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DISSERTATION RESEARCH: The Role of Breeding Hormones in Seasonal Interactions: How Do Birds Meet the Conflicting Demands of Breeding Preparation and Migration?

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Final Report for Period: 07/2011 - 06/2012**Submitted on:** 09/17/2012**Principal Investigator:** Holberton, Rebecca L.**Award ID:** 1011123**Organization:** University of Maine**Submitted By:**

Holberton, Rebecca - Principal Investigator

Title:

DISSERTATION RESEARCH: The Role of Breeding Hormones in Seasonal Interactions: How Do Birds Meet the Conflicting Demands of Breeding Preparation and Migration?

Project Participants

Senior Personnel

Name: Holberton, Rebecca**Worked for more than 160 Hours:** Yes**Contribution to Project:**

1) coordinated and supervised field and laboratory studies that included data collection and analyses, preparing results for publication, presenting results at professional meetings and to general public audiences, etc.; 2) mentored doctoral candidate Christopher Tonra in all aspects of research and professional development, 3) supervised undergraduate students that helped on the project.

Name: Tonra, Christopher**Worked for more than 160 Hours:** Yes**Contribution to Project:**

1) Collected and analyzed field data, performed all laboratory procedures, prepared manuscripts, presented findings at professional meetings and to the general public, 2) interacted with collaborators, and 3) applied for additional funding. Tonra successfully secured additional funds for the project from the Wilson Ornithological Society's Louis Agassiz Fuertes Award (\$2,500) and a University of Maine Graduate Student Government Award (\$800). He also won the 2011 UMaine School of Biology and Ecology Graduate Prize in Animal Biology and the Outstanding Student Presentation Award for presenting research from this grant at the American Ornithologists' Union Annual international meeting in 2011. Tonra was also awarded a Smithsonian Institution Pre-doctoral Fellowship for 2011 (1 yr appointment at the Smithsonian's Migratory Bird Center, \$29,000). Tonra is now a Post-doctoral Fellow at the Smithsonian Conservation Biology Institute.

Post-doc

Graduate Student

Undergraduate Student

Name: Randall, Jeaninne**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Randall assisted Tonra in the field with data collection in 2010. Randall gained valuable professional experience in collecting ecological data, tissue and blood sampling, data management, and participation in scientific discussions. Randall went on to graduate school at the University of Northern British Columbia in 2011.

Name: Rune, Sean**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Rune assisted Tonra in the field with data collection in 2011. Rune gained valuable professional experience in collecting ecological data, tissue and blood sampling, data management, and participating in scientific discussions.

Technician, Programmer

Other Participant**Research Experience for Undergraduates****Organizational Partners**

Jamaica Nat'l Environl Protection Agency

Smithsonian Migratory Bird Center

The Field Museum

Other Collaborators or Contacts

Dr. Peter Marra, Smithsonian Migratory Bird Center

Dr. David Willard, The Field Museum

Activities and Findings**Research and Education Activities:**

Research and Education Activities:

Overview - Previous work from Tonra's dissertation indicated that male American Redstarts (*Setophaga ruticilla*), a long distance migratory bird, begin increasing the gonadal steroid testosterone (hereafter: T) prior to departing from its wintering site in Jamaica, West Indies (Tonra et al. in review). Furthermore, Tonra found that early arriving males at a breeding site in New Hampshire have higher circulating T than late arrivers (Tonra et al. 2011b). This led to Tonra completing a captive experiment on Dark-eyed juncos (*Junco hyemalis*) demonstrating that increasing circulating T early on in the transition from the non-breeding to breeding life history stages advances the timing of migratory preparation (Tonra et al. 2011a).

These results raised questions about the effect such a manipulation would have in wild birds in terms of their ability to migrate earlier than competitors, a major component of breeding success in redstarts. Furthermore, Tonra wished to broaden this study to multiple species, to determine the extent to which these patterns are consistent among species, and to expand the study to the evolution of this migratory life history stage. Our goals for this project were to: 1) examine to what extent the transition between non-breeding and breeding life history stages is modulated by ecological and endogenous factors, and 2) determine if hormones usually associated with breeding readiness can also play an important role in migration by facilitating the ability to arrive earlier at the breeding grounds.

Several predictions arising from the proposed model on the ecophysiological aspects of seasonal interactions in migratory birds were tested through an experiment in which T was manipulated in the American Redstart (*Setophaga ruticilla*) on its wintering grounds in Jamaica, West Indies, during the period leading up to spring departure. Specifically, male redstarts were assigned to one of two treatment groups: implanted with exogenous T to advance and enhance plasma T levels, or implanted with empty implants as controls. The feasibility of using these implants was successfully demonstrated in an earlier pilot study in free-living redstarts. Treatment effects on migratory condition and foraging behavior, including fattening and departure dates, as well as breeding readiness (cloacal protuberance development) were examined during the spring departure period.

To begin to expand the model to other species, measures of breeding readiness and migratory condition were measured in several additional Neotropical migrant species wintering at the same site. These data are supplemented with corresponding museum specimens of migratory songbirds collected at a site en route during spring migration. Feather and/or claw samples were used to link events on the wintering grounds with migration distance to the breeding grounds, as determined by the stable isotope signatures incorporated in these tissues during previous stages. Collectively, the study's intellectual merit lies in the fact that the work has, for the first time, tested hypotheses about relationships among time, energy, breeding preparation, and migration distance across species with different life history constraints, as well as specifically examine the potential dual role of testosterone as a 'migration hormone' as well as a 'breeding hormone'.

Hypotheses, Predictions and Tests

Component I ? Experimental field study of the role that elevated T plays in wintering redstarts

Hypothesis I: As a function of its role in supporting greater energy demand, for migration as well as breeding, through its promotion of skeletal muscle development and red blood cell production, elevated T during the pre-migratory period enhances the rate of migratory preparation while

simultaneously enhancing the rate of breeding preparation on the wintering grounds.

Prediction I-1: Male redstarts with experimentally elevated T during the transition period from wintering to migration will gain mass at a faster rate than control males. Development of the cloacal protuberance (CP), a T-dependent organ, will follow the same pattern. Lastly, both breeding and migratory behavior will be more advanced in males with elevated T.

Tests I-1: In 2010 and 2011, we conducted a T implantation study that included only adult male redstarts. During the first week of April, when we observe males beginning to increase in mass and fat as spring departure approaches (C. Tonra and P. Marra unpubl. data), we captured male redstarts and assigned them to one of two experimental treatments: procedural control or T-implanted. Each male received a subcutaneous silicon implant along the flank. We used a novel "pita" design of hormone implants that reduces friction with the skin and lays flat along the contour of the body (implant size approx. 0.7 X 0.4 X 1.5 mm). To construct implants, we first rolled out a thin layer (< 0.5 mm) of non-toxic 100% silicone (GE? Silicone II TM clear caulking) onto wax paper. After the Silicone sheet dried, we made a "U"-shaped Silicone bead on one-half of the Silicone sheet, with the "open end" of the "U" at the point where the silicone sheet would later be folded over. We carefully filled the interior space of the "U" with the hormone or left it empty. We then folded the thin sheet over the bead, and pressed down along the bead margins to create the "pita" pocket. After allowing the silicone to set for approximately 30 min, we trimmed away excess silicone from around the bead. This resulted in a half-moon shaped implant (approx 0.7 X 0.4 mm) that was thin and flat on one side and slightly bulging (approx. 1.5 mm at its thickest) on the other. Prior to implantation, the implants were punctured once, to facilitate delivery, with a 27-ga needle and stored in sterile saline for > 24 h. We temporarily numbed the area on the bird's flank (under the wing) by holding ice to it for 15-20 s. The area was then gently patted dry. For each implant, we made a small (1-2 mm) incision, using aseptic technique with finely tipped iris scissors, in the skin under the wing with small. We then used a blunt metal probe to gently loosen the skin from the underlying connective tissue, slid the implant under the skin with the flat side against the body, and sealed the incision with a liquid bandage solution (New-Skin?, Prestige Brands, Inc.). The bird was not released until the area was completely sealed and dry. Recaptures of implanted individuals confirmed that healing was completed within 4-5 days. "T-implanted" birds received implants packed with T propionate (product# T-1875; Sigma-Aldrich? Co., St. Louis, MO, USA) and "control" birds received an empty implant. T-implants were intended to elevate T levels enough to effectively "flood" available receptors, and thus produce a response indicative of maximal binding. It should be noted that T levels were well above those observed at this time of year in this species, but as receptor densities change seasonally in birds (Soma et al. 1999), responses to the T treatment demonstrate the full capacity of males to respond physiologically and behaviorally to high circulating T.

Upon first capture, we measured all baseline condition variables and implanted birds, alternating between treatments with each capture. We measured baseline values for body mass, fat score (following Tonra et al. 2011b), and CP diameter, and assigned a pectoral muscle score to measure anabolism. We then attempted to recapture all individuals at least 12 days after implantation (range = 12-17 days). Upon recapture, we re-measured all condition variables. In 2010, we took a 50μL blood sample and measured circulating T as above on both capture occasions to verify that implants were effective.

To test the prediction that T-implanted birds would have more advanced migratory behavior, we conducted foraging observations to estimate prey searching rate as an index of pre-migratory hyperphagia, beginning at least 5 d after implantation. Most (85%) of the observations were completed within 6 h of sunrise. We first identified a focal bird, confirmed that it was foraging, and followed it until it was no longer in line of sight. The observer dictated all movements of the bird into a digital voice recorder. Prey searching movements were categorized as leg powered (hops) or wing powered (flights) movements. As a behavioral index of breeding development, singing behavior was recorded over a longer observation bout, which may have encompassed several foraging observations. We discarded all foraging observations less than 30 s, and all singing observations less than 2 min. All movements and vocalizations were divided by total observation time, and multiple observations for an individual were averaged for each day resulting in average movement or song per minute per individual per day. For foraging and singing behavior, we excluded individuals for whom we did not have at least 7 min over 2 d, and at least 12 min over 3 d, respectively. After excluding these observations, we completed 191 foraging observations totaling 260 min from 17 individuals (9 control, 8 T-implanted), and 106 singing observations totaling 481 min from 19 individuals (9 control, 10 T-implanted).

We used repeated measures linear mixed models (lmer function in R v. 2.11.1; R Core Development Team 1999; Zuur et al. 2009) to test the prediction that, compared to controls, T-implanted birds would have a greater increase in all three migratory (body mass, fat score, breast muscle score) and breeding (CP diameter) preparation metrics. To assess the significance of each variable, we iteratively removed it from the model and compared the reduced model to a model of only the main effects (for testing main effects) or to the full model (for testing interactions) by using a likelihood ratio test (Zuur et al. 2009). We ran preliminary models to screen for any year dependence in the relationship between treatment and capture number, and did not find any effect (capture number*treatment*year: mass, $\chi^2 = 0.006$, $df = 1$, $P = .939$; fat score, $\chi^2 = 0.329$, $df = 1$, $P = .567$; breast muscle score, $\chi^2 = 0.784$, $df = 1$, $P = .376$; CP diameter, $\chi^2 = 1.249$, $df = 1$, $P = .264$), thus we combined years for this analysis. For each model we included individual as the random effect, and treatment (T-implanted, control), capture number (pre-, post-implant), and the treatment by capture number interaction as fixed effects. The number of days between captures was included as a covariate. To compare T and baseline values of dependant variables we used simple non-parametric (for ordinal and small sample size data; Kolmogorov-Smirnov Z, Mann Whitney U) and parametric (t-test) tests. To test the prediction that T-implanted birds would have a greater prey searching and singing rate than controls (i.e. more advanced hyperphagia), we used linear mixed models

Prediction I-2: Male redstarts with experimentally elevated T will depart earlier on spring migration than control males, and control males will depart earlier than T-blocked males.

Test I-2: We estimated departure dates following Studds and Marra (2011) by visiting territories every two days from 1 April to 15 May. We are confident this method reflects migratory departure and not mortality because of high detection probabilities (Studds and Marra 2011).

Further, we performed a pilot study in 2009 where 8 male redstarts were implanted with T in February and 7 of these were re-sighted, without rigorous searching, at least 2 months after implantation.

Alternative outcomes: A result where T-elevated birds prepare for and depart earlier than controls would indicate that T plays an important role in enhancing migratory behavior. However, if T-elevated birds significantly delayed departure compared to controls, this would indicate that such levels are relevant only in a breeding context and not adaptive this early in the migration period. Lastly, a result where control and T-elevated individuals show similar patterns of mass gain and departure schedule would indicate that, if T does enhance migratory preparation, it is probably not the primary driver. T instead may act in concert with other hormones, such as corticosterone (Holberton et al. 2007), which has been shown to regulate migratory preparation.

Component II - Multi-species comparison of breeding hormone activity prior to spring arrival

Hypothesis IIa: Neotropical migrant males begin breeding preparation while on the non-breeding grounds and the level of preparation is greatest for males in superior condition.

Prediction IIa: Male warblers will increase circulating T prior to departing wintering sites, and males in better late-winter condition will have higher T.

Test IIa: We collected blood samples in Jamaica from 2008-2010 from males of the following migrant species: American Redstart, Black and White Warbler (*Mniotilta varia*), Northern Waterthrush (*Seiurus noveboracensis*), and Ovenbird (*Seiurus aurocapillus*). These species represent various points in the continuum of life history and ecological variation in migratory Paruline warblers. We determined age and sex (when possible), measured condition (as above), and collected one tail feather and a plasma T sample. For species that could not be sexed in the field, we sent the cellular portion of the blood to Avian Biotech?, Inc., Tallahassee, FL, a commercial avian sexing company (paid for by grants previously received by Tonra). We first looked at circulating T in early- (15 Jan ? 28 Feb) and late-winter (15 March-30 April) to determine if males show evidence of breeding preparation by increasing circulating breeding hormones. We then compared late-winter circulating T to size-corrected mass in order to determine if breeding preparation was related to body-condition. In redstarts only, we also compared late-winter T to migration distance, and thus likely to be more under endogenous control. As a result of predictable latitudinal patterns in the stable hydrogen isotope content of precipitation, and the uptake of these isotopes into feathers grown prior to departure from breeding areas, measurement of stable hydrogen-isotope ratios ($\delta^2\text{H}$) in feathers can be used to estimate the latitude of a wintering bird's breeding area (Hobson and Wassenaar 1997). Given the prevalence of breeding site fidelity in migratory songbirds, the distance between this estimated location and Jamaica can be inferred as the distance an individual has to migrate. Stable isotope ratios were measured by Isotope Ratio Mass Spectrometry (IRMS; Clark and Fritz 1997) at the Smithsonian Institute Stable Isotope Mass Spectrometry Facility in Suitland, MD, by Tonra. Circulating T was measured by radioimmunoassay by Tonra in the Lab of Avian Biology at University of Maine. We used linear mixed models as above to analyze changes in circulating T while controlling for random individual effects and year, and partial correlation to test for relationships between condition, $\delta^2\text{H}$, and late-winter T.

Alternative outcomes: Results showing no effect of winter period on T in any species, would indicate that breeding preparation is not occurring in migratory males prior to spring departure, and is more likely taking place on migration. A failure to find a relationship between late T and condition or a negative relationship between T and migration distance would indicate that endogenous mechanisms are playing a stronger role in breeding preparation than environmental mechanisms.

Hypothesis IIb: Winter habitat quality influences level of breeding preparation in migrating males.

Prediction IIb: Migrating warblers from high quality breeding habitats will have larger gonads than those from low quality habitats, independent of migration distance.

Test IIb: Through collaboration with the Field Museum, Chicago, IL, we acquired both feather and claw samples from museum specimens. These specimens were collected as a result of mortality on migration caused by collisions with buildings in Chicago in 2010 and 2011.

Deuterium ($\delta^2\text{H}$) in feather samples was used to estimate distance from breeding sites as above. $\delta^{13}\text{C}$ in claw tissue was used to infer non-breeding habitat quality (wetness; Reudink et al. 2009). Breeding preparation was estimated based on the gonad volume (Bauchinger et al. 2009), which was measured in each specimen by the Field Museum. We used an AIC model selection approach to determine the relative contributions of the independent variables migration distance, winter habitat quality, and year to the variation in gonad size on migration.

Alternative outcomes: A result where migration distance is the most important explanatory variable would indicate that endogenous control mechanisms are more important in determining the timing of breeding preparation than non-breeding conditions.

Findings: (See PDF version submitted by PI at the end of the report)

Findings:

Component I- Experimental field study of the role that elevated T plays in wintering redstarts

Hypothesis I: Elevated T during the pre-migratory period enhances migratory preparation while simultaneously enhancing the rate of breeding preparation on the wintering grounds.

Tests I-1: We implanted a total of 28 redstarts in the two years of the study (2010: 4 control, 5 T-implanted; 2011: 9 control, 10 T-implanted). We were able to recapture 10 birds in each treatment group following implantation with either empty or T-packed implants (3 of each in 2010, 7 of each in 2011). Blood sampling from 6 individuals in 2010 (3 in each treatment) before and after implantation revealed that treatments did not differ in circulating T prior to implantation (control: mean \pm SE = 0.39 \pm 0.05 ng/mL, n = 3; T-implanted: mean \pm SE = 0.45 \pm 0.05 ng/mL, n = 3; Mann Whitney U = 2.00, P = 0.275) but T-implanted males had greater circulating T post-implantation (control: mean \pm SE = 0.52 \pm 0.10 ng/mL,

T-implanted: mean \pm SE = 20.97 \pm 7.15 ng/mL; Mann Whitney U = 0.00, P = 0.05).

Experimentally elevating T advanced preparation for both migration and breeding, resulting in earlier departure on spring migration. At the start of the experiment, treatment groups did not differ in mass ($t = -0.177$, $df = 18$, $P = .861$), fat score (Kolmogorov-Smirnov $Z = 0.224$, $n = 20$, $P = 1.00$), breast muscle ($Z = 0.447$, $n = 20$, $P = 0.988$), or CP diameter ($t = -0.917$, $df = 18$, $P = 0.371$). T-implanted birds increased more than controls in every migratory preparation metric (capture number X treatment: mass, $\chi^2 = 7.286$, $df = 1$, $P = 0.007$; fat score, $\chi^2 = 5.860$, $df = 1$, $P = 0.015$; breast muscle score $\chi^2 = 25.126$, $df = 1$, $P < 0.001$; Figure 1a-c) and CP diameter (capture number X treatment: $\chi^2 = 48.175$, $df = 1$, $P < 0.001$; Figure 1d).

Both pre-migratory hyperphagia and breeding behavior were stimulated by elevating T. T-implanted males searched for prey at a higher rate than controls (treatment, $\chi^2 = 5.822$, $df = 1$, $P = 0.016$; year, $\chi^2 = 0.251$, $df = 1$, $P = 0.616$; treatment X year, $\chi^2 = 2.251$, $df = 1$, $P = 0.134$). We did not perform any analysis on singing rate because only T-implanted birds were observed singing during our observations ($n = 10$, mean singing rate \pm SE = 1.34 \pm 0.91 songs/individual/day; 7 of 10 individuals observed singing). However, one control male was observed singing outside of behavioral observations, and two others sang in response to playback when being recaptured.

Test I-2: We were able to model departure dates for 27 males (13 controls 14 T-implanted) males, although 3 males were censored from the Kaplan-Meier model because seasonal flooding on their territories interrupted our departure estimation. As predicted, T-implanted birds departed earlier (Mantel-Cox $\chi^2 = 7.403$, $n = 27$, $P = 0.007$; Figure 2), leaving on spring migration approximately 7 days before controls on average.

These results have been included in a manuscript currently under review in Behavioral Ecology.

Fig 1. Means (\pm SE) for three measures of migratory preparation: a) body mass, b) breast muscle score, and c) fat score, and d) a measure of breeding preparation, cloacal protuberance (CP) diameter, in control (filled circles; $n = 10$) and T-implanted (open circles; $n = 10$), for American Redstarts pre- and post-implantation in late-winter 2010 and 2011 in Jamaica, West Indies.

Component II - Multi-species comparison of breeding hormone activity prior to spring arrival

Hypothesis IIa: Neotropical migrant males begin breeding preparation while on the non-breeding grounds and the level of preparation is greatest for males in superior condition.

Test IIa: For this test we analyzed blood samples collected over three years (2008-2010) from 98 male Northern Waterthrushes and 66 male Ovenbirds, and over two years (2009-2010) from 163 male American Redstarts, and 84 male Black and White Warblers. We did not capture an adequate sample of Black-throated blue warblers for analysis. We pre-screened the dataset for age effects, and only found an effect of age in redstarts, thus we analyzed young and adult males separately for this species.

We found significant increases in circulating T from mid- (15 Jan \pm 28 Feb) to late- winter (15 March - 30 April) in male waterthrushes ($\chi^2 = 19.68$, $df = 1$, $P < 0.001$; Fig 3), Black and White Warblers ($\chi^2 = 6.87$, $df = 1$, $P = 0.01$; Fig 3), and adult American Redstarts ($\chi^2 = 11.92$, $df = 1$, $P = 0.001$; Fig 3). We did not however find increases in male Ovenbirds ($\chi^2 = 0.00$, $df = 1$, $P = 0.99$; Fig 3) or young male redstarts ($\chi^2 = 0.76$, $df = 1$, $P = 0.39$). For the species and ages that exhibited an increase, we only found a relationship between condition (size-corrected mass) and late-winter T in adult redstarts ($r = 0.48$, $n = 56$, $P < 0.001$; waterthrush: $r = -0.18$, $n = 47$, $P = 0.24$; Black and White Warbler: $r = -0.06$, $n = 49$, $P = 0.68$). We then examined the relationship between migration distance (as measured by χ^2) and late-winter T in adult redstart males and found no relationship ($r = 0.02$, $n = 66$, $P = 0.87$). Thus we concluded that preparation for breeding in three of these four species begins prior to departure from wintering sites. Furthermore, there is evidence for substantial variation among species in initiation of breeding preparation and the potential for environmental limitation of development manifest through body condition. The implication of this portion of the study is that studies of breeding limitation need to include a focus on events occurring prior to arrival of migratory birds at breeding sites.

The redstart portion of this test was included in a manuscript currently under review in Behavioral Ecology. We will include the analyses on all of these species in an invited chapter in Studies in Avian Biology featuring studying migratory birds across the full life-cycle.

Test IIb: We received 303 tissue samples from birds collected after collision with buildings in Chicago from spring migration in 2010 and 2011.

The only species with enough sample size for multivariate models was Ovenbird ($n = 101$ males, 81 females). We have also received enough samples for Black and White Warblers ($n = 22$ males, 18 females) and an additional migrant from the family Turdidae, Wood Thrush (*Hylocichla mustelina*; $n = 23$ males, 16 females), to perform some univariate analyses. Our initial analyses, while revealing very interesting patterns, also uncovered substantial annual variation. Therefore we have decided, along with our collaborators at the Field Museum, to analyze another year of samples. This will also potentially give us enough samples from thrushes and Black and White Warblers to perform multivariate analyses on these species. Tonra will be able to perform the isotope analysis at the Smithsonian since he is a Stable-isotope Post-doctoral Fellow there through 2013.

In our analyses thus far, we have found support for both endogenous and exogenous factors explaining variation in breeding preparation. We performed separate model selections for male and female Ovenbirds with models composed of the dependant variable gonad volume and the explanatory variables Julian date, year, breeding latitude (δ), and winter habitat wetness (δ ;13C). The top model explaining variation in male Ovenbird testis size, which had a 57% chance (based on Akaike weight) of being the best model, included δ , δ ;13C, year, and the δ ;13C X year interaction as explanatory variables. The next best model, which included the same explanatory variables except for δ , had only an 18% chance of being the top model. All but two of the top ten models included δ ;13C and all but four included δ . Closer examination of the data revealed that in 2010 δ ;13C, but not δ , was related to testes volume in 2010, but the opposite was true in 2011 (Fig. 4). For female ovenbirds, the top model with a 21% chance (based on Akaike weight) of being the best model, was the null model, indicating that these variables do not do a good job of explaining variation in ovary volume. However, there was a relationship between δ and ovary volume in 2011 ($R^2 = 0.18$, $n = 45$, $P = 0.004$). Thus far, it appears that the factors affecting breeding development in this migratory species can vary year to year, potentially coinciding with variation in environmental conditions. Another year of data will enable us to further examine such annual variation and replicate this model selection in two additional species

Conclusions:

Scientists and naturalists have commonly defined life history stages based on geographic location. For example, a redstart seen in Jamaica is in the non-breeding stage, in Florida the migration stage, in Ontario the breeding stage. However, life history stages are defined by events occurring within the individual not by their movements or locations. Although stages may not completely overlap, one stage may begin physiologically while another is already underway (Ramenofsky and Wingfield 2006) leading to seasonal interactions (e.g. Marra et al. 1998). In fact, demographic parameters of migratory populations can be driven by such interactions between events occurring thousands of kilometers apart (e.g. Marra et al. 1998; Gunnarsson et al. 2005). Thus, determining when transitions between stages begin within individuals is crucial to determining when and where events affecting that stage may occur, and how stages may interact. While a given stage may largely be completed in one location, conditions in a different location, where it commences, could play a critical role in an individual's ability to complete that stage. Thus, assessing what environmental perturbations may have greatest impact on a population requires a detailed understanding of this aspect of an organism's physiology and behavior.

For example, while global climate change models predict increasing precipitation and temperature on redstart breeding grounds, on their wintering grounds the major predicted change is a dramatic decline in precipitation (Neelin et al. 2006). Here in this study, we show that male redstarts are capable of beginning the transition to breeding while still on the wintering grounds, presumably impacting an important determinant of reproductive output: the timing of arrival at breeding sites (e.g. Marra et al. 1998; Reudink et al. 2009; Tonra et al. 2011a). Thus, it would appear for this species that research on adaptation to climate change should focus as much on the impacts of drought on migration phenology and breeding onset on their tropical wintering grounds as on the impacts of warmer temperatures on breeding success on their temperate breeding grounds. This illustrates the critical need for research that focuses on the entire life-cycle of organisms.

Our results also point to substantial species specific and annual variation in these phenomena. This highlights the need for more extensive study across species with variable life-history strategies, ideally spanning many years. Successful full life-cycle management will be critically

dependant on such detailed information.
(a pdf file that includes figures has been uploaded)

Training and Development:

We are committed to disseminating knowledge gained from this research to enhance environmental awareness and scientific understanding. Our field site in Jamaica has hosted several focused research projects as well as an ongoing long-term (LTREB) project established by Marra and T. Sherry. Tonra supervised and trained a large field crew of as many as seven field assistants. Holberton and Tonra mentored undergraduate and graduate assistants in all aspects of educational and professional development. In addition to training assistants in field techniques and data management in Jamaica, Tonra held a weekly reading group in which the field crew discussed current literature. This provided a forum in which assistants related their daily activities to the development and testing of hypotheses exploring ecological phenomena. In addition, this was an opportunity for assistants to develop skills in critical reading and interpreting scientific literature. Tonra worked particularly closely with research volunteers Randall and Rune, whom he trained in multiple ecological field methods. He frequently discussed this research and broader topics with them throughout the project. Tonra maintains contact with previous assistants and continues to provide them with advice on employment directions and applying to graduate programs. Randall went on to the graduate program at the University of Northern British Columbia following her time on this project. In Jamaica, Tonra interacted with the local community and conveyed the goals and importance of the research. He also interacted with the National Environmental Protection Agency of Jamaica, as well as the Petroleum Company of Jamaica, which is the landowner of the field site. Holberton continues to present the work on this project to local and regional Audubon and US Fish & Wildlife agencies to encourage greater partnerships for managing migratory bird populations.

Outreach Activities:

Tonra participated in a demonstration of field techniques for studying wild birds open to the general public as part of the 2011 International Migratory Bird Day celebration at Rock Creek National Park, Washington D.C. He has co-led two field trips for Smithsonian Associates during which he made presentations about migratory bird research in general, as well as his research activities. He also participated in conservation meetings with Marra regarding the preservation of our Jamaica field site. These meetings were generally held with local business owners and members of local NGOs.

Journal Publications

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Product Type:

presentations

Product Description:

Product Type: Oral Presentation (Tonra)

Ecological Society of America Annual Conference, Pittsburgh, PA (2010) Balancing the demands of migration and the physiological transition to breeding in Neotropical songbirds: a dual role for testosterone? (P.P. Marra and R.L. Holberton, co-authors).

Product Description: Tonra presented preliminary results from the current study, as well as results from his previous dissertation research, to a broad audience of ecologists and evolutionary biologists as part of a session on animal migration

Abstract:

Migratory birds face a conflicting set of energetic demands between surviving the winter, preparing to migrate and preparing to breed. Physiological mechanisms that minimize conflicts, perhaps through diversifying hormone function, would, if achieved, be advantageous to both survival and breeding success. We hypothesized that the primary male breeding hormone, testosterone, enhances both migratory and breeding preparation prior to departure on spring migration, resulting in early arrival and higher breeding success for males with high testosterone. We

measured circulating testosterone in non-breeding male American redstarts (*Setophaga ruticilla*) in Jamaica, West Indies throughout winter and during arrival at breeding territories in New Hampshire, U.S.A. Further, we experimentally manipulated testosterone during migratory preparation in captive male dark-eyed juncos (*Junco hyemalis*). Our results from free living redstarts demonstrate that testosterone increases prior to spring departure from winter quarters and that male redstarts with higher testosterone arrive at the breeding grounds earlier than those with lower testosterone and early arrivers are more likely to successfully breed. Further, early arriving males were more likely to be from high quality winter habitats (as measured by stable-carbon isotopes in claws). Our experimental study indicated that testosterone enhances migratory preparation through its anabolic and erythropoietic effects. Males with experimentally elevated testosterone achieved peak migratory condition (measured by mass, food intake, Zugunruhe, haematocrit, and fat stores) and peak breeding condition (measured by cloacal protuberance development) approximately six days before controls. Males in which the ability of testosterone to bind to receptors was blocked did not begin migratory or breeding preparation until twelve days after controls. This work reveals a potential physiological mechanism by which winter to breeding seasonal interactions occur in migratory birds.

Sharing Information:

this was presented at an national ornithological meeting

Product Type:

presentations

Product Description:

Product Type: Upcoming Oral Presentation (Tonra)

American Ornithologist's Union Annual Conference, Jacksonville, FL (July 26-29, 2011) The role of testosterone in seasonal interactions: Observational and experimental studies in the American redstart (P.P. Marra and R.L. Holberton, co-authors).

Product Description: Tonra will be presenting cumulative results from his dissertation research to a broad ornithological audience as part of a session on behavior. He has entered this presentation for a Student Presentation Award.

Abstract:

Migratory birds face a conflicting set of energetic demands between preparing to migrate and preparing to breed. Physiological mechanisms that minimize conflicts, perhaps through diversifying hormone function, would be advantageous to both survival and breeding success. We sought to determine if variation in production of testosterone (T) could mediate winter to breeding carry-over effects in male migratory birds. In an observational study, we demonstrated that male American Redstarts (*Setophaga ruticilla*) increase T production before departure from Jamaica, and that males in better late winter condition have higher T. Further, we demonstrated that, compared to late arrivers, early arriving males at a breeding site in New Hampshire had higher T, were from higher quality winter habitat, were in superior migratory condition, and ultimately were more likely to successfully breed. In a hormone manipulation experiment, we showed that males with elevated T in late winter prepare to migrate faster (measured by change in mass, fat, and breast muscle) and depart on migration earlier. These results demonstrate that early elevation of T could facilitate early arrival at breeding areas, ultimately increasing fitness.

Sharing Information:

This is scheduled to be presented at a national meeting.

Product Type:

manuscript in review

Product Description:

Tonra, C. M., Marra, P. P., and Holberton, R. L. In review. The overlap and interaction of multiple life history stages: observational and experimental studies in a migratory bird. *Behavioral Ecology*. (This manuscript includes all of Hypothesis I and the redstart portions of Hypothesis IIa.)

Sharing Information:

This manuscript will be published in a peer-reviewed journal.

Product Type:

Book chapter

Product Description:

Tonra, C.M., Marra, P.P. and Holberton, R.L. In prep. The nexus of non-breeding, migratory, and breeding life history stages in migratory birds. For a *Studies in Avian Biology* issue dedicated to full life-cycle biology. (This chapter will be a synthesis of many of the topics from Tonra's dissertation, a detailed literature review and will feature the results from Hypothesis IIa as well as a review of previously published results, including those from Hypothesis Ia, once published elsewhere.)

Sharing Information:

This chapter will be published in a broadly read book.

Product Type:**Presentation at scientific meeting****Product Description:**

Invited Symposium Presentation (Tonra)

5th North American Ornithological Conference, Vancouver, BC (2012) The Nexus of Non-breeding, Migratory, and Breeding Life History Stages in Migratory Songbirds (P.P. Marra, D. Willard, and R.L. Holberton, co-authors). Symposium: Hot Topics in the Tropics: Research to Support Full Life-cycle Conservation of Migrants.

Abstract:

In spring, migratory birds are at the juxtaposition of three life history stages in which they must complete the non-breeding stage, initiate and complete migration, and initiate breeding. For many species, this transitional period is critical because the timing of arrival at breeding areas can influence reproductive output. We sought to determine a) when physiological preparation for breeding begins, b) if variation in breeding preparation is better explained by endogenous or environmental factors, and c) if breeding preparation can influence migration phenology. We found that male American Redstarts (*Setophaga ruticilla*) wintering in Jamaica show evidence of breeding preparation prior to departure from wintering grounds while females do not. In both these male redstarts and migrating Ovenbirds (*Seiurus aurocapilla*) collected in Chicago, we found that variation in breeding preparation (measured by circulating testosterone, T, and testis size) was better explained by environmental factors (condition, winter habitat wetness measured by stable-carbon isotopes) than endogenous timing (distance to breeding site measured by stable-hydrogen isotopes). Lastly, we demonstrated experimentally that male redstarts who elevate T earlier than competitors are more likely to depart earlier on migration, presumably enabling them to arrive earlier to breed, a strong determinant of reproductive success. These results demonstrate that life history stages greatly overlap for migratory birds and can interact with one another to potentially influence fitness. Our findings have implications for the ability of these species to respond to changing environments.

Sharing Information:

Tonra presented cumulative results from his doctoral research in the context of managing populations across their entire life-cycle. His portion of the symposium focused on how multiple life-history stages overlap and interact with one another, highlighting the need to understand such interactions to know when and where particular vital rates may be regulated and limited.

Product Type:**Presentation at scientific meeting****Product Description:**

Oral Presentation (Tonra)

Ecological Society of America Annual Conference, Pittsburgh, PA (2010) Balancing the Demands of Migration and the Physiological Transition to Breeding in Neotropical Songbirds: A Dual Role for Testosterone? (P.P. Marra and R.L. Holberton, co-authors).

Abstract:

Migratory birds face a conflicting set of energetic demands between surviving the winter, preparing to migrate and preparing to breed. Physiological mechanisms that minimize conflicts, perhaps through diversifying hormone function, would, if achieved, be advantageous to both survival and breeding success. We hypothesized that the primary male breeding hormone, testosterone, enhances both migratory and breeding preparation prior to departure on spring migration, resulting in early arrival and higher breeding success for males with high testosterone. We measured circulating testosterone in non-breeding male American Redstarts (*Setophaga ruticilla*) in Jamaica, West Indies throughout winter and during arrival at breeding territories in New Hampshire, U.S.A. Further, we experimentally manipulated testosterone during migratory preparation in captive male dark-eyed juncos (*Junco hyemalis*). Our results from free living redstarts demonstrate that testosterone increases prior to spring departure from winter quarters and that male redstarts with higher testosterone arrive at the breeding grounds earlier than those with lower testosterone and early arrivers are more likely to successfully breed. Further, early arriving males were more likely to be from high quality winter habitats (as measured by stable-carbon isotopes in claws). Our experimental study indicated that testosterone enhances migratory preparation through its anabolic and erythropoietic effects. Males with experimentally elevated testosterone achieved peak migratory condition (measured by mass, food intake, Zugunruhe, haematocrit, and fat stores) and peak breeding condition (measured by cloacal protuberance development) approximately six days before controls. Males in which the ability of testosterone to bind to receptors was blocked did not begin migratory or breeding preparation until twelve days after controls. This work reveals a potential physiological mechanism by which winter to breeding seasonal interactions occur in migratory birds.

Sharing Information:

Tonra presented preliminary results from the current study, as well as results from his previous dissertation research, to a broad audience of ecologists and evolutionary biologists as part of a session on animal migration

Product Type:**Presentation at scientific meeting****Product Description:**

Oral Presentation (Tonra)

American Ornithologists' Union Annual Conference, Jacksonville, FL (2011) The Role of Testosterone in Seasonal Interactions: Observational and Experimental Studies in the American Redstart (P.P. Marra and R.L. Holberton, co-authors).*

Abstract:

Migratory birds face a conflicting set of energetic demands between preparing to migrate and preparing to breed. Physiological mechanisms that minimize conflicts, perhaps through diversifying hormone function, would be advantageous to both survival and breeding success. We sought to determine if variation in production of testosterone (T) could mediate winter to breeding carry-over effects in male migratory birds. In an observational study, we demonstrated that male American Redstarts (*Setophaga ruticilla*) increase T production before departure from Jamaica, and that males in better late winter condition have higher T. Further, we demonstrated that, compared to late arrivers, early arriving males at a breeding site in New Hampshire had higher T, were from higher quality winter habitat, were in superior migratory condition, and ultimately were more likely to successfully breed. In a hormone manipulation experiment, we showed that males with elevated T in late winter prepare to migrate faster (measured by change in mass, fat, and breast muscle) and depart on migration earlier. These results demonstrate that early elevation of T could facilitate early arrival at breeding areas, ultimately increasing fitness.

Sharing Information:

Tonra presented cumulative results from his dissertation research to a broad ornithological audience as part of a session on behavior.

* Tonra was awarded the Outstanding Student Research Presentation Award for this presentation

Contributions**Contributions within Discipline:**

This project is the first to study underlying mechanisms (integrating physiological, ecological, behavioral) of migration phenology and seasonal interactions in a migratory bird. This approach was novel, and has broad applicability to many other taxa, avian and non-avian.

Contributions to Other Disciplines:

The concept of seasonal interactions and carry-over effects is broadly applicable to not only evolutionary biology and ecological physiology, but also has implications for conservation and understanding biodiversity (via different life history strategies).

Public programs

- Tonra participated in a demonstration of field techniques for studying wild birds open to the general public as part of the 2011 International Migratory Bird Day celebration at Rock Creek National Park, Washington D.C. There he was able to engage local bird enthusiasts and discuss the findings of this research with them, while also demonstrating some of the research methods he employed.

- Tonra co-led two field trips with Smithsonian Associates during which he gave presentations to the participants from the public about migratory bird research and conservation.

- Throughout 2010, Holberton discussed this project and its findings to members of Maine's government and Maine's Governor's Ocean Energy Task Force group and subcommittees during the development of state regulations for offshore energy development.

Contributions to Human Resource Development:

We are committed to disseminating knowledge gained from this research to enhance environmental awareness and scientific understanding.

Our collaborative research program involves a national and international training component (post-docs, field assistants, wildlife biologists and governmental and NGOs in Jamaica) as well as graduate and undergraduate education. We continue to work with officials at the National Environmental Protection Agency of Jamaica, training wildlife officials in basic and applied research approaches to studies on migratory birds.

The activities of this particular work provided new experiences and opportunities to develop new skills for the research volunteers, J. Randall and S. Rune. Tonra traveled to the Smithsonian Institute and learned how to perform mass spectrometry analysis on bird claws and feathers to determine $\delta^{13}C$. In addition, Tonra attended the annual meeting of the Ecological Society of America in 2010, the American Ornithologists Union in 2011, and the 5th North American Ornithological Conference in 2012. At all these meetings he was able to make multiple contacts with researchers working on similar questions, but in widely ranging taxa, including beyond birds. The cumulative experience of this research has enabled Tonra to begin forming ideas about his future research goals beyond his degree and post-doctoral program.

Contributions to Resources for Research and Education:**Public programs**

- Tonra participated in a demonstration of field techniques for studying wild birds open to the general public as part of the 2011 International Migratory Bird Day celebration at Rock Creek National Park, Washington D.C. There he was able to engage local bird enthusiasts and discuss the findings of this research with them, while also demonstrating some of the research methods he employed.

- Throughout 2010, Holberton discussed this project and its findings to members of Maine's government and Maine's Governor's Ocean Energy Task Force group and subcommittees during the development of state regulations for offshore energy development.

The data from this project is being incorporated into the extensive Migratory Bird Database at the Laboratory of Avian Biology at University of Maine (Holberton). Plans are underway to make this an online searchable database, comprising ecophysiological data for over 3500 birds, available to the public. The work is also instrumental in illustrating, by Holberton, the importance of life cycle conservation to public education and policy makers.

Contributions Beyond Science and Engineering:

Understanding the factors that affect individual survival and reproduction is central to resource management and conservation. The 'redstart study system' has provided many opportunities for students who are not tracked in a science career to have a better understanding of natural systems, and the challenges facing resource managers. The work has been instrumental in providing a system for continued development of technology needed to track movements of small individual songbirds across the annual cycle. Holberton is currently working with developers of nano-tag radiotelemetry equipment to track songbirds along the Atlantic Flyway.

Conference Proceedings

Categories for which nothing is reported:

Any Journal

Any Book

Any Web/Internet Site

Any Conference

Findings:

Component I- Experimental field study of the role that elevated T plays in wintering redstarts

Hypothesis I: Elevated T during the pre-migratory period enhances migratory preparation while simultaneously enhancing the rate of breeding preparation on the wintering grounds.

Tests I-1: We implanted a total of 28 redstarts in the two years of the study (2010: 4 control, 5 T-implanted; 2011: 9 control, 10 T-implanted). We were able to recapture 10 birds in each treatment group following implantation with either empty or T-packed implants (3 of each in 2010, 7 of each in 2011). Blood sampling from 6 individuals in 2010 (3 in each treatment) before and after implantation revealed that treatments did not differ in circulating T prior to implantation (control: mean \pm SE = 0.39 ± 0.05 ng/mL, $n = 3$; T-implanted: mean \pm SE = 0.45 ± 0.05 ng/mL, $n = 3$; Mann Whitney U = 2.00, $P = 0.275$) but T-implanted males had greater circulating T post-implantation (control: mean \pm SE = 0.52 ± 0.10 ng/mL, T-implanted: mean \pm SE = 20.97 ± 7.15 ng/mL; Mann Whitney U = 0.00, $P = 0.05$).

Experimentally elevating T advanced preparation for both migration and breeding, resulting in earlier departure on spring migration. At the start of the experiment, treatment groups did not differ in mass ($t = -0.177$, $df = 18$, $P = .861$), fat score (Kolmogorov-Smirnov $Z = 0.224$, $n = 20$, $P = 1.00$), breast muscle ($Z = 0.447$, $n = 20$, $P = 0.988$), or CP diameter ($t = -0.917$, $df = 18$, $P = 0.371$). T-implanted birds increased more than controls in every migratory preparation metric (capture number X treatment: mass, $\chi^2 = 7.286$, $df = 1$, $P = 0.007$; fat score, $\chi^2 = 5.860$, $df = 1$, $P = 0.015$; breast muscle score $\chi^2 = 25.126$, $df = 1$, $P < 0.001$; Figure 1a-c) and CP diameter (capture number X treatment: $\chi^2 = 48.175$, $df = 1$, $P < 0.001$; Figure 1d).

Both pre-migratory hyperphagia and breeding behavior were stimulated by elevating T. T-implanted males searched for prey at a higher rate than controls (treatment, $\chi^2 = 5.822$, $df = 1$, $P = 0.016$; year, $\chi^2 = 0.251$, $df = 1$, $P = 0.616$; treatment X year, $\chi^2 = 2.251$, $df = 1$, $P = 0.134$). We did not perform any analysis on singing rate because only T-implanted birds were observed singing during our observations ($n = 10$, mean singing rate \pm SE = 1.34 ± 0.91 songs/individual/day; 7 of 10 individuals observed singing). However, one control male was observed singing outside of behavioral observations, and two others sang in response to playback when being recaptured.

Test I-2: We were able to model departure dates for 27 males (13 controls 14 T-implanted) males, although 3 males were censored from the Kaplan-Meier model because seasonal flooding on their territories interrupted our departure estimation. As predicted, T-implanted birds departed earlier (Mantel-Cox $\chi^2 = 7.403$, $n = 27$, $P = 0.007$; Figure 2), leaving on spring migration approximately 7 days before controls on average.

These results have been included in a manuscript currently under review in Behavioral Ecology.

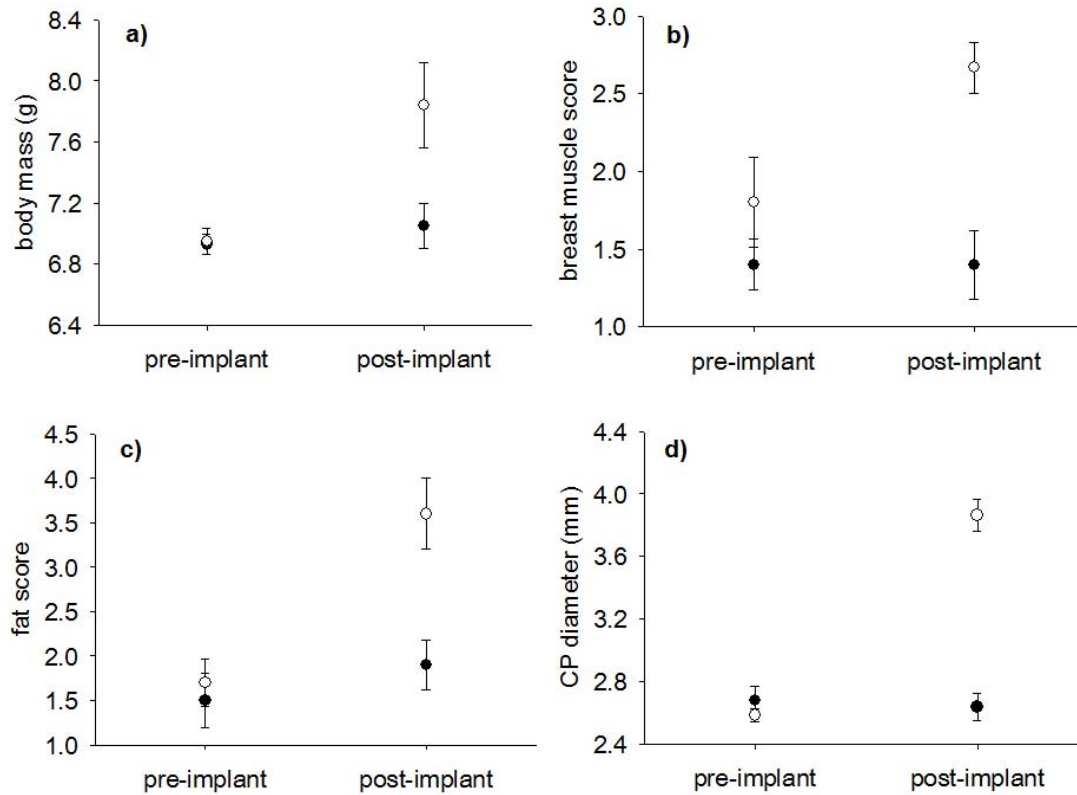


Fig 1. Means (\pm SE) for three measures of migratory preparation: a) body mass, b) breast muscle score, and c) fat score, and d) a measure of breeding preparation, cloacal protuberance (CP) diameter, in control (filled circles; $n = 10$) and T-implanted (open circles; $n = 10$), for American Redstarts pre- and post-implantation in late-winter 2010 and 2011 in Jamaica, West Indies.

Component II - Multi-species comparison of breeding hormone activity prior to spring arrival

Hypothesis IIa: Neotropical migrant males begin breeding preparation while on the non-breeding grounds and the level of preparation is greatest for males in superior condition.

Test IIa: For this test we analyzed blood samples collected over three years (2008-2010) from 98 male Northern Waterthrushes and 66 male Ovenbirds, and over two years (2009-2010) from 163 male American Redstarts, and 84 male Black and White Warblers. We did not capture an adequate sample of Black-throated blue warblers for analysis. We pre-screened the dataset for age effects, and only found an effect of age in redstarts, thus we analyzed young and adult males separately for this species.

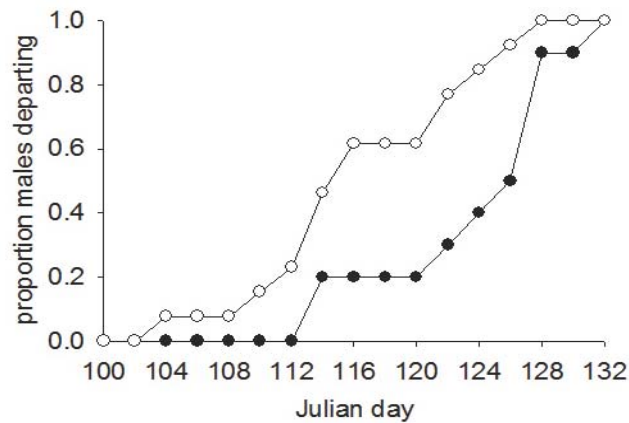


Fig 2. Proportion of control (n= 10; filled circles) and T-implanted (n=14; empty circles) male redstarts departing on spring migration by Julian day, from Jamaica, West Indies, 2010 and 2011

We found significant increases in circulating T from mid- (15 Jan – 28 Feb) to late- winter (15 March - 30 April) in male waterthrushes ($\chi^2 = 19.68$, $df = 1$, $P < 0.001$; Fig 3), Black and White Warblers ($\chi^2 = 6.87$, $df=1$, $P= 0.01$; Fig 3), and adult American Redstarts ($\chi^2 = 11.92$, $df = 1$, $P = 0.001$; Fig 3). We did not however find increases in male Ovenbirds ($\chi^2 = 0.00$, $df = 1$, $P = 0.99$; Fig 3) or young male redstarts ($\chi^2 = 0.76$, $df = 1$, $P= 0.39$). For the species and ages that exhibited an increase, we only found a relationship between condition (size-corrected mass) and late-winter T in adult redstarts ($r = 0.48$, $n = 56$, $P < 0.001$; waterthrush: $r = -0.18$, $n = 47$, $P = 0.24$; Black and White Warbler: $r = -0.06$, $n = 49$, $P = 0.68$). We then examined the relationship between migration distance (as measured by δD) and late-winter T in adult redstart males and found no relationship ($r = 0.02$, $n = 66$, $P = 0.87$). Thus we concluded that preparation for breeding in three of these four species begins prior to departure from wintering sites. Furthermore, there is evidence for substantial variation among species in initiation of breeding preparation and the potential for environmental limitation of development manifest through body condition. The implication of this portion of the study is that studies of breeding limitation need to include a focus on events occurring prior to arrival of migratory birds at breeding sites.

The redstart portion of this test was included in a manuscript currently under review in Behavioral Ecology. We will include the analyses on all of these species in an invited chapter in Studies in Avian Biology featuring studying migratory birds across the full life-cycle.

Test IIb: We received 303 tissue samples from birds collected after collision with buildings in Chicago from spring migration in 2010 and 2011. The only species with enough sample size for multivariate models was Ovenbird ($n = 101$ males, 81 females).

We have also received enough samples for Black and White Warblers (n = 22 males, 18 females) and an additional migrant from the family Turdidae, Wood Thrush (*Hylocichla mustelina*; n = 23 males, 16 females), to perform some univariate analyses. Our initial analyses, while revealing very interesting patterns, also uncovered substantial annual variation. Therefore we have decided, along with our collaborators at the Field Museum, to analyze another year of samples. This will also potentially give us enough samples from thrushes and Black and White Warblers to perform multivariate analyses on these species. Tonra will be able to perform the isotope analysis at the Smithsonian since he is a Stable-isotope Post-doctoral Fellow there through 2013.

In our analyses thus far, we have found support for both endogenous and exogenous factors explaining variation in breeding preparation. We performed separate model selections for male and female Ovenbirds with models composed of the dependant variable gonad volume and the explanatory variables Julian date, year, breeding latitude (δD), and winter habitat wetness ($\delta^{13}C$). The top model explaining variation in male Ovenbird testis size, which had a 57% chance (based on Akaike weight) of being the best model, included δD , $\delta^{13}C$, year, and the $\delta^{13}C$ X year interaction as explanatory variables. The next best model, which included the same explanatory variables except for δD , had only an 18% chance of being the top model. All but two of the top ten models included $\delta^{13}C$ and all but four included δD . Closer examination of the data revealed that in 2010 $\delta^{13}C$, but not δD , was related to testes volume in 2010, but the opposite was true in 2011 (Fig. 4). For female ovenbirds, the top model with a 21% chance (based on Akaike weight) of being the best model, was the null model, indicating that these variables do not do a good job of explaining variation in ovary volume. However, there was a relationship between δD and ovary volume in 2011 ($R^2 = 0.18$, $n = 45$, $P = 0.004$). Thus far, it appears that the factors affecting breeding development in this migratory species can vary year to year, potentially coinciding with variation in environmental conditions. Another year of data will enable us to further examine such annual variation and replicate this model selection in two additional species

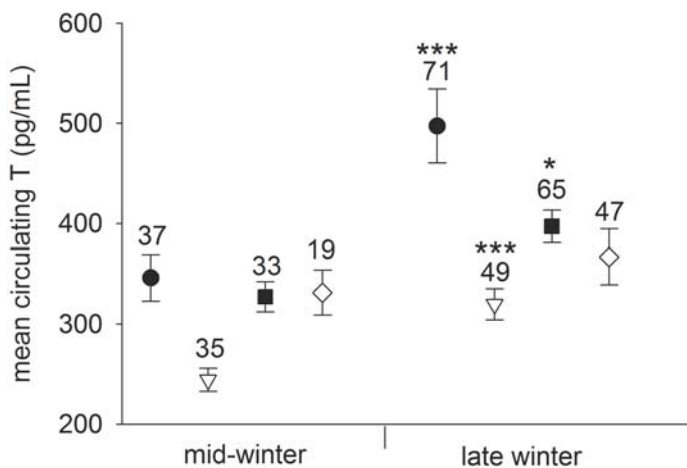


Fig 3. Means (\pm SE) of circulating testosterone (T) in four species of migratory warbler: American Redstart (adults only shown; circles), Black and White Warbler (inverted triangles), Northern Waterthrush (squares), and ovenbird (diamonds) in mid- and late-winter in Jamaica, West Indies.

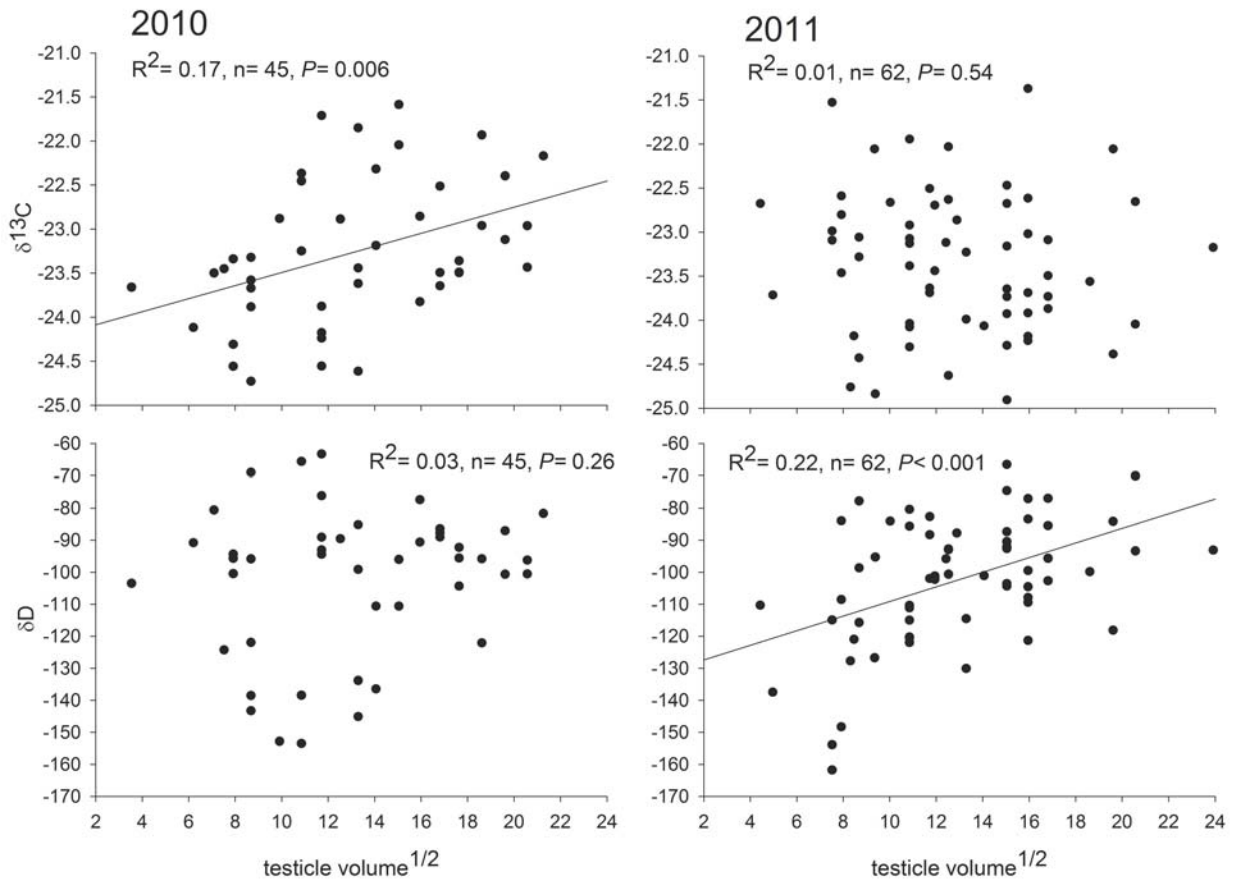


Fig 4. Linear relationships between stable-carbon isotopes ($\delta^{13}\text{C}$) and stable-hydrogen isotopes (δD) and testis volume (square-root transformed) in male Ovenbirds collected after collision with buildings in Chicago, IL during Spring 2010 and 2011. More negative $\delta^{13}\text{C}$ values are indicative of wetter winter habitat while more negative δD values are associated with more northern breeding latitudes, and thus longer migration distance.

Conclusions:

Scientists and naturalists have commonly defined life history stages based on geographic location. For example, a redstart seen in Jamaica is in the non-breeding stage, in Florida the migration stage, in Ontario the breeding stage. However, life history stages are defined by events occurring within the individual not by their movements or locations. Although stages may not completely overlap, one stage may begin physiologically while another is already underway (Ramenofsky and Wingfield 2006) leading to seasonal interactions (e.g. Marra et al. 1998). In fact, demographic parameters

of migratory populations can be driven by such interactions between events occurring thousands of kilometers apart (e.g. Marra et al. 1998; Gunnarsson et al. 2005). Thus, determining when transitions between stages begin within individuals is crucial to determining when and where events affecting that stage may occur, and how stages may interact. While a given stage may largely be completed in one location, conditions in a different location, where it commences, could play a critical role in an individual's ability to complete that stage. Thus, assessing what environmental perturbations may have greatest impact on a population requires a detailed understanding of this aspect of an organism's physiology and behavior.

For example, while global climate change models predict increasing precipitation and temperature on redstart breeding grounds, on their wintering grounds the major predicted change is a dramatic decline in precipitation (Neelin et al. 2006). Here in this study, we show that male redstarts are capable of beginning the transition to breeding while still on the wintering grounds, presumably impacting an important determinant of reproductive output: the timing of arrival at breeding sites (e.g. Marra et al. 1998; Reudink et al. 2009; Tonra et al. 2011a). Thus, it would appear for this species that research on adaptation to climate change should focus as much on the impacts of drought on migration phenology and breeding onset on their tropical wintering grounds as on the impacts of warmer temperatures on breeding success on their temperate breeding grounds. This illustrates the critical need for research that focuses on the entire life-cycle of organisms.

Our results also point to substantial species specific and annual variation in these phenomena. This highlights the need for more extensive study across species with variable life-history strategies, ideally spanning many years. Successful full life-cycle management will be critically dependant on such detailed information.