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Patterns of testosterone in three Nearctic–Neotropical migratory songbirds during spring passage

Kristen M. Covino [a,b,*], Sara R. Morris [b,c], Frank R. Moore [a]

[a] Department of Biological Sciences, University of Southern Mississippi, 118 College Drive Box 5018, Hattiesburg, MS 39406, USA
[b] Shoals Marine Laboratory, 102 Chase Ocean Engineering Lab, 24 Colovos Rd, Durham, NH 03824, USA
[c] Biology Department, Canisius College, 2001 Main St., Buffalo, NY 14208, USA

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ABSTRACT

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

1. Introduction

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

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* Corresponding author at: Department of Biological Sciences, University of Southern Mississippi, 118 College Drive Box 5018, Hattiesburg, MS 39406, USA.
E-mail address: kristen.covino@eagles.usm.edu (K.M. Covino).

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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; 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,b; 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,b; 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° 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 min.

We obtained a blood sample via brachial puncture from each individual within 10 min of extraction from a net (mean ± 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 min. Plasma was extracted and stored at −20 °C until analyzed. Red blood cells were placed in approximately 500 μl of lysis buffer (50 mM TRIS, 10 mM 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 0.01 g) 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 provided 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 μl 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 μl of ethanol and 300 μl of the assay buffer provided in the EIA kit. Recoveries were determined with 100 μl from each reconstituted sample. Samples were run in duplicate with 100 μl 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