell us? *Behav. Ecol.* 25, 685–699. doi:10.1093/beheco/aru019

483 Greenberg, R., Marra, P.P. (Eds.), 2005. Birds of Two Worlds: The Ecology and Evolution of
484 Migration. John Hopkins University Press, Baltimore, MD.

485 Greenberg, R., Reitsma, R., Angon, A.C., 1996. Interspecific Aggression by Yellow Warblers in
486 a Sun Coffee Plantation. *Condor* 98, 640–642.

487 Griffiths, R., Daan, S., Dijkstra, C., 1996. Sex identification in birds using two CHD genes. *Proc.*
488 *Biol. Sci.* 263, 1251–1256.

489 Griffiths, R., Double, M.C., Orr, K., Dawson, R.J.G., 1998. A DNA test to sex most birds. *Mol.*
490 *Ecol.*

491 Hahn, T.P., Watts, H.E., Cornelius, J.M., Brazeal, K.R., MacDougall-Shackleton, S. a., 2009.
492 Evolution of environmental cue response mechanisms: Adaptive variation in
493 photorefractoriness. *Gen. Comp. Endocrinol.* 163, 193–200.
494 doi:10.1016/j.ygcen.2009.04.012

495 Hau, M., Romero, L.M., Brawn, J.D., Van't Hof, T.J., 2002. Effect of polar day on plasma profiles
496 of melatonin, testosterone, and estradiol in high-Arctic Lapland Longspurs. *Gen. Comp.*
497 *Endocrinol.* 126, 101–112. doi:10.1006/gcen.2002.7776

498 Helms, C., Drury, W., 1960. Winter and migratory weight and fat: field studies on some North
499 American buntings. *Bird-Banding* 31, 1–40.

500 Jacobs, J.D., Wingfield, J.C., 2000. Endocrine control of life-cycle stages: a constraint on
501 response to the environment? *Condor* 102, 35–51. doi:10.1650/0010-
502 5422(2000)102[0035:ECOLCS]2.0.CO;2

503 Jawor, J.M., 2007. Testosterone in Northern Cardinals (*Cardinalis cardinalis*): Possible
504 Influence of Prolonged Territorial Behavior (*Testosterona en Cardinalis cardinalis* :
505 Posible Influencia del Comportamiento Territorial Prolongado) Author (s): Jodie M .
506 Jawor and A. *Auk* 124, 331–338.

507 Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E. a., Bentley, G.E., Ketterson,
508 E.D., 2007. Testosterone response to GnRH in a female songbird varies with stage of
509 reproduction: implications for adult behaviour and maternal effects. *Funct. Ecol.* 21, 767–
510 775. doi:10.1111/j.1365-2435.2007.01280.x

511 Kellam, J.S., Wingfield, J.C., Lucas, J.R., 2004. Nonbreeding season pairing behavior and the
512 annual cycle of testosterone in male and female downy woodpeckers, *Picoides pubescens*.
513 *Horm. Behav.* 46, 703–714. doi:10.1016/j.yhbeh.2004.06.014

514 Kempenaers, B., Peters, A., Foerster, K., 2008. Sources of individual variation in plasma
515 testosterone levels. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 363, 1711–1723.
516 doi:10.1098/rstb.2007.0001

517 Ketterson, E.D., Nolan, V., Sandell, M., 2005. Testosterone in females: mediator of adaptive
518 traits, constraint on sexual dimorphism, or both? *Am. Nat.* 166 Suppl , S85–S98.
519 doi:10.1086/444602

520 King, J.R., Farner, D.S., 1962. The relationship of fat deposition to Zugunruhe and migration.
521 *Condor* 65, 200–223.

522 Lynn, S., Houtman, A., Weathers, W., Ketterson, E., Nolan, V., 2000. Testosterone increases
523 activity but not daily energy expenditure in captive male dark-eyed juncos, *Junco hyemalis*.
524 *Anim. Behav.* 60, 581–587. doi:10.1006/anbe.2000.1510

525 Marra, P.P., 2000. The role of behavioral dominance in structuring patterns of habitat
526 occupancy in a migrant bird during the nonbreeding season. *Behav. Ecol.* 11, 299–308.
527 doi:Article

528 Morton, M.L., Mewaldt, L.R., 1962. Some Effects of Castration on a Migratory Sparrow. *Physiol.*
529 *Zool.* 35, 237–247.

530 Norris, D.O., 1997. *Vertebrate Endocrinology*, 3rd ed. Academic Press, London.

531 Ornat, A.L., Greenberg, R., 1990. Sexual Segregation by Habitat in Migratory Warblers in
532 Quintana Roo, Mexico. *Auk* 107, 539–543.

533 Owen, J.C., Garvin, M.C., Moore, F.R., 2014. Elevated testosterone advances onset of
534 migratory restlessness in a nearctic-neotropical landbird. *Behav. Ecol. Sociobiol.* 68, 561–
535 569. doi:10.1007/s00265-013-1671-x

536 Owen, J.C., Moore, F.R., 2006. Seasonal differences in immunological condition of three
537 species of thrushes. *Condor* 108, 389–398.

538 Paxton, K., Moore, F., 2015. Carry-over effects of winter habitat quality on en route timing and
539 condition of a migratory passerine during spring migration. *J. Avian Biol.*

540 Peters, a., Astheimer, L.B., Cockburn, a., 2001. The annual testosterone profile in cooperatively
541 breeding superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity. *Behav.*
542 *Ecol. Sociobiol.* 50, 519–527. doi:10.1007/s002650100403

543 Piersma, T., van Gils, J.A., 2011. *The Flexible Phenotype*. Oxford University Press, New York.

544 Poole, A., 2005. *The Birds of North America Online* [WWW Document]. Cornell Lab. Ornithol.
545 URL <http://bna.birds.cornell.edu.bnaproxy.birds.cornell.edu/BNA/>

546 Pyle, P., 1997. *Identification Guide to North American Birds: Part 1*. Braun-Brumfield Inc., Ann
547 Arbor, Michigan.

548 Quay, W.B., 1986. Timing and Location of Spring Sperm Release in Northern Thrushes. *Wilson*
549 *Bull.* 98, 526–534.

550 Quay, W.B., 1985a. Cloacal Sperm in Spring Migrants: Occurrence and Interpretation. *Condor*
551 87, 273. doi:10.2307/1366894

552 Quay, W.B., 1985b. Sperm release in migrating wood-warblers (Parulinae) nesting at higher
553 latitudes. *Wilson Bull.* 97, 283–295.

554 Ramenofsky, M., 2011. Hormones in migration and reproductive cycles of birds, in: Norris, D.,
555 Lopez, K. (Eds.), *Hormones and Reproduction in Vertebrates Volume 8*. Academic Press,
556 pp. 205–236.

557 Ramenofsky, M., Németh, Z., 2014. Regulatory mechanisms for the development of the
558 migratory phenotype: Roles for photoperiod and the gonad. *Horm. Behav.* 66, 148–158.
559 doi:10.1016/j.yhbeh.2014.04.012

560 Ramenofsky, M., Savard, R., Greenwood, M.R.C., 1999. Seasonal and diel transitions in
561 physiology and behavior in the migratory dark-eyed junco. *Comp. Biochem. Physiol. Part A*
562 *Mol. Integr. Physiol.* 122, 385–397. doi:10.1016/S1095-6433(99)00013-6

563 Ramenofsky, M., Wingfield, J.C., 2006. Behavioral and physiological conflicts in migrants: the
564 transition between migration and breeding. *J. Ornithol.* 147, 135–145. doi:10.1007/s10336-
565 005-0050-4

566 Rosvall, K. a., 2013. Proximate perspectives on the evolution of female aggression: good for the
 567 gander, good for the goose? *Philos. Trans. R. Soc. B Biol. Sci.* 368, 20130083.
 568 doi:10.1098/rstb.2013.0083

569 Rowan, W., 1925. Relation of light to bird migration and developmental changes. *Nature* 115,
 570 494–495.

571 Schwabl, H., Farner, D.S., 1989a. Endocrine and Environmental Control of Vernal Migration in
 572 Male White-Crowned Sparrows, *Zonotrichia leucophrys gambelii*. *Physiol. Zool.* 62, 1–10.

573 Schwabl, H., Farner, D.S., 1989b. Dependency on Testosterone of Photoperiodically-Induced
 574 Vernal Fat Deposition in Female White-Crowned Sparrows. *Condor* 91, 108–112.

575 Schwabl, H., Schwabl-Benzinger, I., Goldsmith, A.R., Farner, D.S., 1988. Effects of ovariectomy
 576 on long-day-induced premigratory fat deposition, plasma levels of luteinizing hormone and
 577 prolactin, and molt in white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *Gen.*
 578 *Comp. Endocrinol.* 71, 398–405. doi:10.1016/0016-6480(88)90268-7

579 Staub, N.L., De Beer, M., 1997. The role of androgens in female vertebrates. *Gen. Comp.*
 580 *Endocrinol.* 108, 1–24. doi:10.1006/gcen.1997.6962

581 Stetson, M.H., Erickson, J.E., 1972. Hormonal control of photoperiodically induced fat
 582 deposition in White-crowned Sparrows. *Gen. Comp. Endocrinol.* 19, 355–362.

583 Studds, C.E., Kyser, T.K., Marra, P.P., 2008. Natal dispersal driven by environmental conditions
 584 interacting across the annual cycle of a migratory songbird. *Proc. Natl. Acad. Sci. U. S. A.*
 585 105, 2929–2933. doi:10.1073/pnas.0710732105

586 Stutchbury, B.J.M., Tarof, S. a, Done, T., Gow, E., Kramer, P.M., Tautin, J., Fox, J.W.,
 587 Afanasyev, V., 2009. Tracking long-distance songbird migration by using geolocators.
 588 *Science* 323, 896. doi:10.1126/science.1166664

589 Swett, M.B., Breuner, C.W., 2008. Interaction of testosterone, corticosterone and corticosterone
 590 binding globulin in the white-throated sparrow (*Zonotrichia albicollis*). *Comp. Biochem.*
 591 *Physiol. A. Mol. Integr. Physiol.* 151, 226–31. doi:10.1016/j.cbpa.2008.06.031

592 Tonra, C.M., Marra, P.P., Holberton, R.L., 2013. Experimental and observational studies of
 593 seasonal interactions between overlapping life history stages in a migratory bird. *Horm.*
 594 *Behav.* 64, 825–832. doi:10.1016/j.yhbeh.2013.10.004

595 Tonra, C.M., Marra, P.P., Holberton, R.L., 2011a. Migration phenology and winter habitat quality
 596 are related to circulating androgen in a long-distance migratory bird. *J. Avian Biol.* 42, 397–
 597 404. doi:10.1111/j.1600-048X.2011.05333.x

598 Tonra, C.M., Marra, P.P., Holberton, R.L., 2011b. Early elevation of testosterone advances
 599 migratory preparation in a songbird. *J. Exp. Biol.* 214, 2761–7. doi:10.1242/jeb.054734

600 Weise, C.M., 1967. Castration and Spring Migration in the White-Throated Sparrow. *Condor* 69,
 601 49–68.

602 Wikelski, M., Lynn, S., Breuner, J.C., Wingfield, J.C., Kenagy, G.J., 1999. Energy metabolism,
603 testosterone and corticosterone in white-crowned sparrows. *J. Comp. Physiol. A Sensory,*
604 *Neural, Behav. Physiol.* 185, 463–470. doi:10.1007/s003590050407

605 Wikelski, M., Tarlow, E.M., Rain, A., Diehl, R.H., Larkin, R.P., Visser, G.H., 2003. Costs of
606 migration in free-flying songbirds. *Nature* 423, 704.

607 Wingfield, J.C., 2008. Organization of vertebrate annual cycles: implications for control
608 mechanisms. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 363, 425–441.
609 doi:10.1098/rstb.2007.2149

610 Wingfield, J.C., Farner, D.S., 1978a. The Endocrinology of a Natural Breeding Population of the
611 White-Crowned Sparrow (*Zonotrichia leucophrys pugetensis*). *Physiol. Zool.* 51, 188–205.

612 Wingfield, J.C., Farner, D.S., 1978b. The annual cycle of plasma irLH and steroid hormones in
613 feral populations of the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. *Biol.*
614 *Reprod.* 19, 1046–1056.

615 Wingfield, J.C., Hegner, R.E., Dufty, A., Ball, G., 1990. The “ Challenge Hypothesis ”:
616 Theoretical Implications for Patterns of Testosterone Secretion , Mating Systems , and
617 Breeding Strategies. *Am. Nat.* 136, 829–846.

618 Wingfield, J.C., Lynn, S.E., Soma, K.K., 2001. Avoiding the “costs” of testosterone: Ecological
619 bases of hormone-behavior interactions. *Brain. Behav. Evol.* 57, 239–251.
620 doi:10.1159/000047243

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623 Figure Captions

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625 Fig. 1. Breeding ranges for (A) Swainson's Thrushes, (B) Northern Waterthrushes, and (C)
626 Magnolia Warblers. Sampling locations in Louisiana and Maine are indicated by filled triangles.
627 Breeding range data were provided by BirdLife-International and NatureServe (2014).

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630 Fig. 2. Circulating testosterone levels in male (circles) and female (triangles) Swainson's
631 Thrushes, Northern Waterthrushes, and Magnolia Warblers sampled at a southern and a
632 northern site during vernal migration. Data points are back-transformed means and error bars
633 represent ± 1 SE.

634

635

636 Fig. 3. Circulating testosterone levels and corticosterone levels in (A) Swainson's Thrushes and
637 (B) Northern Waterthrushes for bird sampled within three minutes of capture (squares) and
638 those in which precise capture time is unknown (circles). Data points are back-transformed
639 means and error bars represent ± 1 SE.

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641

TABLE 1. Number of birds sampled in Louisiana and Maine during spring migration by age and sex.

Species	Site	Male			Female		
		SY	AS	AH	SY	ASY	AHY
Swainson's Thrush	Louisian	57	55	6	57	33	8
	Maine	22	20	2	18	23	1
Northern	Louisian	19	24	17	21	10	5
	Maine	13	19	2	24	19	4
Magnolia Warbler	Louisian	19	20	0	21	6	0
	Maine	34	21	0	29	17	0

TABLE 2. Linear mixed model analysis of the effects of age, sex, and sampling location on testosterone. Model effect estimates and standard error is given for each species and variable. Chi-squared statistics derive from likelihood ratio tests that compared a model with the variable in question to the null model. Asterisks denote statistical significance of a variable.

Species	Fixed	Model Statistics		Comparison to Null Model	
		Estimat	SE	X ²	P
Swainson's Thrush	Age	0.89	0.02	0.76	0.39
	Sex	0.008	0.01	0.42	0.52
	Location	0.03	0.02	2.77	0.10
Northern	Age	-0.11	0.14	0.58	0.45
	Sex	0.23	0.13	3.22	0.07
	Location	-0.29	0.14	3.87	0.049*
Magnolia Warbler	Age	-0.17	0.12	1.81	0.18
	Sex	-0.13	0.12	13.16	0.001*
	Location	-0.49	0.13	13.20	0.0003

TABLE 3. Linear mixed model analysis of the effects of energetic condition, handling time, sampling date, and time of day on testosterone. Model effect estimates and standard error is given for each species and variable. Chi-squared statistics derive from likelihood ratio tests that compared a model with the variable in question to the null model. Asterisks denote statistical significance of a variable.

Species	Fixed Effect	Model Statistics		Comparison to Null Model	
		Estimate	SE	χ^2	<i>P</i>
Swainson's Thrush	Energetic	0.003	0.002	0.46	0.50
	Handling Time	-0.0009	0.003	0.10	0.76
	Date	0.0004	0.0005	0.65	0.42
	Time of Day	0.003	0.002	0.74	0.39
Northern	Energetic	0.005	0.04	0.01	0.91
	Handling Time	0.07	0.04	5.00	0.03*
	Date	0.01	0.005	5.18	0.02*
	Time of Day	-0.003	0.02	0.02	0.90
Magnolia Warbler	Energetic	0.007	0.07	0.01	0.92
	Handling Time	0.008	0.03	3.69	0.06
	Date	0.02	0.004	10.53	0.001*
	Time of Day	-0.008	0.02	0.25	0.62

TABLE 4. Linear mixed model analysis of testosterone and foraging competition. Testosterone was compared to the total number of other migrants and to the number of migrants within each species' foraging group (see methods). Statistics derive from likelihood ratio tests that compared a model with the variable in question to the null model.

Species	Comparison	Louisiana		Maine	
		χ^2	<i>P</i>	χ^2	<i>P</i>
Swainson's Thrush	Total	0.26	0.61	1.52	0.22
	Foraging Group	0.05	0.82	1.13	0.29
Northern Waterthrush	Total	0.33	0.57	3.30	0.07
	Foraging Group	0.14	0.71	1.14	0.29
Magnolia Warbler	Total	0.33	0.57	0.14	0.71
	Foraging Group	0.37	0.54	0.35	0.55

Figure 1

Figure 2

Figure 3