

**EFFECTS OF MIGRATION SCHEDULES ON PHYSIOLOGICAL  
CONDITION AND TIMING OF BREEDING IN WOOD THRUSH  
(*HYLOCICHLA MUSTELINA*)**

**Maggie P. MacPherson**

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN BIOLOGY  
YORK UNIVERSITY  
TORONTO, ONTARIO

March, 2014

© Maggie MacPherson, 2014

**Abstract**

I examine for the first time whether individual migration strategy affects physiological condition upon arrival and how arrival condition influences reproductive effort in a migratory songbird. Migration duration was predicted by departure date from the wintering grounds, arrival date to the northern Gulf coast, and arrival date to breeding grounds using geolocators, but not by sex (n=15 males, 6 females). There was a significant negative relationship between the number of days spent on migration and arrival mass index in males ( $p=0.027$ ), with B-OH concentrations ( $p=0.013$ ), and a positive correlation with baseline CORT ( $p=0.0026$ ). High baseline CORT was significantly correlated with later mate acquisition dates for males ( $p<0.001$ ). Arrival date predicted first egg date ( $p=0.012$ ). Males had significantly higher adrenocortical responses and differences between adrenocortical responses and baseline CORT than females. My study suggests that migration behaviour could have carry-over effects of poor stopover habitat on arrival condition and reproductive success.

**Keywords:** CORT, adrenocortical response, B-OH, physiological condition, geolocator, migration, migratory ecology, migration strategy, Wood Thrush

## Acknowledgements

I would first like to sincerely thank my friends and colleagues of the Stutchbury lab at York University. Most especially the Wood Thrush (WOTH) crew during my course of study: Tyler Done, Callie Stanley, and Elizabeth Gow, and later Emily McKinnon and Kevin Fraser. The friendships formed on the WOTH crew of 2008 have been lasting and helped me to complete this degree. Since Callie and I began our studies with the Stutchbury lab together in 2009, we have continued to work together through completing these degrees with Dr. Bridget Stutchbury and also gone on to support one another during Ph.D. programs in the U. S. Tyler Done has also moved to the U.S. and is a regular inspiration as he follows his musical career. The ideas building this thesis came largely from learning physiological measuring techniques from Tyler and conversations with Callie during our summer nights as field techs at the King Road House in 2008.

Secondly, the intensive fieldwork required of this thesis would have been impossible without the numerous field staff and volunteers. I would be remiss if I didn't list the names of those who devoted their time to helping me find WOTH with geolocators in the early spring, catch them in a timely manner, and then track their reproductive progress across the breeding season. **2009:** Matt Brady (staff), Rebecca Blakey, Katy Buchanan, Rachael Derbyshire, Paul DesBrisay, Julie Hildebrand, Andrea Hitchon, Ross Kresnik, Matthew Madigan, Dugan Maynard, Emily McKinnon, Megan Rasmussen, Tim Sneider, Abiya Saeed, Callie Stanley, Michael Wells; **2010:** Dominic Cormier, Nikole Freeman, Andrea Hitchon (staff), Catie Ivy, Sarah Manderstrom (staff), Peter Mills, Carly Rivard, Alex Sutton, Nicole Vella-Geldart, Warren Verina, Brenna Wells, Michael Wells; **2011:** Joseph Addesi, Joshua Addesi, Paulson DesBrisay, Meghan Douglas, Cordy DuBois, Megan Freeman, Nikole Freeman, Megan Geddes, Athena Gubbe, Lisa Harris, Sarah Manderstrom (staff), Peter Mills, Matt Strimas-Mackey, Luke Powell, Franca Schwaerma, Alex Sutton, Michael Wells, Lucy Welsh.

Lastly, I am forever grateful for the enduring support of Dr. Bridget Stutchbury, my family in northern Ontario, and my PhD advisor and committee member: Dr. Caz Taylor and Dr. Alex Jahn. Each of these people have brought me up from the depths of confusion with statistics, writing style, and time/project management. Thank you to all of you for standing by me throughout the completion of this work.

## Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
Table of Contents.....	iv
List of Figures.....	v
Introduction.....	1
Methods.....	10
Results.....	16
Discussion.....	22
References.....	28
Figures.....	36
Appendix A.....	45



## List of Figures

Figure 1. Physiological predictions of migration pace in Wood Thrush.....	36
Figure 2. Spring migration strategy and arrival date for Wood Thrush breeding in the same Pennsylvania population.....	37
Figure 3. Birds that take longer on spring migration arrive late to the north shore of the Gulf of Mexico and the breeding grounds.....	38
Figure 4. Scaled mass index after arrival to the breeding grounds versus days spent on spring migration.....	39
Figure 5. Spring migration duration and physiological condition after arrival at the breeding site.....	40
Figure 6. Regression for estimating arrival dates for non-geolocator males.....	41
Figure 7. Pairing date depends on arrival baseline CORT concentrations while first egg depends on arrival date.....	42
Figure 8. The difference between adrenocortical response and baseline CORT is significantly predicted by B-OH levels in Wood Thrush upon arrival to the breeding grounds in Pennsylvania.....	43
Figure 9. Summary of results.....	44

## **Introduction**

In North America roughly 80% of breeding birds are migrants, flying to northern latitudes in the spring for the breeding season (Morton 1992, Rappole 1995). Many long distance migratory songbirds have suffered steep population declines since the 1960s (Sauer et al. 2012), leading to a large literature on the effects of habitat fragmentation on nesting success, body condition and residency at stopover sites en route, and the effects of habitat quality at wintering grounds on individual survival and body condition (Faaborg et al. 2010). The key piece of missing information needed to understand the population dynamics of these species is migration itself. The vast majority of these migrants are small passerines and so until recently had been impossible to track because satellite or GPS units were too heavy. The recent development of small geolocators (< 1.5g) has allowed the direct study of migratory behaviour in small birds to assess migratory connectivity between wintering and breeding grounds and enabled ornithologists to better understand large-scale movements of migratory birds (see reviews McKinnon et al. 2013, and Bridge et al. 2013).

The new technology has led to an explosion of novel information about songbird migration. The discovery of new wintering grounds (e.g. Beason et al. 2012, Stach et al. 2012), patterns of loop migration in migrant passerines (Tøttrup et al. 2012), patterns of rapid spring migration (Stutchbury et al. 2009), and prolonged spring stopovers (Callo et al. 2013) are amongst the key new developments that are helping us to better understand migrant species throughout their annual cycle. My thesis research has made important contributions to a series of collaborative geolocator papers on Wood Thrushes (see Appendix A) that address a wide range of questions that are separate from this thesis. Our

research has shown that 1) breeding late in the summer delays fall migration but not arrival at the winter territory in Central America (Stutchbury et al. 2010), 2) repeat-tracked individuals are consistent from year to year in departure date from the tropics (Stanley et al. 2012), 3) geolocators are reasonably accurate for estimating geographic location in tropical forests (McKinnon et al. 2013) and 4) range-wide migration tracking can be used to create a migratory network and estimate the species-level importance of stopover and wintering regions (Stanley et al. in review).

My thesis focuses on the link between spring migration behaviour in Wood Thrushes and its consequences to individuals when they arrive at the breeding grounds. Our collaborative research has examined how timing of breeding affects fall migration (Stutchbury et al. 2010) but not how spring migration timing, pace, and route affect birds upon arrival to the breeding territory. Breeding success of migrant passerines has long been associated with arrival date at breeding sites (Perrins 1970, Price et al. 1988, Møller 1994, Lozano et al. 1996, Sandberg and Moore 1996, Stolt and Fransson 1995). In the short breeding seasons of northern latitudes, early spring arrival for male songbirds may allow a competitive advantage for the acquisition of high-quality territories and mates resulting in earlier nest attempts (e.g. Myers 1981, Price 1984, Newton and Marquiss 1984, Murphy 1986, Møller 1994). For double-brooding species, early and successful first nest attempts can increase the likelihood that a female can produce two, or even three, successful nests per season. Early nest attempts can also be associated with low predator abundance in some species (Holmes et al. 1992); therefore the ability to produce young earlier in the breeding season may increase seasonal reproductive success of males and females.

There is also evidence that lowered reproductive success can be attributed to poor physiological condition of late-arriving individuals (Verboven and Visser 1998, Marra et al. 1998). Spring migration schedules and routes could thus incur carry-over effects onto breeding success through delays in arrival and through poor arrival condition. Carry-over effects refer to processes occurring during one part of the annual cycle that influence an individual's success in the other parts of the cycle (Harrison et al. 2011). Studies of American Redstarts (*Setophaga ruticilla*) have linked wintering ground habitat quality with spring arrival date to breeding grounds (Marra et al. 1998) and seasonal reproductive success (Norris et al. 2003). By sampling muscle tissue (Marra et al. 1998) and red blood cells (Norris et al. 2003) of newly arrived birds to their breeding grounds, these authors inferred the habitat in which birds had lived in the tropics several weeks earlier by using habitat-specific stable carbon isotope signatures. However, they did not know the migration schedules (i.e. departure dates, migration pace) or routes the birds had used prior to arrival.

There is consistent support for a pattern of lean (i.e. no or few fat stores) passerines caught on stopover spending more time on stopover than individuals arriving in better energetic condition (Gannes 2002, Goymann et al. 2010). Furthermore, theoretical work of Hedenström and Ålerstam (1997) calculated that the overall cost of stopover is twice that of migratory flight in the currency of energy. The cost of stopover in terms of time is calculated to be seven times that of migratory flight (Hedenström and Ålerstam 1997). Since a lean individual arriving at a given stop requires a longer stopover time to refuel, a bird with a slow overall start-to-finish migration should experience a more energetically demanding migration because of more individual flights

and frequent and prolonged refueling stops. For songbirds, little is known about the overall cost of start-to-finish migration, what determines the overall pace of an individual, and how that pace affects events on the breeding grounds. With direct tracking technology we can finally assess whether migratory behaviour itself has important carry-over effects on spring arrival date, condition and seasonal reproductive behaviour.

Here I examine for the first time whether individual migration strategy affects physiological condition upon arrival and how arrival condition influences reproductive effort in Wood Thrush (*Hylocichla mustelina*). The schedules (timing of departure from wintering grounds, duration of stopovers, timing when to cross the Gulf of Mexico, migration route, timing of arrival to breeding grounds) that individual songbirds employ during spring migration should reflect a trade-off between balancing the energetic needs of migration with the energetic needs upon arrival to acquire mates and begin breeding. First, I test the prediction that a fast migration pace (longer migratory flights, less time refueling, shorter duration stopovers) should result in birds arriving in better energetic condition. Following this, birds with a slower pace of migration should arrive in poor condition due to the high costs of prolonged stopovers. Second, I test if better energetic condition upon arrival increases subsequent reproductive success.

#### *Determining energetic condition from physiological measures*

The condition (or health) of a bird is often assessed by measuring morphological features such as visible fat stores or the size of breast muscles, but these measures are imprecise predictors of a birds true condition except when at extreme highs or lows

(Marra and Holberton 1998). Physiological measures such as the concentration of circulating stress hormones and plasma metabolites are more informative about the current condition of the individual and how it is responding to its environment (Marra and Holberton 1998). The following predictions of how differing migratory strategies may influence physiology upon arrival to breeding grounds follow from both theoretical and empirical studies of stopover ecology and physiology of migratory passerines (on stopover ecology see Newton 2006, Hedenström and Alerstam 1997, Wikelski et al. 2003; on physiology of migrants see Astheimer et al. 1992, Wingfield 1994, Marra and Holberton 1998, Smith and Moore 2003, Long and Holberton 2004, Wilson and Holberton 2004, Wilson and Holberton 2007, Covino and Holberton 2011).

Corticosterone (CORT) has been implicated in promoting migration (reviewed in Wingfield et al. 1990), increasing foraging behaviour (Berdanier 1989, Gray et al. 1990, Astheimer et al. 1992, Breuner et al. 1998) and redirecting the metabolism of birds to use lipids or proteins from skeletal muscle for energy when carbohydrate food sources are not available (Holmes and Phillips 1976). CORT is the main glucocorticoid found in birds (Holmes and Phillips 1976), and can be released in response to low food availability or during times of high energetic demand (Wingfield et al. 1983, Wingfield 1994).

Physiological profiling using plasma metabolites and CORT can be used to assess refueling rates of birds following a migration flight. The role of the stress hormone CORT has been implicated in promoting refueling on stopover in birds (Berdanier 1989, Gray et al. 1990, Astheimer et al. 1992, Breuner et al. 1998) and has also been implicated in regulating metabolite pathways away from using skeletal muscle for energy on stopover (Long and Holberton, 2004). In their study, lean individuals were those with the

highest baseline and 30-minute adrenocortical response concentrations of CORT (Long and Holberton, 2004). Songbirds that have just completed migration should have high circulating (baseline) levels of CORT in comparison to other life history stages since they are arriving to relatively food-limited breeding grounds early in the spring and stored fuel sources have been largely exhausted during migratory flight. Individual birds with a start-to-finish more energetically demanding migration – more refueling stops, longer stopover durations - are expected to arrive in relatively lean condition which should be reflected by a higher circulating baseline CORT concentrations. Higher arrival CORT presumably should promote foraging activity and food intake rates in preparation for competition for territories and mates, and subsequent nesting activities.

Similarly, we can also measure adrenocortical responses to stress in individuals by documenting the increase in plasma concentrations of CORT to the acute stress of capture and handling (see Done et al. 2011, Long and Holberton 2004, Wingfield et al. 1992, Marra and Holberton 1998). Acute increases in CORT to environmental perturbations (such as predator attacks simulated by capture and handling of individuals, or short-term food shortages in early spring cold snaps) are seen as adaptive responses to promote behaviours that lead to increased survival (Wingfield 1994, Wingfield et al. 1995, Wingfield and Kitaysky 2002). Later in the breeding season, acute CORT secretion has been shown to be suppressed in individuals when it is adaptive to decrease sensitivity to perturbations, for instance to increase nest attendance and nestling feeding rates (Wilson and Holberton 2004, Wilson and Holberton 2007). For birds that have recently completed spring migration, higher adrenocortical response to capture and handling is

likely to be indicative of poor physiological condition. Thus slow-paced migrants are expected to have a greater acute CORT response to handling stress.

Plasma metabolites can be used to determine an individual's true energetic state as it relates to recovery from spring migration and preparation for breeding. Specifically, the concentration of circulating plasma metabolites that reflect the production, absorption and consumption of lipids have been used to determine refueling performance and stopover duration during migration (Guglielmo et al. 2005), as well as indicate rates of mass change (Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Jenni and Schwilch 2001, Seaman et al. 2005). Here I assume that hyperphagic refueling upon return to breeding sites is necessary to prepare individuals for reproductive behaviours (territory acquisition, mate acquisition and mate guarding). Three plasma metabolites, triglyceride (TRIG), glycerol (GLYC) and  $\beta$ -hydroxybutyrate (B-OH) can be used to determine physiological state upon arrival, following Guglielmo et al. (2005). TRIG increases when individuals are absorbing fats from food or synthesizing them de novo in the liver for use in muscle function. TRIG should be highest in birds arriving in good condition. Plasma B-OH and GLYC concentrations increase when birds are not refueling rapidly, or during mass loss, implying that birds are in poor condition. Measuring these three plasma metabolites provides a metabolic profile that can be used to assess fat metabolism and hence physiological condition upon arrival.

#### *Sex differences in the consequences of poor arrival condition*

The potential consequences from carry-over effects of migration on reproduction are expected to differ for males and females because of differing sex roles, the strength



and direction of sexual selection, and differing costs of reproduction. The arrival of female songbirds is typically difficult to assess because females do not advertise as males do (females do not sing) and are often more camouflaged in appearance. As discussed earlier, selection favours early arriving females (relative to other females) who could gain an offspring advantage by nesting early and selecting the best males on the best territories. There is balancing selection for arrival date, however, because females that arrive too early encounter colder temperatures and less arthropod food resources. Similarly, selection should favour males who arrive relatively early to the breeding grounds as they can secure higher quality territories and attract mates (Myers 1981, Oring and Lank 1982, see 'intra-sexual competition hypothesis' in table 2.2 of Rappole 2013). Since sex ratios seem to be male-biased in songbirds (Stewart and Aldrich 1951, Ficken and Ficken 1967, Proctor-Gray and Holmes 1981, Holmes et al. 1989, Van Horn et al. 1995, Wunderle 1995, Latta and Faaborg 2002), females should not be under as intense intra-sexual competition as males (see Francis and Cooke 1986). Carry-over effects of arrival condition on territory and mate acquisition should be relatively weak for females since females can more easily obtain a mate and do not undergo the energetic costs of singing. However, carry-over effects of poor arrival condition on timing of nesting should be greater for females due to the energetic costs of egg production that in turn should delay nest building, egg laying, and incubation. I expect females arriving in lean condition will have a greater increase in baseline and stress-induced CORT compared with males (sampled during the same interval post individual arrival dates), to better promote increased foraging in preparation for egg production. The consequences of arriving in poor physiological condition (high CORT, high B-OH, high GLYC and low

TRIG) will be greater for females and should be correlated with later first egg dates (Schoech et al. 2009, Angelier et al. 2007, Bonier et al. 2009a, Bonier et al. 2009b).

*Determining energetic condition from physiological measures: Summary of Predictions*

The objective of this research is to document the physiological consequences resulting from variation in migration strategies and whether the physiological condition after arrival was correlated with timing of nesting. Sampling blood from individuals immediately following spring migration allowed for a detailed assessment of arrival physiology. Assuming that slower paced migrations are more energetically costly and have a carry-over effect on arrival condition, individuals with slower pace are expected to have (Fig. 1) (1) lower mass:body size ratios, (2) higher baseline CORT concentrations, and (3) higher adrenocortical responses to acute stress (response to capture stress after 30-minutes). Slow-paced migrants are also expected to have (4) lower TRIG than fast-paced migrants and (5) high B-OH and GLYC concentrations. I expect that individuals arriving in poorer physiological condition (low mass:body size ratio, high baseline CORT, high adrenocortical response, low TRIG, high B-OH, high GLYC) to take longer to obtain territories and mates, and to have delayed first egg dates in comparison to individuals arriving in better condition.

## Methods

### *Field Methods*

Wood Thrushes are large *Hylocichla* thrushes (45-52g) in the family Turdidae that breed in the forests of eastern North America and winter in Central America. Wood Thrushes are omnivores and often forage for arthropods on the ground; they also eat fruit.

The study was conducted from late April-early August of 2009-2011 in a northern Carolinian forest 120ha field site in northwestern Pennsylvania (41.8°N, 79.9°W). The field site - Hemlock Hill Field Station – is a mixed forest dominated by Eastern Hemlock (*Tsuga canadensis*), Red Maple (*Acer rubrum*), American Beech (*Fagus grandifolia*), and Oak (*Quercus sp.*). Many of the Wood Thrush at the site had previously been banded by researchers at this location (Stutchbury et al. 2009, Stutchbury et al. 2010, Done et al. 2011, Gow et al. 2011, Gow and Stutchbury 2013) and migration data from geolocator birds returning in 2007 and 2008 were included in some of my analyses (though physiological data were not available for these individuals). Myself, at least one highly trained field assistant, and numerous student volunteers (names listed in opening acknowledgements) were responsible for finding, capturing, and monitoring individuals. Over three years I achieved 272 captures of 151 Wood Thrush from within the study area. On average 40 pairs were monitored into the beginning of each breeding season representing all of the pairs in the study area that I was able to find.

Each year, we arrived at the field site each year by April 20<sup>th</sup> prior to Wood Thrush arrival. The earliest sighting date for Wood Thrush at Hemlock Hills was April 24<sup>th</sup>. Each morning, transects were surveyed for Wood Thrush along the 50x50m grid

marked on trees for accurate territory mapping. Wood Thrush songs were played every 100m to solicit a response from skulking birds otherwise undetected. The identification of each individual was obtained using unique colour band combinations.

To capture, sample and band individual Wood Thrush as close to their arrival dates as possible we set up between 2-18 mist nets in the area where they were first seen (6m and 12m black nylon mist nets with 30mm mesh). All hormone and metabolite information in this thesis is from birds captured within twenty days of arrival and prior to egg laying on the territory in question. Nets were opened pre-dawn in areas where Wood Thrushes were previously observed. All birds were sampled between 6a.m.-11a.m. in order to minimize variation associated with daily changes in hormone secretions. Mist nets were closely monitored from within 50m and once a bird was captured in the mist net, I or my field assistant would extract the bird and take a 75 $\mu$ L blood sample in less than three minutes from the moment of capture (hereafter referred to as the ‘speed bleed’).

All blood sampling was done from the brachial vein using 27-gauge needles. Blood was captured using 25 $\mu$ L heparinized microcapillary tubes (hereafter ‘cap tubes’). Blood was immediately expelled from cap tubes into 0.5mL eppendorf tubes which were labeled and deposited into a Ziploc bag and placed into a cooler filled with ice packs until later processing. The bird was then banded with a USFWS aluminum band and 3 auxiliary colour bands used to visually identify individuals in the field for the remainder of the project. We then took standard morphological measures (tarsus length, wing length, tail length, weight), fit each bird with a radio-transmitter and then a second 75 $\mu$ L

blood sample was drawn from the right brachial vein at precisely thirty minutes post capture in order to assess CORT levels after inducing the stress of capture and handling.

Radio-transmitters (1.5g, BD-2, Holohil Systems Inc.) were used to more effectively monitor individuals and find nests. Across three years thirty-two individuals were radio-tracked, although many were dropped from hormone analysis when later geolocator information revealed they had not been caught within twenty days of arrival to the breeding grounds (birds radio-tracked after returning with geolocators: fourteen in 2009, nine in 2010, and nineteen in 2011). Capturing birds soon after arrival proved to be the most logistically challenging part of the study and greatly reduced sample sizes. Radio-tagged birds were monitored daily and their behaviour recorded. Each tracking session lasted a minimum of one hour and would be increased to two hours if the bird was observed for fewer than 10 minutes out of the hour. I specifically focused on trying to estimate precise pairing dates for each bird and then first egg dates. In the event that nests were discovered after a complete clutch of eggs had been laid (3-4 eggs), then we would count back 15 days from the date of hatch to determine the approximate first egg date. When these nests were predated prior to hatching, they were excluded from analyses.

#### *Lab Methods*

Since Wood Thrushes were only caught during morning hours, all samples were processed at noon each day. Samples were centrifuged for 11 minutes at 10,000 r.p.m. in a tabletop centrifuge. Plasma and red blood cells (hereafter 'RBCs') were separated and stored in a -40°C freezer. In 2009, centrifuged samples were immediately divided into

6 $\mu$ L aliquots of plasma and red blood cells. In 2010 and 2011, centrifuged samples were divided into 12 $\mu$ L aliquots instead of 6 $\mu$ L to help prevent dehydration of samples during storage. All samples were double-bagged in Ziploc bags to reduce dehydration within the freezer. CORT and plasma metabolite assays were conducted at the University of Western Ontario. Samples from 2009 were run in November of that year and samples from 2010 and 2011 were run together in August of 2011; samples were kept frozen using dry ice during transport.

CORT assays followed the manufacturer's protocol for a double-antibody radioimmunoassay protocol that has been modified for avian plasma (MP Biomedicals No. 07-120103, see Washburn et al. 2002) in the lab of Dr. Elizabeth Hampson. The standard CORT sample was diluted to 12.5ng/mL and so concentrations of CORT lower than 12.5ng/mL measured from Wood Thrush plasma could not be precisely measured. For this reason, samples were run in duplicate and if both samples fell outside of the standard curve they were excluded from further analyses. Averaging across low and high pools, the intra-assay coefficient of variation was 12.0% for the 2009 samples and 7.84% for the 2010/2011 samples.

Plasma metabolites were assayed in the lab of Dr. Christopher Guglielmo and follow the protocol for metabolite profiling (see Guglielmo et al. 2005). All metabolites measured from plasma were taken from blood samples drawn within three minutes of capture. I diluted all samples 1:2 in 0.9% NaCl saline solution to bring metabolite concentrations into the range of the standard curve for these assays. I used 400 $\mu$ L flat-bottomed microplates (Nunc Brand Products, Rochester, NY) in a microplate spectrophotometer. GLYC and TRIG were measured in a single endpoint assay (Sigma

Glycerol reagent F6428, Triglyceride Reagent T2449) and B-OH was measured by a kinetic endpoint assay (R-Biopharm, Marshall, MI, USA). All samples were run in duplicate and two samples per plate were run again to ensure inter-plate variability was negligible (coefficient of variation <6%).

### *Geolocator Analysis*

Determining departure date, arrival date and the intermediate number of days spent on spring migration from geolocator information followed the method outlined in McKinnon et al. (2012). I used the ‘threshold method’ with an assigned sun elevation angle of -2.10, which is the calculated average for the Pennsylvania breeding site from ground-truthing data. Since estimates of longitude have less error than those of latitude (see McKinnon et al. 2012), movements during migration were primarily determined by sustained longitudinal shifts (e.g. a shift from east to west across the Central American land bridge or from the Yucatan peninsula to the mouth of the Mississippi River without a later jump back to the original longitude). In this way I was able to assess departure date from the wintering grounds, the number of days spent between wintering and breeding locations and the spring arrival date to the breeding grounds.

### *Statistical Analysis*

Variables were tested for normality and I then used either simple linear regression or general linear mixed-effect modelling (GLMM) to test for relationships between variables. All statistics were performed using R Statistical Computing Software (version

3.0.0, R Development Core Team, R Foundation for Statistical Computing, <http://www.r-project.org/>), and all graphics were made with Microsoft Excel.

To most accurately adjust mass for body size in this population of Wood Thrush, I pooled all individuals caught within the months of April and May across all years and followed Peig and Green (2009). Tarsus length did not differ between the sexes (n=39 males, 21 females,  $t=-0.96$ ,  $df=44.24$ ,  $p=0.341$ ), but females were significantly heavier than males during this time (n=40 males, 21 females, mean female mass=53.5g, mean male mass=47.0g,  $t=4.67$ ,  $df=22.104$ ,  $p=0.00014$ ). For this reason mass:body size calculations were assessed separately for each sex. Following Peig and Green (2009), I calculated the adjusted mass for each individual using the equation below where  $\hat{M}_i$  is the scaled mass index,  $M_i$  is the mass of the individual,  $L_0$  is the population mean tarsus length calculated from each set of birds ( $L_0$  for males was 31.28mm and  $L_0$  for females was 30.85mm),  $L_i$  is the tarsus length of the individual, and  $b_{SMA}$  is the scaling exponent estimated by the standard major axis (SMA) regression on ln-transformed data (Step 2 from Peig and Green, 2009):

$$\hat{M}_i = M_i \left[ \frac{L_0}{L_i} \right]^{b_{SMA}}$$



## Results

### *Spring migration strategy and arrival date for Pennsylvania population*

Stanley et al. (2012; see Appendix A) combined geolocator data from breeding and wintering populations of Wood Thrush, including the Pennsylvania population studied here, and showed that arrival date at the breeding grounds was strongly dependent on departure date from the winter territory in Central America. They did not test the extent to which departure date predicted within-population variation in arrival date. Within my Pennsylvania population, males had significantly earlier mean arrival date than females (general linear mixed-effects model comparing slopes of regressions:  $t = -4.57$ ,  $df = 16$ ,  $p = 0.012$ ; Fig. 2a). The mean arrival date for males was April 26<sup>th</sup> (day 116), and the mean arrival date for females was May 7<sup>th</sup> (day 127). For both males and females, birds departing late from Central America arrived late at the Pennsylvania breeding site (linear regression:  $n = 21$ ,  $F = 16.82$ ,  $df = 1$  and  $19$ ,  $R^2 = 0.47$ ,  $p = 0.00061$ ; Fig. 2a). Late departing individuals tended to have faster migration pace (linear regression:  $n = 22$ ,  $F = 17.3$ ,  $df = 1$  and  $20$ ,  $R^2 = 0.46$ ,  $p = 0.00048$ ; Fig. 2b). Most Wood Thrush from this population winter in the same region of eastern Honduras and Nicaragua, and take a similar route north in spring via the Yucatan Peninsula and across the Gulf of Mexico to the Mississippi River delta (see Figure 1 and 2 in Stanley et al. in review; Appendix A). Therefore, migration distance is similar for most individuals. Winter departure date significantly predicted spring migration duration, with individuals who left earlier from the wintering grounds spending more days migrating (linear regression:  $n = 22$ ,  $F = 5.434$ ,  $df = 1$  and  $20$ ,  $R^2 = 0.73$ ,  $p = 0.03$ ; Fig. 2c).

Despite the difference in the timing of migration between males and females (males depart wintering grounds earlier; t-test:  $n=15$  males, 6 females,  $t=-2.58$ ,  $df=20$ ,  $p=0.018$ ; males arrive earlier at breeding grounds stated in preceding paragraph), there was no effect of sex on migration duration (GLMM:  $n=15$  males, 6 females,  $t=-0.58$ ,  $df=16$ ,  $p=0.57$ ). While still accounting for sex as a factor in the GLMM, late arrival date to the north shore of the Gulf of Mexico (getting around the Gulf of Mexico is presumed to be the major ecological barrier in Wood Thrush migration) predicted more days spent on spring migration (GLMM:  $n=15$  males, 6 females,  $t=2.22$ ,  $df=16$ ,  $p=0.038$ ; Fig. 3a). Likewise, arrival date to the breeding grounds was positively correlated with birds taking longer to get there from the wintering grounds (GLMM:  $n=15$  males, 6 females,  $t=2.43$ ,  $df=16$ ,  $p=0.025$ ; Fig. 3b).

#### *Migration Pace/Duration and Arrival Condition*

A subset of all geolocator-tagged birds were captured soon after arrival and their physiological condition assessed to determine if slow migration pace (i.e. longer migration duration for a given distance) incurs a carry-over cost of arriving in poor condition. There was a significant negative relationship between the number of days spent on migration and arrival scaled mass index (SMI) in male Wood Thrush caught within 20 days of arrival to breeding grounds and prior to pairing (linear regression:  $n=8$ ,  $F=8.463$ ,  $df= 1$  and  $6$ ,  $R^2=0.58$ ,  $p=0.027$ ; Fig. 4). The number of geolocator females caught after arrival, and prior to egg-laying, was too small for statistical analysis ( $n=4$ , Fig. 4).

There was no relationship found between plasma TRIG levels after arrival and spring migration duration (linear regression:  $n=8$ ,  $F=0.057$ ,  $df=1$  and  $6$ ,  $R^2=0.28$ ,  $p=0.82$ ) and there were no sex differences in mean arrival TRIG (t-test:  $n=5$  males, 3 females,  $t=-1.14$ ,  $df=2.0$ ,  $p=0.37$ ; Fig. 5a). One female had a TRIG level  $>15\text{mmol/L}$  and was excluded as an outlier because TRIG in breeding Wood Thrush is  $<5\text{mmol/L}$  (see Done et al. 2011). There was no difference in B-OH concentrations when comparing males and females (t-test:  $n=5$  males, 4 females,  $t=-1.40$ ,  $df=3.15$ ,  $p=0.25$ ). The number of days spent on spring migration and the B-OH concentrations was positively correlated (linear regression:  $n=9$ ,  $F=11.01$ ,  $df=1$  and  $7$ ,  $R^2=0.61$ ,  $p=0.013$ ; Fig. 5b). There was no sex difference between GLYC concentrations (t-test:  $n=5$  males, 4 females,  $t=-1.20$ ,  $df=4.18$ ,  $p=0.29$ ) and no relationship between GLYC and the number of days migrating (linear regression:  $n=9$ ,  $F=2.62$ ,  $df=1$  and  $7$ ,  $R^2=0.12$ ,  $p=0.15$ ).

There was no difference in baseline CORT measures between males and females (t-test:  $n=5$  males, 4 females,  $t=-0.87$ ,  $df=4.764$ ,  $p=0.42$ ). Individuals spending longer on migration arrived with higher baseline CORT concentrations up to 20 days post arrival (linear regression:  $n=9$ ,  $F=20.66$ ,  $df=1$  and  $7$ ,  $R^2=0.75$ ,  $p=0.0026$ ; Fig. 5c). There was no difference between the sexes in their adrenocortical response levels from blood samples taken 30 minutes post capture (t-test:  $n=5$  males, 4 females,  $t=-0.358$ ,  $df=3.16$ ,  $p=0.74$ ). Likewise, there was no relationship between the adrenocortical response and the number of days spent migrating when males and females were pooled (linear regression:  $n=9$ ,  $F=1.638$ ,  $df=1$  and  $4$ ,  $R^2=0.16$ ,  $p=0.27$ ). Lastly, the difference between response and baseline CORT measures was not significantly correlated with the number of days spent on spring migration (linear regression:  $n=9$ ,  $F=5.341$ ,  $df=1$  and  $7$ ,  $R^2=0.43$ ,  $p=0.054$ ; Fig.

5d) and there was no sex difference in mean CORT values (t-test:  $n=5$  males, 4 females,  $t=-1.53$ ,  $df=3.57$ ,  $p=0.21$ ).

#### *Arrival Date and Condition versus Timing of Breeding*

For testing the effects of arrival date and condition on timing of nesting, I first analyzed data only from birds carrying geolocators ( $n=9$ ). Sample sizes were unavoidably small owing to the small number of birds returning with geolocators, the difficulty of catching individuals soon after arrival, and the requirement that blood be sampled within 3 minutes of the bird being caught. I also obtained visual estimates of arrival date from daily surveys of occupied territories, and used geocator birds to determine the relationship between first visual sighting and actual arrival date. The average number of days following geocator arrival to seeing an individual bird was 6 days (0-12 day range,  $n=8$ ). I regressed estimated arrival dates for geocator males based on visual sightings against the number of days it took to discover these birds after true arrival (based on geocator assignment of arrival date);  $n=8$ ,  $F=10.02$ ,  $df=1$  and 6,  $R^2=0.62$ ,  $p=0.019$ ; (Fig. 6). Females were not included since they have less conspicuous behaviour upon arrival compared with singing males. This allowed me to estimate true arrival date for non-geocator birds, and increase the sample size for testing the relationship between arrival date and physiological variables.

Mate acquisition was significantly later for males that had high baseline CORT upon arrival (linear regression:  $n=15$ ,  $F=32.33$ ,  $df=1$  and 13,  $R^2=0.71$ ,  $p<0.001$ ; Fig. 7a). However, baseline CORT showed no relationship with first egg date (linear regression:  $n=18$ ,  $F=0.8852$ ,  $df=1$  and 16,  $R^2=0.05$ ,  $p=0.36$ ; Fig. 7b). First egg date of the male's

mate was correlated with male arrival date (linear regression:  $n=14$ ,  $F=8.621$ ,  $df=1$  and  $12$ ,  $R^2=0.42$ ,  $p=0.012$ ; Fig. 7c).

High CORT and high B-OH are both indicators of poor body condition, and thus should be closely related to each other for a given bird. I compared all measures of arrival CORT (baseline, adrenal response, and the difference between adrenal response and baseline), with each of the plasma metabolites (TRIG, GLYC, and B-OH). I found that the adrenal response CORT was significantly related to B-OH concentrations in the plasma (linear regression:  $n=15$ ,  $F=7.854$ ,  $df=1$  and  $14$ ,  $R^2=0.36$ ,  $p=0.014$ ; Fig. 8).

#### *Sex differences in arrival condition*

There was a significant difference in mean adrenocortical response between male and female Wood Thrush caught prior to the egg laying stage (t-test:  $n=46$  males,  $24$  females,  $t=4.49$ ,  $df=59.48$ ,  $p<0.00001$ ) with the average adrenocortical response in males being  $83.36$  ng/mL ( $sd=58.67$  ng/mL) and females  $40.96$  ng/mL ( $sd=18.43$  ng/mL). Additionally, the difference between the adrenocortical response and baseline measure of CORT was higher for males ( $86.31$  ng/mL,  $sd=62.20$  ng/mL,  $n=25$ ) than females ( $36.74$  ng/mL,  $sd=19.25$  ng/mL,  $n=22$ ; t-test:  $t=-3.78$ ,  $df=29.33$ ,  $p<0.001$ ). There was no difference in baseline CORT between males and females ( $n=45$  males,  $n=22$  females, t-test:  $t=-1.26$ ,  $df=44.54$ ,  $p=0.21$ ).

In males, baseline CORT did not differ between individuals carrying geolocators versus those without ( $n=26$  non-geolocator,  $11$  geolocator, t-test:  $t=-1.71$ ,  $df=11.15$ ,  $p=0.12$ ), and neither did adrenocortical response CORT measures ( $n=29$  non-geolocator,  $11$  geolocator males, t-test:  $t=1.10$ ,  $df=10.38$ ,  $p=0.296$ ). While sample sizes are too small

for statistical comparison of females, those fit with geolocators (n=3) had higher baseline CORT (11.5-33.71 ng/mL (mean= 20.5ng/mL) compared with 0.21-33.70ng/mL (mean= 5.05ng/mL)), and higher difference between baseline CORT and the adrenocortical response measure (11.75-75.07ng/mL (mean=34.90 ng/mL) versus 2.64-36.24ng/mL (mean=16.41 ng/mL)), in comparison to females not carrying geolocators (n=15).

*Summary of energetic condition results with respect to days spent on spring migration:*

To sum, in accordance with the predictions (Fig. 1), mass (SMI) was significantly negatively correlated with the duration of migration, however, TRIG was not (summary of results shown in Fig. 9). Baseline CORT and B-OH were both positively correlated with migration duration, but there were no relationships with either the adrenocortical response or GLYC levels. The predicted differences between males and females were not observed, although comparisons were difficult to make given unavoidably small sample sizes of females. Contrary to prediction, males showed significantly higher values than females in both the adrenocortical response and the difference between adrenocortical response and baseline CORT measures.

## **Discussion**

Using geolocators I was able to show that the longer an individual spends on spring migration, the poorer its arrival physiological condition. Increased migration duration resulted in significantly lower mass (SMI), higher B-OH, and higher baseline CORT. Each of these is an indicator of poor condition in birds, and thus provides support for a carry-over effect. Lean passerines on stopover spend more time on stopover than those individuals arriving at a stopover site in better condition (Gannes 2002, Goymann et al. 2010), and the energetic costs of stopover are more taxing than migratory flight (Hedenström and Ålerstam 1997). Migration is thought to be the most limiting portion of the annual cycle (Sillett and Holmes 2002) in that most mortality occurs on migration. This is the first evidence in songbirds of a link between difficult migration strategies – longer duration migration – and arrival condition.

Geolocator analysis paired with measuring physiological condition in this study provides support for a continuum of migration strategies in Wood Thrush. From the geolocator information, birds that left early from the wintering grounds took longer to get to the breeding location, and yet could still be amongst the first to arrive. Blood testing showed that long migration to the breeding grounds was associated with poor physiological condition upon arrival. Conversely, birds that left later from the wintering grounds arrived late to the breeding grounds but did not spend as much time on spring migration. These individuals thus arrived late, but in good physiological condition and obtained mates faster. Kokko (1999) describes how older males may be able to afford

arriving late to the breeding grounds if they are dominant and can simply usurp territories from other individuals regardless of when they arrive. This implies that carry-over effects will be less severe for older individuals since they do not have to compete as intensely for territories. Alternatively, later arriving individuals (regardless of age or status) could arrive in better condition from shorter duration spring migrations and into better climatic conditions a few days later in the spring when food is more abundant. In this way physiological recovery from spring migration could be achieved at a faster pace.

Physiological condition upon arrival was important in determining pairing date, but was not correlated with first egg dates. Upon arrival to the breeding grounds I found that baseline CORT was a significant correlate of mate acquisition date, with higher levels of arrival baseline CORT predicting later mate acquisition dates in both sexes. First egg date, however, was not related to physiological measures but was correlated with arrival date to the breeding grounds. Carry-over effects from migration to breeding were examined for males only because sample size for females was low. Arrival date of females was estimated less reliably as they do not sing. Mate condition does not directly affect egg-laying date, but may indirectly affect female preparation for egg-laying via his singing and mate guarding behaviour (on the genetic mating system of Wood Thrush see Evans et al. 2008).

Arrival date has been used as a proxy for individual condition in songbirds (Hatch et al. 2010, and see ‘condition-dependent arrival hypothesis’ in Kokko 1999), since evidence supporting arrival date as important for predicting onset of nesting behaviour is plentiful in the literature (e.g. Perrins 1970, Price et al. 1988, Møller 1994, Lozano et al. 1996, Sandberg and Moore 1996, Stolt and Fransson 1995). It is likely that early arrival



to the breeding grounds allows a longer recovery time for individuals and so they are physiologically prepared to begin breeding sooner regardless of their original arrival condition. There may be other benefits conferred to birds arriving in better condition not measured in this study such as increased investment in higher quality eggs, nestlings, or nestling provisioning that could increase an individuals' fitness beyond that of individuals able to nest earlier in the spring. Since I did not test how seasonal reproductive output was affected by arrival condition, future research should focus on documenting this potential carry-over effect of how migration strategies may shape population dynamics by either lowering the number or quality of offspring.

Opposite to predictions (Fig. 1), males had significantly higher adrenocortical responses and differences between adrenocortical responses and baseline CORT measures than females. I assumed that arrival condition should be more important for females to better promote increased foraging in preparation for egg production, a task that males do not have to prepare for. This was supported by evidence that increased CORT secretions are important in promoting increased foraging behaviour (Berdanier 1989, Gray et al. 1990, Astheimer et al. 1992, Breuner et al. 1998). This unexpected result may be due to differing timing between males and females in when, during the pre-breeding period, they undergo the physiological changes that prepare them for breeding. While there is evidence supporting that early spring condition is a determinant of laying date in songbirds (Davies and Lundberg 1985, Daan et al. 1989), and that female birds may both travel with more fat and accumulate more fat during stopover (e.g. Drent et al. 2006), there may exist differing tradeoffs between males and females in terms of being more 'income' versus 'capital' based breeders. If females are able to begin egg production

using pre-stored materials ('breeding performance hypothesis' in Sandberg and Moore 1996), then they could arrive with a lower requirement for refueling than males (i.e. females are more 'capitol' breeders, Drent and Daan 1980) and thus show a lower CORT response if CORT is a true signal of promoting the high-level refueling suspected to be required to recover from spring migration and prepare for breeding. In this scenario, males should be more 'income' breeders, and early arrival to breeding grounds to compete for territories may be more important than condition upon arrival. The possibility of this scenario would also be heightened when territories and mates are limited, as is suggested in songbird mating systems with male-biased sex ratios (Stewart and Aldrich 1951, Ficken and Ficken 1967, Proctor-Gray and Holmes 1981, Van Horn et al. 1995, Wunderle 1995, Latta and Faaborg 2002) and where habitat is limited.

Alternatively, the consequences of suffering high CORT upon arrival may be too high for females, but tolerable for males during this phase of the annual cycle. Studies assessing the relationship between CORT and breeding behaviour have shown that acute CORT secretion can be suppressed in individuals when it may be adaptive to decrease sensitivity to perturbations, such as to increase nest attendance and nestling feeding rates (Wilson and Holberton 2004, Wilson and Holberton 2007). While there are detrimental effects of prolonged elevated CORT (Harvey et al. 1984), CORT secretion is widely accepted to be an adaptive response, modulated with respect to short-term stress (Wingfield et al. 1983, Meddle et al. 2003, Long and Holberton 2004). Since the difference between adrenocortical response and baseline CORT levels were positively correlated with B-OH levels (a sign of mass loss and poor physiological condition), it may be that either males are arriving in poorer condition than females or that females –

even those in poor physiological condition – are directing behaviours away from those associated with increased CORT secretion in order to facilitate successful reproductive behaviours or survival.

Studies assessing the effects of attaching geolocators to migratory passerines have been both supportive and cautionary. For Wood Thrush across both the wintering and breeding ranges, return rates for geocator-tagged birds were higher or equal to return rates for banded birds (14-38% depending on site and sex Stutchbury, Stanley, and McKinnon unpubl. data) indicating that geocator-tagged birds have equal survival to banded individuals. However, Arlt et al. (2013) showed that Northern Wheatears suffered consequences of carrying geolocators on both migratory performance and reproduction. Likewise, in their review, Bridge et al. (2013) made a summary comparing return rates of geocator individuals versus banded individuals with aerial insectivores and small-bodied species ( $\leq 35\text{g}$ ) being most compromised in their return rates after being fit with geolocators.

In my study, female Wood Thrush fit with geolocators returned with slightly higher baseline and difference between baseline and adrenocortical response CORT measures when compared to females not fit with geolocators (although small sample sizes here preclude testing this). The levels of baseline CORT and adrenocortical response CORT levels are much higher in Wood Thrush arriving to the breeding grounds than during other times in the annual cycle (arrival to breeding grounds: this study; during the breeding season: Done et al. 2010; on the wintering grounds: Stanley unpubl data). If high CORT levels are indicative of poor condition – as is suggested by the positive correlation with B-OH levels shown here – then spring migration could be

considered the most stressful period during the annual cycle of migratory passerines. If male and female Wood Thrush are under different stressors during the course of spring migration, females fit with geolocators arrive in poorer condition relative to females not fit with geolocators, and arrival condition is an important determinant of breeding success then research is needed to test potential consequences of tag use on migrating females. To better test the potential of sex differences in carry-over effects of spring migration into breeding future research should focus on improving arrival data from females and monitoring reproductive success across the entire breeding season.

### *Conclusion*

This study found evidence that individual migration strategy has significant influence on arrival condition in Wood Thrush with individuals that spent more days on spring migration arriving in the poorest condition. Because the date of reaching the northern Gulf of Mexico was positively correlated with migration duration, this indicated that the variation in migration duration within this population could be accounted for with travel prior to reaching North America. If poor quality of stopover sites along this migratory path can act to prolong migration or increase the physiological costs of migration, then conservation of forest corridors for declining migrant forest birds should be of high priority. Arrival condition of males was important in determining date of mate acquisition, and arrival date was important in determining first egg dates. Since this study shows that early reproductive behaviours such as mate acquisition and first egg date can be linked to migration strategy (namely duration), there may be reproductive benefits of stopover habitat conservation for migratory passerines. More research is needed to link

arrival condition of females to timing of nesting and measures of egg and nestling quality  
in order to fully understand how arrival condition can affect population dynamics.

## References

- Angelier, F., Weimerskirch, H., Dano S., and Chastel, O. 2007. Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behavioral Ecology and Sociobiology*. 61:611-621.
- Arlt, D., Low, M., and Pärt, T. 2013. Effect of Geolocators on Migration and Subsequent Breeding Performance of a Long-Distance Passerine Migrant. *PLoS ONE*. 8(12): e82316. DOI:10.1371/journal.pone.0082316.
- Astheimer, L.B., Buttemer, W. A., and Wingfield, J.C. 1992. Interactions of Corticosterone with Feeding, Activity and Metabolism in Passerine Birds. *Ornis Scandinavica*. 23(3):355-365.
- Barron, D.G., Brawn, J.D., and Weatherhead, P.J. 2010. Meta-analysis of transmitter effects on avian behavior and ecology. *Methods in Ecology and Evolution*. 1:180-187.
- Beason, J.P., Gunn, C., Potter, K.M., Sparks, R.A., and Fox, J.W. 2012. The Northern Black Swift: Migration Path And Wintering Area Revealed. *The Wilson Journal of Ornithology*. 124(1):1-8.
- Berdanier, C.D. 1989. Role of glucocorticoids in the regulation of lipogenesis. *The Journal of the Federation of American Societies for Experimental Biology*. 3:2179-2183.
- Bonier, F., Martin, P.R., Moore, I.T., and Wingfield, J.C. 2009a. Do baseline glucocorticoids predict fitness? *Trends in Ecology and Evolution*. 24(11):634-642.
- Bonier, F., Moore, I.T., Martin, P.R., and Robertson, R.J. 2009. The relationship between fitness and baseline glucocorticoids in a passerine bird. *General and Comparative Endocrinology*. 163:208-213.
- Breuner, C.W., Greenbert, A.L., and Wingfield, J.C. 1998. Non-Invasive Corticosterone Treatment Rapidly Increases Activity in Gambel's White-Crowned Sparrow (*Zonotrichia leucophrys gambelii*). *General and Comparative Endocrinology*. 111:386-394.
- Bridge, E.S., Kelly, J.F., Contina, A., Gabrielson, R.M., MacCurdy, R.B., and Winkler, D.W. 2013. Advances in tracking small migratory birds: a technical review of light-level geolocation. *Journal of Field Ornithology*. 84(2):121-137.
- Callo, P.A., Morton, E.S., and Stutchbury, B.J.M. 2013. Prolonged spring migration in the Red-eyed Vireo (*Vireo olivaceus*) *The Auk*. 130:240-246.
- Covino, K.M., and Holberton, R.L. 2011. The Influence of Energetic Condition on Flight Initiation and Orientation of Migratory Songbirds in the Gulf of Maine Region. *The Auk*. 128(2):313-320.

- Daan, S., Dijkstra, C., Drent, R.H., and Meijer, T. 1989. Food supply and the annual timing of avian reproduction. *Acta XIX Congressus Internationalis Ornithologici*. Pp. 392-407. University of Ottawa Press, Ottawa.
- Davies, N.B., and Lundgerb, A. 1985. The influence of food on time budgets and timing of breeding in the Dunnock *Prunella modularis*. *Ibis*. 127:100-110.
- Done, T., Gow, E.A., and Stutchbury, B.J.M. 2011. Corticosterone stress response and plasma metabolite levels during breeding and molt in a free-living migratory songbird, the wood thrush (*Hylocichla mustelina*). *General and Comparative Endocrinology*. DOI:10.1016/j.ygcen.2011.01.006.
- Drent, R.H., and Daan, S. 1980. The Prudent Parent: Energetic Adjustments in Avian Breeding. *Ardea*. 68:225-252.
- Drent, R.H., Fox, A.D., and Stahl, J. 2006. Travelling to breed. *Journal of Ornithology*. 147:122-134.
- Evans, M.L., Stutchbury, B.J.M., and Woolfenden, B.E. 2008. Off-Territory Forays and Genetic Mating System of the Wood Thrush (*Hylocichla mustelina*). *The Auk*. 125(1):67-75.
- Faaborg, J., Holmes, R.T., Anders, A.D., Bildstein, K.L., Dugger, K.M., Gauthreaux, S.A. Jr., Heglund, P., Hobson, K.A., Jahn, A.E., Johnson, D.H., Latta, S.C., Levey, D.J., Marra, P.P., Merkord, C.L., Nol, E., Rothstein, S.I., Sherry, T.W., Sillett, T.S., Thompson, F.R.III, Warnock, H. 2010. Recent advances in understanding migration systems of New World land birds. *Ecological Monographs*. 80(1):3-48.
- Ficken, M.S., and Ficken, R.W. 1967. Age-specific differences in the breeding behavior and ecology of the American Redstart. *The Wilson Bulletin*. 79:188-199.
- Francis, C.M., and Cooke, F. 1986. Differential Timing of Spring Migration in Wood Warblers (Parulinae). *The Auk*. 103(3):548-556.
- Gannes, L.Z. 2002. Mass change pattern of blackcaps refueling during spring migration: evidence for physiological limitations to food assimilation. *Condor*. 104:231-239.
- Goymann, W., Spina, F., Ferri, A., and Fusani, L. 2010. Body fat influences departure from stopover sites in migratory birds: evidence from whole-island telemetry. *Biology Letters*. 6:478-481.
- Gow, E.A., Done, T.W., and Stutchbury, B.J.M. 2011. Radio-tags have no behavioral or physiological effects on a migratory songbird during breeding and molt. *Journal of Field Ornithology*. 82(2):193-201.

- Gow, E.A., and Stutchbury, B.J.M. 2013. Within-season Nesting Dispersal and Molt Dispersal are Linked to Habitat Shifts in a Neotropical Migratory Songbird. *The Wilson Journal of Ornithology*. 125(4):696-708.
- Gray, J.M., Yarian, D., and Ramenofsky, M. 1990. Corticosterone, Foraging Behavior, and Metabolism in Dark-Eyed Juncos, *Junco hyemalis*. *General and Comparative Endocrinology*. 79:375-384.
- Guglielmo, C.G., Cerasale, D.J. and Eldermire, C. 2005. A Field Validation of Plasma Metabolite Profiling to Assess Refueling Performance of Migratory Birds. *Physiological and Biochemical Zoology*. 78(1):116-125.
- Harrison, X.A., Blound, J.D., Inger, R., Norris, D.R., and Bearhop, S. 2011. Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology*. 80:4-18.
- Harvey, S., Phillips, J. G., Rees, A., and Hall, R. 1984. Stress and Adrenal Function. *The Journal of Experimental Zoology*. 232:633-645.
- Hatch, M.I., Smith, R.J., and Owen, J.C. 2010. Arrival timing and hematological parameters in Gray Catbirds (*Dumetella carolinensis*). *Journal of Ornithology*. 151:545-552.
- Hedenström, A., and Ålerstam, T. 1997. Optimum Fuel Loads in Migratory Birds: Distinguishing Between Time and Energy Minimization. *Journal of Theoretical Biology*. 189:227-234.
- Holmes, W.N., and Phillips, J.G. 1976. The adrenal cortex of birds –In: Chester-Jones, I. and Henderson, I.W. (eds). *General and comparative endocrinology of the adrenal cortex*. Academic Press, New York. Pp. 293-420.
- Holmes, R.T., Sherry, T.W. and Reitsma, L. 1989. Population structure, territoriality, and overwinter survival of two migrant warbler species in Jamaica. *Condor*. 91:545-561.
- Holmes, R.T., Sherry, T.W., Marra, P.P., and Petit, K.E. 1992. Multiple Brooding and Productivity of a Neotropical Migrant, the Black-Throated Blue Warbler (*Dendroica caerulescens*), in an Unfragmented Temperate Forest. *The Auk*. 109(2):321-333.
- Jenni-Eiermann, S., and Jenni, L. 1994. Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the garden warbler. *The Auk*. 112:888-899.
- Jenni, L. and Schilch, R. 2001. Plasma metabolite levels indicate changes in body mass in reed warblers *Acrocephalus scirpaceus*. *The International Journal of Avian Science*. 1:55-65.



- Kokko, H. 1999. Competition for Early Arrival in Migratory Birds. *Journal of Animal Ecology*. 68(5):940-950.
- Latta, S.C., and Faaborg, J. 2002. Demographic and population responses of cape may warblers wintering in multiple habitats. *Ecology*. 83:2502-2515.
- Long, J.A., and Holberton, R.L. 2004. Corticosterone Secretion, Energetic Condition, and a Test of the Migration Modulation Hypothesis in the Hermit Thrush (*Catharus guttatus*), a Short-Distance Migrant. *The Auk*. 121(4):1094-1102.
- Lozano, G.A., Perreault, S., and Lemon, R.E. 1996. Age, arrival date and reproductive success of male American Redstarts *Setophaga ruticilla*. *Journal of Avian Biology*. 27:164-170.
- Marra, P.P., Hobson, K.A., and Holmes, R.T. 1998. Linking Winter and Summer Events in a Migratory Bird by Using Stable-Carbon Isotopes. *Science*. 282:1884-1886.
- Marra, P.P., and Holberton, R.L. 1998. Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia*. 116:284-292.
- McKinnon, E.A., Stanley, C.Q., Fraser, K.C., MacPherson, M.P., Casbourn, G., Marra, P.P., Studds, C.E., Diggs, N., and Stutchbury, B.J.M. 2012. Estimating geolocator accuracy for a migratory songbird using live ground-truthing in tropical forest. *Animal Migration*. DOI: 10.2478/ami-2013-0001. Pp. 31-38.
- McKinnon, E.A., Fraser, K.C., and Stutchbury, B.J.M. 2013. New Discoveries in Landbird Migration Using Geolocators, And a Flight Plan for the Future. *The Auk*. 130(2):211-222.
- Meddle, S.L., Owen-Ashley, N.T., Richardson, M.I., and Wingfield, J.C. 2003. Modulation of the Hypothalamic-Pituitary-Adrenal Axis of an Arctic-Breeding Polygynandrous Songbird, the Smith's Longspur, *Calcarius pictus*. *Proceedings: Biological Sciences*. 270(1526):1849-1856.
- Møller, A.P. 1994. Phenotype-dependent arrival time and its consequences in a migratory bird. *Behavioral Ecology and Sociobiology*. 35:115-122.
- Morton, E.S. 1992. What do we know about the future of migrant landbirds? Pp. 579-589 In: *Ecology and conservation of neotropical landbirds* (J.M. Hagen and D.W. Johnson, Eds). Smithsonian Institution Press, Washington.
- Murphy, M.T. 1986. Temporal Components of Reproductive Variability in Eastern Kingbirds (*Tyrannus tyrannus*). *Ecology*. 67(6):1483-1492.

- Myers, J.P. 1981. A test of three hypotheses for latitudinal segregation of the sexes in wintering birds. *Canadian Journal of Zoology*. 59:1527-1534.
- Newton, I. and Marquiss, M. 1984. Seasonal trend in the breeding performance of sparrowhawks. *Journal of Animal Ecology*. 53:809-829.
- Newton, I. 2006. Can conditions experienced during migration limit the population levels of birds? *Journal of Ornithology*. 147:146-166.
- Norris, D.R., Marra, P.P., Kyser, T.K., Sherry, T.W., and Ratcliffe, L.M. 2003. Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proceedings of the Royal Society –B*. 271:59-64.
- Oring, L.W., and Lank, D.B. 1982. Sexual selection, arrival times, philopatry and site fidelity in the polyandrous Spotted Sandpiper. *Behavioural Ecology and Sociobiology*. 10:185-191.
- Peig, J., and Green, A.J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*. 118:1883-1891.
- Perrins, C.J. 1970. The timing of birds' breeding seasons. *Ibis*. 112:242-255.
- Price, T.D. 1984. The Evolution of Sexual Size Dimorphism in Darwin's Finches. *The American Naturalist*. 123(4):500-518.
- Price, T.D., Kirkpatrick, M., and Arnold, S.J. 1988. Directional selection and the evolution of breeding date in birds. *Science*. 240:798-799.
- Proctor-Gray, E., and Holmes, R.T. 1981. Adaptive significance of delayed attainment of plumage in male American Redstarts: tests of two hypotheses. *Evolution*. 35:742-751.
- Rappole, J.H. 1995. *The ecology of migrant birds: a Neotropical perspective*. Smithsonian Institution Press, Washington, D.C.
- Rappole, J.H. 2013. *The Avian Migrant: The Biology of Bird Migration*. Columbia University Press, New York.
- Sandberg, R., and Moore, F.R. 1996. Fat Stores and Arrival on the Breeding Grounds: Reproductive Consequences for Passerine Migrants. *Oikos*. 77(3):577-581.
- Sauer, J.R., Himes, J.E., Fallon, J.E., Pardieck, K.L., Ziolkowski, D.J. Jr., and Link, W.A. 2012. *The North American Breeding Bird Survey, Results and Analysis 1966-2010*. Version 12.07.2012. USGS Patuxent Wildlife Research Center, Laurel, MD.

- Schoech, S.J., Rensel, M.A., Bridge, E.S., Boughton, R.K. and Wilcoxon, T.E. 2009. Environment, glucocorticoids, and the timing of reproduction. *General and Comparative Endocrinology*. 163:201-207.
- Seaman, D.A., Guglielmo, C.G., and Williams, T.D. 2005. Effects of physiological state, mass change and diet on plasma metabolite profiles in the western sandpiper *Calidris mauri*. *The Journal of Experimental Biology*. 208:761-769.
- Sillett, T.S., and Holmes, R.T. 2002. Variation in survivorship of a migratory songbird throughout its annual cycle. *Journal of Animal Ecology*. 71:296-308.
- Smith, R.J., and Moore, F.R. 2003. Arrival fat and reproductive performance in a long-distance passerine migrant. *Oecologia*. 134:325-331.
- Stach, R., Jakobsson, S., Kullberg, C., and Fransson, T. 2012. Geolocators reveal three consecutive wintering areas in the thrush nightingale. DOI: 10.2478/ami-2012-0001.
- Stanley, C.Q., MacPherson, M., Frazer, K.C., McKinnon, E.A., and Stutchbury, B.J.M. 2012. Repeat Tracking of Individual Songbirds Reveals Consistent Migration Timing but Flexibility in Route. *PLoSOne*. 7(7):e40688.
- Stanley, C.Q., McKinnon, E., Fraser, K., MacPherson, M., Casbourn, G., Friesen, L., Marra, P., Studds, C., Diggs, N., and Stutchbury, B. Creating species-level and regional migratory connectivity networks by tracking a declining forest songbird over the annual cycle. *Conservation Biology*. *In press*.
- Stewart, R.E., and Aldrich, J.W. 1951. Removal and repopulation of breeding birds in a spruce-fir forest community. *The Auk*. 68:471-482.
- Stolt, B.O., and Fransson, T. 1995. Body mass, wing length and spring arrival of the Ortolan Bunting *Emberiza hortulana*. *Ornis Fennica*. 72:14-18.
- Stutchbury, B.J.M., Tarof, S.A., Done, T., Gow, E., Kramer, P.M., Tautin, J., Fox, J.W., and Afanasyev, V. 2009. Tracking Long-Distance Songbird Migration by Using Geolocators. *Science*. 323:896.
- Stutchbury, B.J.M., Gow, E.A., Done, T., MacPherson, M., Fox, J.W. and Afanasyev, V. 2010. Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics. *Proceedings of the Royal Society –B*. DOI: 10.1098/rspb.2010.1220.
- Tøttrup, A.P., Klassen, R.H.G., Stransberg, R., Thorup, K., Kristensen, M.W., Jørgensen, P.S., Fox, J., Afanasyev, V., Rahbek, C., and Alerstam, T. 2012. The annual cycle of a trans-equatorial Eurasian-African passerine migrant: different spatio-temporal strategies for autumn and spring migration. *Proceedings of the Royal Society –B*. 279:1008-1016.

- Van Horn, M.A., Gentry, R.M., and Faaborg, J. 1995. Patterns of Ovenbird (*Seiurus aurocapillus*) pairing success in Missouri forest tracks. *The Auk*. 112:98-106.
- Verboven, N., and Visser, M.E. 1998. Seasonal Variation in Local Recruitment of Great Tits: The Importance of Being Early. *Oikos*. 81(3):511-524.
- Washburn, B.E., Morris, D.L., Millspaugh, J.J., Faaborg, J., and Schulz, J.H. 2002. Using commercially available radioimmunoassay to quantify corticosterone in avian plasma. *Condor*. 104:558-563.
- Wikelski, M., Tarlow, E.M., Raim, A., Diehl, R.H., Larkin, R.P., and Visser, G.H. 2003. Costs of migration in free-flying songbirds. *Nature*. 423:704.
- Williams, T.D., Guglielmo, C.G., Egeler, O., Martyniuk, C.J. 1999. Plasma lipid metabolites provide information on mass change over several days in captive western sandpipers. *The Auk*. 116:994-1000.
- Wilson, C.M., and Holberton, R.L. 2004. Individual Risk Versus Immediate Reproductive Success: A Basis for Latitudinal Differences in the Adrenocortical Response to Stress in Yellow Warblers (*Dendroica petechia*). *The Auk*. 121(4):1238-1249.
- Wilson, C.M., and Holberton, R.L. 2007. Are Nestlings the Cue for Reduction of the Adrenocortical Response to Stress in Male Yellow Warblers Breeding at High Latitude? *The Condor*. 109:675-679.
- Wingfield, J.C. 1983. Environmental and endocrine control of reproduction: an ecological approach. In "Avian Endocrinology: Environmental and Ecological Aspects" (S.-I. Mikami and M. Wada, Eds.), pp. 205-288. Japanese Scientific Societies Press, Tokyo, and Springer-Verlag, Berlin.
- Wingfield, J.C., Schwabl, J., and Mattock, P.W. Jr., 1990. Endocrine mechanisms of migration. In: Gwinner, E. (Ed.), *Bird Migration: Physiology and Ecophysiology*. Springer-Verlag, New York, pp. 232-256.
- Wingfield, J.C., Vleck, C.M., and Moore, M.C. 1992. Seasonal changes in the adrenocortical response to stress in birds of the Sonoran Desert. *Journal of Experimental Zoology*. 264:419-428.
- Wingfield, J.C. 1994. Modulation of the adrenocortical response to stress in birds. Pages 520-528 In "Perspectives in Comparative Endocrinology" (K.G. Davey, R.E. Peter, and S.S. Tobe, Eds.), pp. 520-528. National Research Council Canada, Ottawa.
- Wingfield, J.C., O'Reilly, K.M., and Astheimer, L.B. 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. *American Zoologist*. 35:285-294.

Wingfield, J.C., and Kitaysky, A.S. 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integrative and Comparative Biology*. 42:600-602.

Wunderle, J.M. 1995. Population characteristics of Black-throated Blue Warblers wintering in three sites on Puerto Rico. *The Auk*. 112:931-946.

## Figures

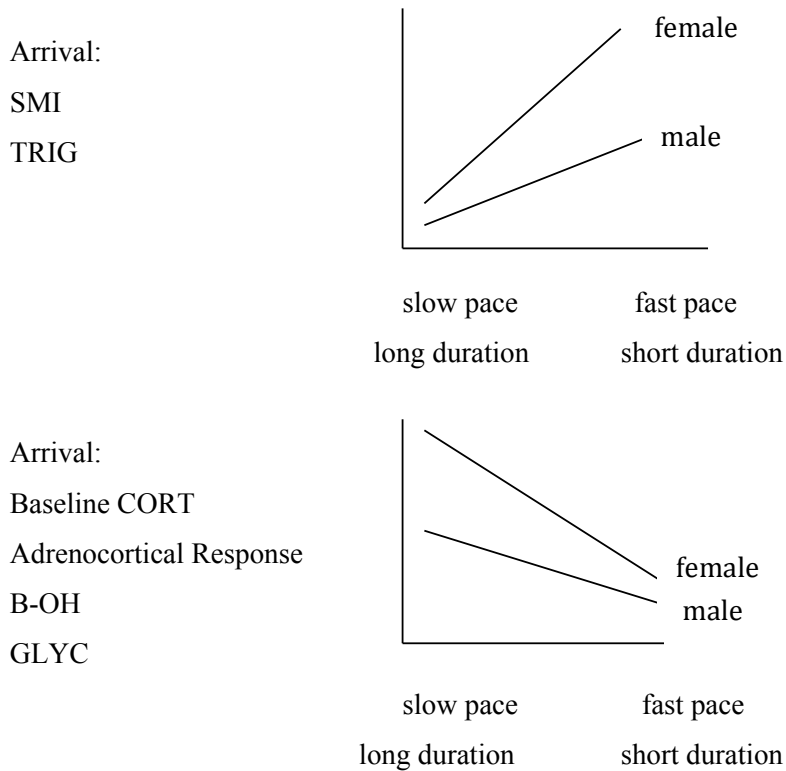


Figure 1. Physiological predictions of migration pace in migrant passerines. Slow-paced migrants are expected to arrive in poorer condition than fast-paced migrants. The potential consequences from carry-over effects of migration on arrival condition are expected to differ for males and females because of differing sex roles upon arrival.

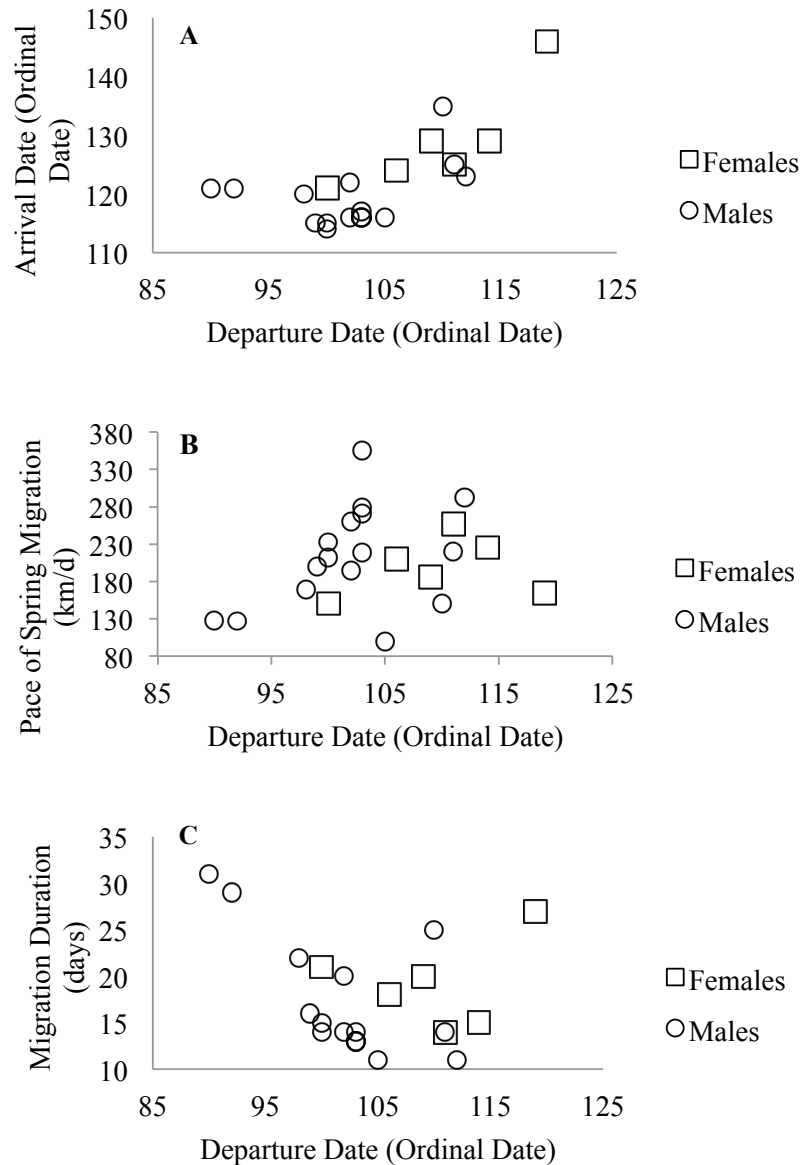


Figure 2. Spring migration strategy and arrival date for individual Wood Thrush breeding in the same Pennsylvania population. A) Arrival date to breeding grounds versus departure date from wintering grounds. B) Overall pace (km/day) of spring migration versus departure date from the wintering grounds. C) Migration duration (days) versus departure date from wintering grounds. Geolocator data from males (open circles) and females (open squares) were from those retrieved from 2008-2011 (n=6 females, 16 males).

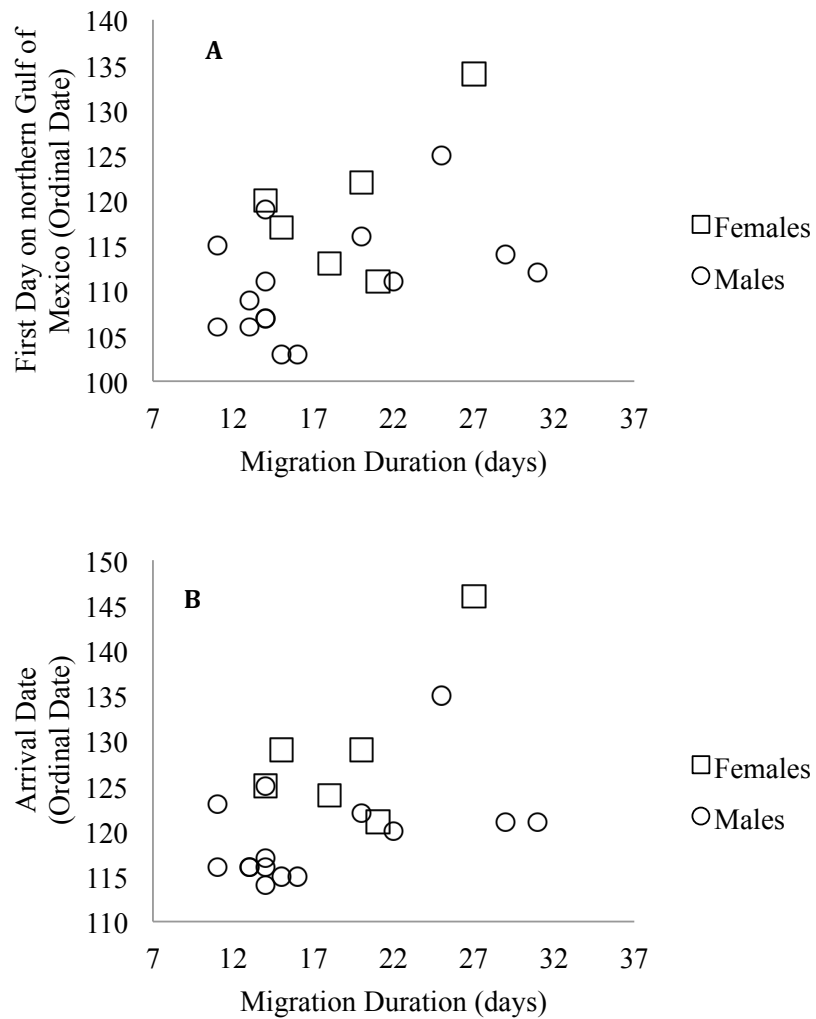


Figure 3. Individuals that take longer on spring migration, arrive late to the north shore of the Gulf of Mexico and the breeding grounds. A) Migration duration (days) versus the first day spent on the northern Gulf of Mexico. B) Migration duration (days) versus arrival date to Pennsylvania breeding grounds. Males are shown as open circles and females as open squares.



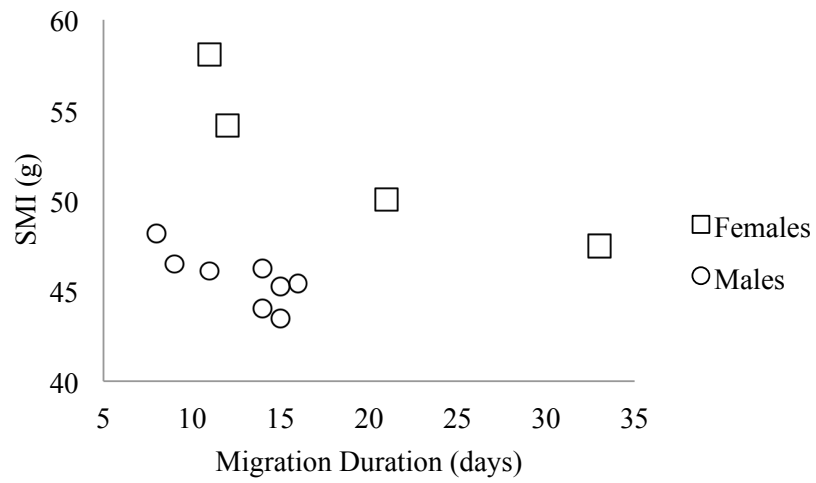


Figure 4. Scaled mass index of individuals after arrival to the breeding grounds versus days spent on spring migration. Males are shown as open circles and females as open squares.

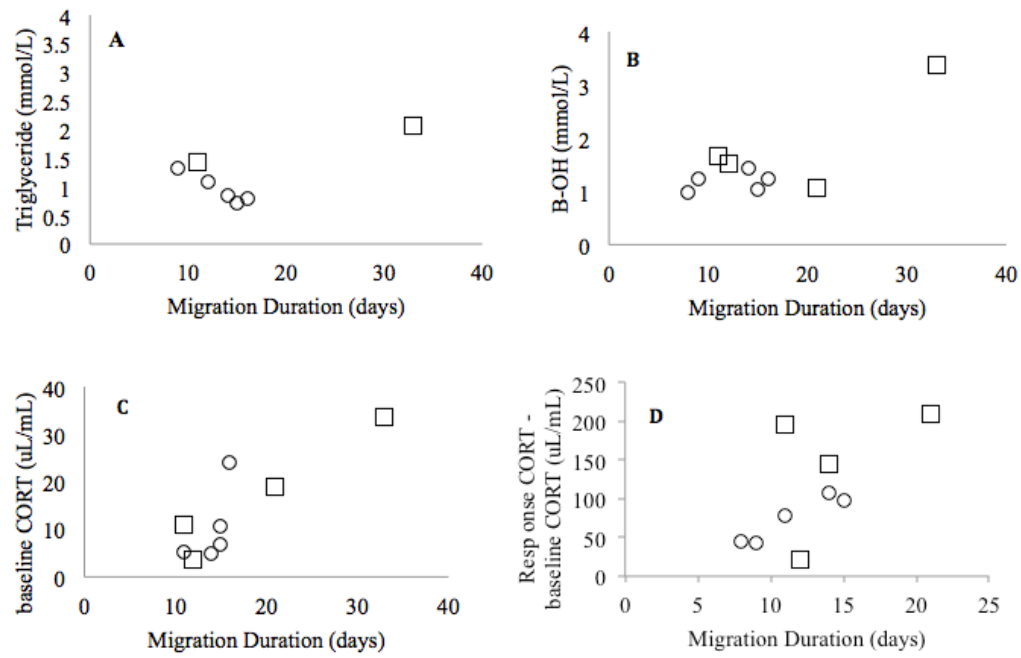


Figure 5. Spring migration duration and physiological condition after arrival at the breeding site (males represented by open circles, females by open squares) for A) triglyceride, B) B-OH, C) baseline CORT, and D) difference between stress-induced CORT and baseline CORT.

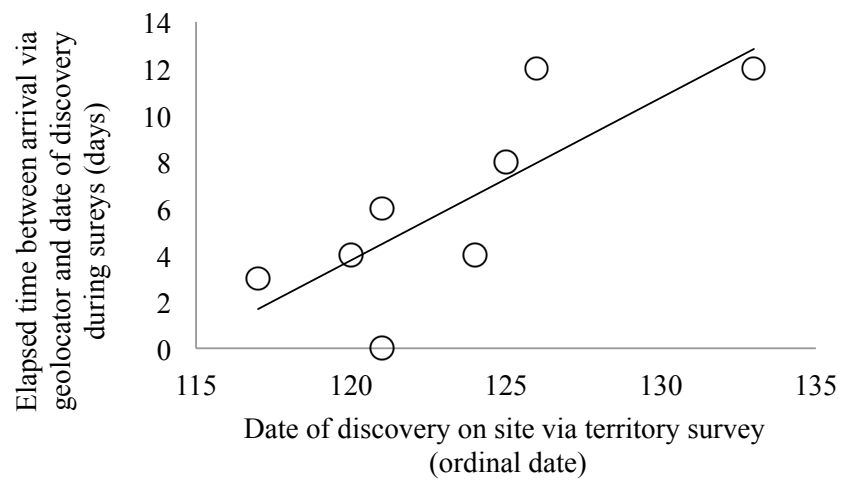


Figure 6. Regression for estimating arrival dates for non-geolocator males (n=8 males;  $R^2=0.626$ ,  $y=0.6971x-79.875$ ).

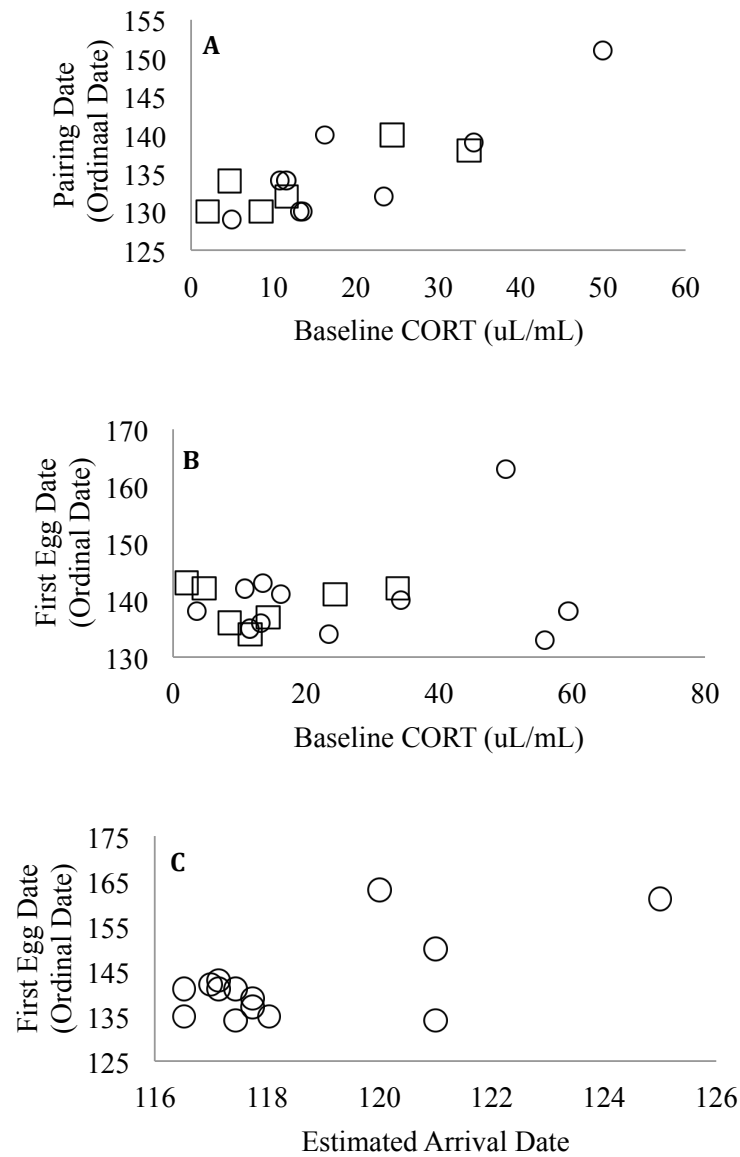


Figure 7. Pairing date depends on arrival baseline CORT concentrations while first egg date depends on arrival date. Males are represented by open circles, females by open squares. A) Pairing date depends on baseline CORT, B) first egg date is unrelated to baseline CORT, C) first egg date depends on arrival date.

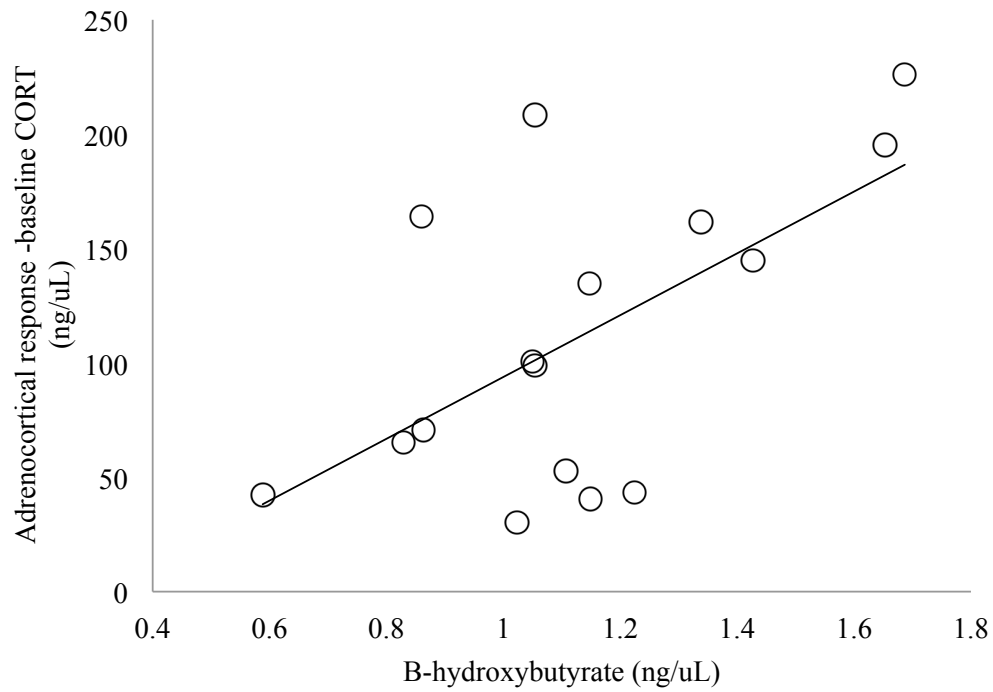


Figure 8. The difference between adrenocortical response and baseline CORT is significantly predicted by B-hydroxybutyrate levels in wood thrush upon arrival to the breeding grounds in Pennsylvania (n=16 males;  $R^2=0.36$ ,  $y=135.12x-41.279$ ).

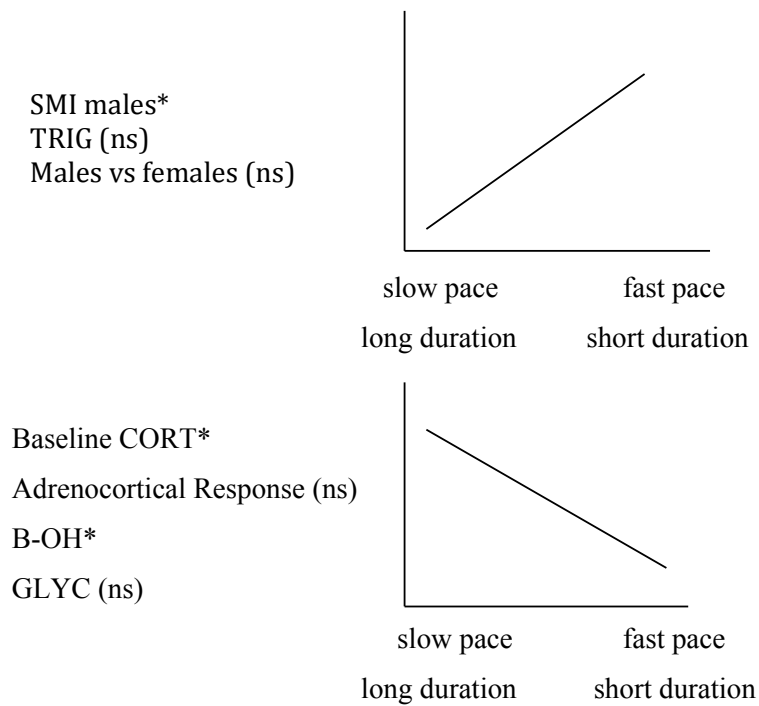


Figure 9. Summary of results. The variables on the x-axis shown with an asterisk are significant relationships that agreed with a priori predictions (see Fig. 1). Those listed with (ns) next to them were not significant. The predicted differences between males and females were not seen, although comparisons were difficult to make given unavoidably small sample sizes of females.

## Appendix A

### Table of Contents

Stutchbury, B.J.M., Gow, E.A., Done, T., **MacPherson, M.**, Fox, J.W. and Afanasyev, V. 2010. Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics. *Proceedings of the Royal Society –B*. DOI: 10.1098/rspb.2010.1220.

Stanley\*, C.Q., **MacPherson\*, M.**, Frazer, K.C., McKinnon, E.A., and Stutchbury, B.J.M. 2012. Repeat Tracking of Individual Songbirds Reveals Consistent Migration Timing but Flexibility in Route. *PLoSOne*. 7(7):e40688.

*\*These authors contributed equally to this work.*

McKinnon, E.A., Stanley, C.Q., Fraser, K.C., **MacPherson, M.P.**, Casbourn, G., Marra, P.P., Studds, C.E., Diggs, N., and Stutchbury, B.J.M. 2012. Estimating geolocator accuracy for a migratory songbird using live ground-truthing in tropical forest. *Animal Migration*. DOI: 10.2478/ami-2013-0001. Pp. 31-38.

Stanley, C.Q., McKinnon, E., Fraser, K., **MacPherson, M.**, Casbourn, G., Friesen, L., Marra, P., Studds, C., Diggs, N., and Stutchbury, B. Creating species-level and regional migratory connectivity networks by tracking a declining forest songbird over the annual cycle. *Conservation Biology*. *In press*.

## Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics

Bridget J. M. Stutchbury, Elizabeth A. Gow, Tyler Done, Maggie MacPherson, James W. Fox and Vsevolod Afanasyev

*Proc. R. Soc. B* published online 21 July 2010  
doi: 10.1098/rspb.2010.1220

---

### Supplementary data

"Data Supplement"

<http://rsbp.royalsocietypublishing.org/content/suppl/2010/07/21/rspb.2010.1220.DC1.html>

### References

**This article cites 33 articles, 10 of which can be accessed free**

<http://rsbp.royalsocietypublishing.org/content/early/2010/07/21/rspb.2010.1220.full.html#ref-list-1>

### P<P

Published online 21 July 2010 in advance of the print journal.

### Subject collections

Articles on similar topics can be found in the following collections

[behaviour](#) (1359 articles)

### Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

---

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

---

To subscribe to *Proc. R. Soc. B* go to: <http://rsbp.royalsocietypublishing.org/subscriptions>

---



## Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics

Bridget J. M. Stutchbury<sup>1,\*</sup>, Elizabeth A. Gow<sup>1</sup>, Tyler Done<sup>1</sup>,  
Maggie MacPherson<sup>1</sup>, James W. Fox<sup>2</sup> and Vsevolod Afanasyev<sup>2</sup>

<sup>1</sup>Department of Biology, York University, Toronto, Ontario, Canada M3J 1P3

<sup>2</sup>British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, UK

Each autumn billions of songbirds migrate between the temperate zone and tropics, but little is known about how events on the breeding grounds affect migration to the tropics. Here, we use light level geolocators to track the autumn migration of wood thrushes *Hylocichla mustelina* and test for the first time if late moult and poor physiological condition prior to migration delays arrival on the winter territory. Late nesting thrushes postponed feather moult, and birds with less advanced moult in August were significantly farther north on 10 October while en route to the tropics. Individuals in relatively poor energetic condition in August (high  $\beta$ -Hydroxybutyrate, low triglyceride, narrow feather growth bars) passed into the tropics significantly later in October. However, late moult and poor pre-migratory condition did not result in late arrival on the winter territory because stopover duration was highly variable late in migration. Although carry-over effects from the winter territory to spring migration may be strong in migratory songbirds, our study suggests that high reproductive effort late in the season does not impose time constraints that delay winter territory acquisition.

**Keywords:** migration; geocator; neotropical migrant; plasma metabolite; moult

### 1. INTRODUCTION

Carry-over effects between different stages of the life cycle are important for understanding the evolution of life-history traits but are difficult to study in migratory animals where seasonal interactions often occur across different continents. In nearctic–neotropical migratory songbirds, carry-over effects from the non-breeding ‘wintering’ season to the breeding season have been well documented using stable carbon isotopes to infer tropical habitat quality occupied prior to spring migration [1]. Habitat quality in the tropics affects an individual’s body condition and consequently the timing of spring migration, arrival time on the breeding territory, future breeding success and annual survival [2–4]. However, few studies have examined how breeding season events carry-over into autumn migration [5] because the key variables, reproductive effort and timing do not have isotopic signatures that can be measured on the wintering grounds. Only recently has it become possible to track small songbirds over thousands of kilometres [6].

The short breeding season of migratory birds creates time and energetic trade-offs between reproductive effort, feather moult and migration. Nesting late in the breeding season can increase annual reproductive success but may also delay moult because of the costs of overlapping parental care and moult [7–9]. One of the most energetically demanding periods in a songbird’s annual

cycle is the post-breeding moult of flight and body feathers that can increase energy expenditure by over 30 per cent [10] and impose energetic constraints on pre-migratory fattening [11]. Despite the importance of the moult period in avian life cycles, it is rarely addressed in studies of carry-over effects. Norris *et al.* [5] found that male American redstarts with high reproduction effort late in the season were more likely to moult tail feathers during migration, resulting in reduced carotenoid content of the feather.

A possible carry-over effect of late breeding and moult is delayed migration and arrival on the winter territory. In many species, individuals complete moult, or nearly so, prior to the initiation of autumn migration [12,13] so birds are expected to face trade-offs between timing of nesting, moult and migration. Competition for high-quality territories in the tropics is intense [14–16] and if late breeding delays arrival on the winter territory this could constrain reproductive effort of migratory songbirds [8,9]. Reproductive effort and late nesting may also reduce post-breeding energetic condition, [17–19] which in turn could result in a slower migration [20,21]. Stopover site studies assess short-term migration decisions and have shown that poor energetic condition and stopover habitat quality delay resumption of migration [21–23].

Many migratory birds follow an overall time-minimization strategy [24] where migration behaviour can be viewed as a series of optimal refueling stops such that birds should travel as fast as possible given their energetic condition, stopover habitat quality and weather conditions. Under a time-minimization model, late

\* Author for correspondence ([bstutch@yorku.ca](mailto:bstutch@yorku.ca)).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2010.1220> or via <http://rsph.royalsocietypublishing.org>.

departure and poor physiological condition prior to departure should be hard to overcome and result in later arrival on the winter territory. Here, we use light level geolocators to track the autumn migration of wood thrushes *Hylocichla mustelina* and test for the first time in a migratory songbird if late moult and poor physiological condition prior to migration delays migration and arrival on the winter territory.

## 2. MATERIAL AND METHODS

### (a) Study site and species

We studied a breeding population of 40–50 pairs of wood thrushes in a 150 ha forest at the Hemlock Hill Biological Research Area in Crawford County, PA, USA (41.8° N, 79.9° W). Birds (47–55 g) were captured using mist nets and banded with a numbered U.S. Geological Service aluminum band and a unique colour-band combination. We aged birds as first-time breeders (second year) or older (after second year) based on plumage characteristics [25]. Most birds were fitted with a 1.6 g radio-transmitter (Model BD-2, 14 week battery; Holohil, Inc.) in late May or early June to obtain complete nesting histories and to aid in capturing adults during the moult period in August, at which point the radio-transmitter was replaced with a geolocator (see below). Almost half (49%) of females attempted a second brood after fledging young from their first nest and average number of young fledged per female was  $2.7 \pm 0.30$  (range 0–10,  $n = 39$ ). Nesting completion date was defined as the day of fledging, or day of nest loss, of an individual's last nesting attempt of the season and could only be accurately determined if an individual had been radio-tagged owing to high, concealed nests and occasional territory switching between nesting attempts.

### (b) Geolocator deployment

We used archival light level geolocators to reconstruct the timing and route of migration of individual wood thrushes [6]. In 2007 and 2008, 47 adult wood thrushes (23 females, 24 males; 18 second year, 29 after second year) were fitted with an Mk14S geolocator (1.6 g, British Antarctic Survey) using a leg-loop backpack harness. Most geolocators (35 of 47) were deployed in August, but 12 were deployed in 25 June–29 July on nesting birds. We retrieved geolocators in May or June of subsequent years from 12 different males and two females. Two of these birds were tracked in multiple years but only the first was used for analysis. We observed but could not capture four other returning geolocator birds (three males, one female). The annual return rate for geolocator birds (16 of 24 males, 66% males; three of 23 females, 13%) was not lower than for non-geolocator birds (11 of 26 males, 42%; four of 35 females, 11%).

Latitude and longitude are inferred from day length and sunrise times, respectively. Day-to-day error was estimated by the standard deviation while a bird was on its territory (electronic supplementary material; breeding: 230 km latitude, 105 km longitude; wintering: 390 km latitude, 140 km longitude). Movements away from the breeding site, and from one stopover location to another, were defined as long distance movements (greater than 250 km latitude, greater than 150 km longitude) that were consistent with autumn migration. We used the term 'regional' stopover to describe birds that interrupted migration for one or more

days, though birds could have moved short distances within a given region.

While in the tropics, wood thrushes defend feeding territories from conspecifics and remain on their winter territory from late autumn until early spring [26]. The birds in our study were initially sampled on their breeding territory, had already spent at least one winter in the tropics, and were probably returning to the same winter territory occupied the year before. Arrival date on the winter territory was defined as when the latitude and longitude ceased to shift in a direction consistent with autumn migration, fluctuated around a narrow range of values consistent with a stationary bird, and remained similar until the onset of northward migration in spring. During presumed stopovers and while stationary during winter, location was determined by calculating average latitude and longitude during the period.

### (c) Energetic condition during moult

We captured wood thrushes from 9 to 24 August to determine the extent of moult and obtain a blood sample to assess physiological condition. Most birds (17 of 20) were captured after their nesting completion date ( $22 \pm 2.6$  days later, range 6–43); two of three birds sampled 0–5 days prior to fledging their final brood had begun moult. All birds were sampled 06.00–11.00 EST by taking a 100  $\mu$ l blood sample via brachial venipuncture within 3 min of capture.

Blood plasma metabolites have been widely used with migratory birds to assess individual energetic state [17,22,23,27].  $\beta$ -Hydroxybutyrate levels increase during fasting and mass loss, while triglyceride levels increase during feeding and fat deposition. Plasma metabolites were assayed on a microplate spectrophotometer in 400  $\mu$ l flat-bottom microplates. All samples were diluted 1:2 in 0.9 per cent NaCl saline solution to bring concentrations within the set of standards that produced the standard curve. Triglyceride was measured in a single endpoint assay (Triglyceride Reagent T2449; [28]) and  $\beta$ -hydroxybutyrate was measured by kinetic endpoint assay (R-Biopharm, Marshall, MI, USA; [22]). All samples were run in duplicate. Samples where both assays yielded a concentration that fell outside of the standard curve were excluded.

We quantified the extent of flight feather moult by scoring the nine innermost primaries, six secondaries, three tertials and six rectrices from the left side of all birds. We assigned each of the 24 feathers a moult score of 0–5 based on its stage of growth (0: old feather; 1: missing feather or pin; 2: less than 1/4 grown; 3: 1/4 to 1/2 grown; 4: 1/2–3/4 grown; 5: 3/4 grown to full feather). The average moult score of geolocator birds was  $11.0 \pm 2.4$  (range 0–26; maximum possible 120) and nine of 10 individuals had no visible fat. Full flight feather moult in wood thrushes lasts an average of 38 days [13].

We obtained moult score and blood metabolite data for 20 individuals and 10 subsequently returned with geolocators. Four geolocator birds were not sampled during moult in August but we retrieved a tail feather after migration. We removed the third rectrix (tail feather) from returning birds to measure its rate of feather growth during the previous moult using daily growth bars; narrow growth bars indicate the feather grew slowly and thus a lower nutritional state of the bird [29]. We measured growth bar width by taking digital black and white images using a gel documentation system (Alpha-Innotech, San Leandro, CA, USA; [30]). A digital

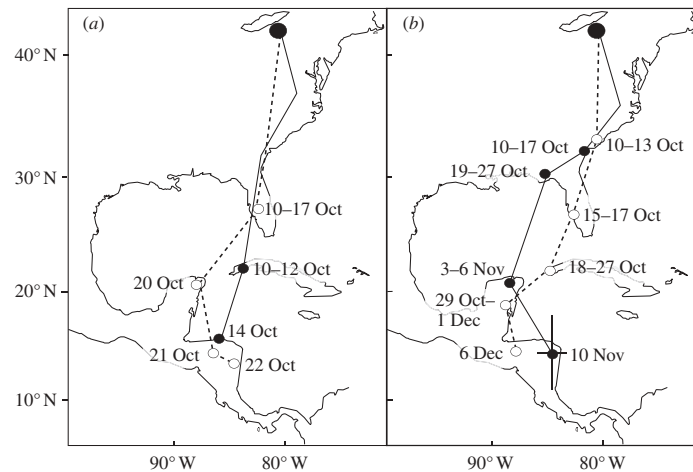


Figure 1. Autumn migration of four male wood thrushes illustrating (a) two birds (solid versus dashed line) that crossed into the tropics (23.4° N) relatively early and arrived on the winter territory by 14 and 22 October, respectively, and (b) two relatively slow birds that were still north of 32° N on 10 October and arrived on the winter territory 10 November and 6 December, respectively. The daily location error, based on average standard deviation for individuals on the wintering grounds, is shown for one individual with error bars. Lines connect daily positions, but do not necessarily reflect actual flight paths.

ruler was positioned at the centre of the most visible dark band in the image proximate to the base of the feather and the cumulative length of nine growth bars distal to it were measured. Growth bar width was the total length divided by the number of growth bars; each feather was measured three times and averaged.

### 3. RESULTS

The date on which individuals first entered the tropics (23.4° N), about 2000 km south of the breeding site, spanned three weeks (9 October–1 November) and estimated arrival date on the winter territory was even more variable (14 October–6 December; figures 1 and 2). Autumn migration routes were similar as all birds remained east (less than 86° W) as they moved south through the USA, with 10 of 14 birds continuing south through Florida and Cuba while four crossed the Gulf of Mexico from Florida to Mexico. Most individuals (13 of 14) over-wintered in Honduras or Nicaragua, and flew an average of 3460 km ( $\pm 126$ ) to their winter territory.

Nesting completion date spanned almost two months (2 July–24 August) and wood thrushes that nested late into the season produced more fledglings (Spearman rank correlation,  $r_s = 0.62$ ,  $n = 23$ ,  $p = 0.002$ ). Moulting score in August was negatively correlated with nesting completion date (figure 2a) and individuals in an advanced stage of moulting in mid-August were those that had finished nesting in early July. The departure date for autumn migration was unknown because latitude cannot be inferred from sunrise and sunset times within two weeks of the autumn equinox in September, and all our birds left the breeding site (41.8° N) during this period. By 10 October geolocator birds ranged in latitude from 16 to 36° N and birds that were farther north had lower moulting scores when captured six to eight weeks earlier (figure 2b). Late moulting birds also tended to cross

into the tropics later ( $r_s = -0.52$ ,  $n = 10$ ,  $p = 0.13$ ) but contrary to prediction did not arrive significantly later on their winter territory (figure 2c).

Individuals varied greatly in their pace of autumn migration after 10 October ( $110 \pm 15.8$  km d<sup>-1</sup>, range 42–212 km d<sup>-1</sup>). Seven of 14 birds had a regional stopover greater than 10 days long, and two of these birds stopped in the tropics greater than three weeks. For example, one male was in southern Florida 15–17 October, stopped in western Cuba for about 9 days and then stopped in northern Belize for about four weeks before completing migration (figure 1b). Early migrants were not more likely to spend more time on stopovers, as latitude on 10 October was not correlated with subsequent number of stopover days ( $r_s = 0.11$ ,  $n = 13$ ,  $p = 0.73$ ). Arrival date on the winter territory was determined largely by the cumulative number of stopover days (figure 2d) and was only weakly associated with an individual's latitude on 10 October ( $r_s = 0.37$ ,  $n = 14$ ,  $p = 0.19$ ).

Relatively poor post-breeding energetic condition was significantly correlated with later migration. Thrushes with relatively high  $\beta$ -hydroxybutyrate and low triglyceride in August entered the tropics significantly later in October (figure 3a,b). As expected, plasma  $\beta$ -hydroxybutyrate concentration of individuals was negatively correlated with triglyceride concentration ( $r_s = -0.51$ ,  $n = 33$ ,  $p = 0.003$ ). Slow growth of tail feathers (narrow bars) was also strongly associated with later migration into the tropics (figure 3c). Post-breeding energetic condition showed similar, but in some cases non-significant, patterns with latitude on 10 October ( $\beta$ -hydroxybutyrate:  $r_s = 0.64$ ,  $p = 0.05$ ; triglyceride:  $r_s = -0.43$ ,  $p = 0.21$ ; growth bar:  $r_s = 0.42$ ,  $p = 0.14$ ).

The relationship between blood metabolites and timing of migration could occur if metabolite profiles change with progression of moulting; so we tested if high moulting score was associated lower  $\beta$ -hydroxybutyrate and

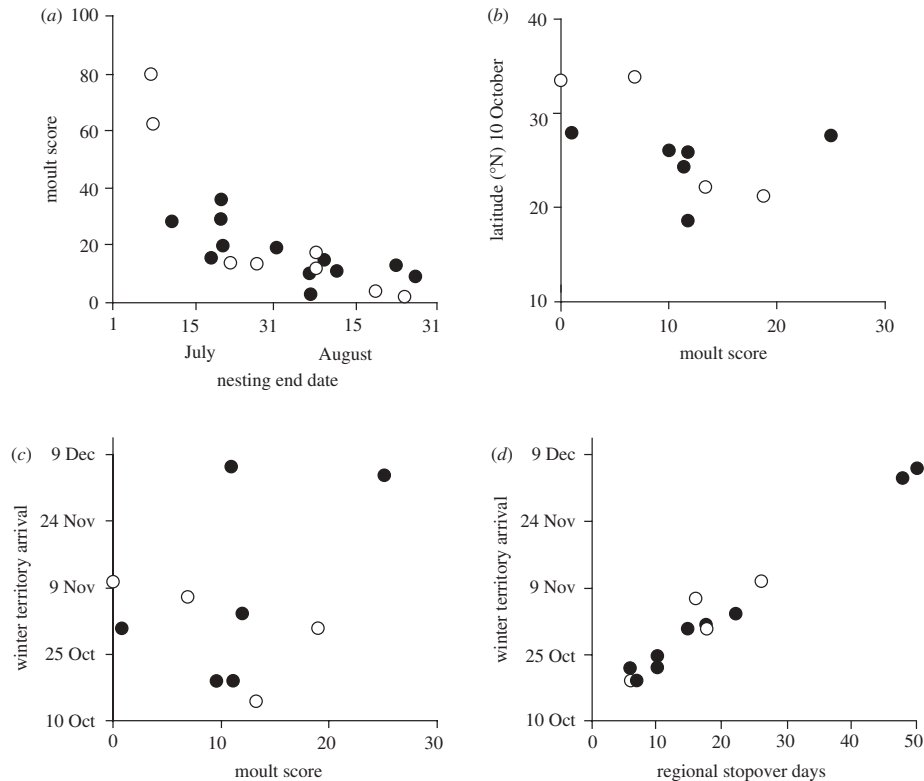


Figure 2. Relationship between moulting score in August and (a) completion date of the individual's last nest, (b) latitude on 10 October while migrating, and (c) date of arrival on winter territory. Open symbols are 1-year-old breeders, closed symbols are 2 years or older. (d) Arrival date on the winter territory versus estimated number of stopover days after 10 October, defined as individuals remaining in a region for one or more days after arrival. One individual that arrived its winter territory less than a week after 10 October was not included. (a)  $r_{\text{ss}} = -0.89$ ;  $p < 0.001$ . (b)  $r_{\text{ss}} = -0.64$ ;  $p = 0.047$ . (c)  $r_{\text{ss}} = -0.15$ ;  $p = 0.67$ . (d)  $r_{\text{ss}} = 0.95$ ;  $p < 0.001$ .

higher triglyceride. We also tested the prediction that high reproductive effort and late breeding reduce energetic condition during moult. We used a general linear model with metabolite concentration (log transformed) as the dependent variable, nest completion date, annual reproductive success (total number of fledglings) and moulting score as covariates and age class as a random factor. For  $\beta$ -hydroxybutyrate the overall model was significant (figure 4a;  $F_{1,15} = 16.43$ ,  $n = 21$ ,  $p = 0.002$ ; reproductive success:  $p = 0.61$ ; last nest date:  $p = 0.31$ ; moulting score:  $p = 0.44$ ; age class:  $p = 0.02$ ). For triglyceride the overall model was not significant (figure 4b; whole model:  $F_{1,15} = 0.96$ ,  $n = 21$ ,  $p = 0.35$ ; reproductive success:  $p = 0.74$ ; last nest date:  $p = 0.21$ ; moulting score:  $p = 0.12$ ; age class:  $p = 0.035$ ). There was a significant age class effect and first-time breeders tended to have lower  $\beta$ -hydroxybutyrate and higher triglyceride than older birds.

#### 4. DISCUSSION

We tested for the first time if late nesting and moult imposes carry-over effects on timing of autumn migration and arrival on the winter territory. Late nesting wood

thrushes had higher annual reproductive success and delayed moult and in early October, while en route to the tropics, were farther north suggesting that they probably departed the breeding grounds later. Despite this initial delay in autumn migration, late moulting birds did not arrive later on their winter territories.

Long-distance migration of birds is divided into alternating phases of stopover and flight, and overall migration speed is thought to be driven by fuel deposition rate at stopover sites [24,31]. Late breeding thrushes could, in theory, have mitigated time constraints imposed by late moult and migration departure by increasing fuel deposition rates and shortening stopover duration. However, birds that were farther north in early October did not subsequently increase their migration speed. Several individuals had long (greater than 14 days) stopovers on autumn migration, prior to or after entering the tropics, which were probably longer than necessary to simply refuel [20]. Mortality during migration can be high [32] and prolonged stopovers could benefit individuals by reducing short-term mortality risks via improved flight performance, lower predation or reduced physiological costs of endurance flights [33,34].

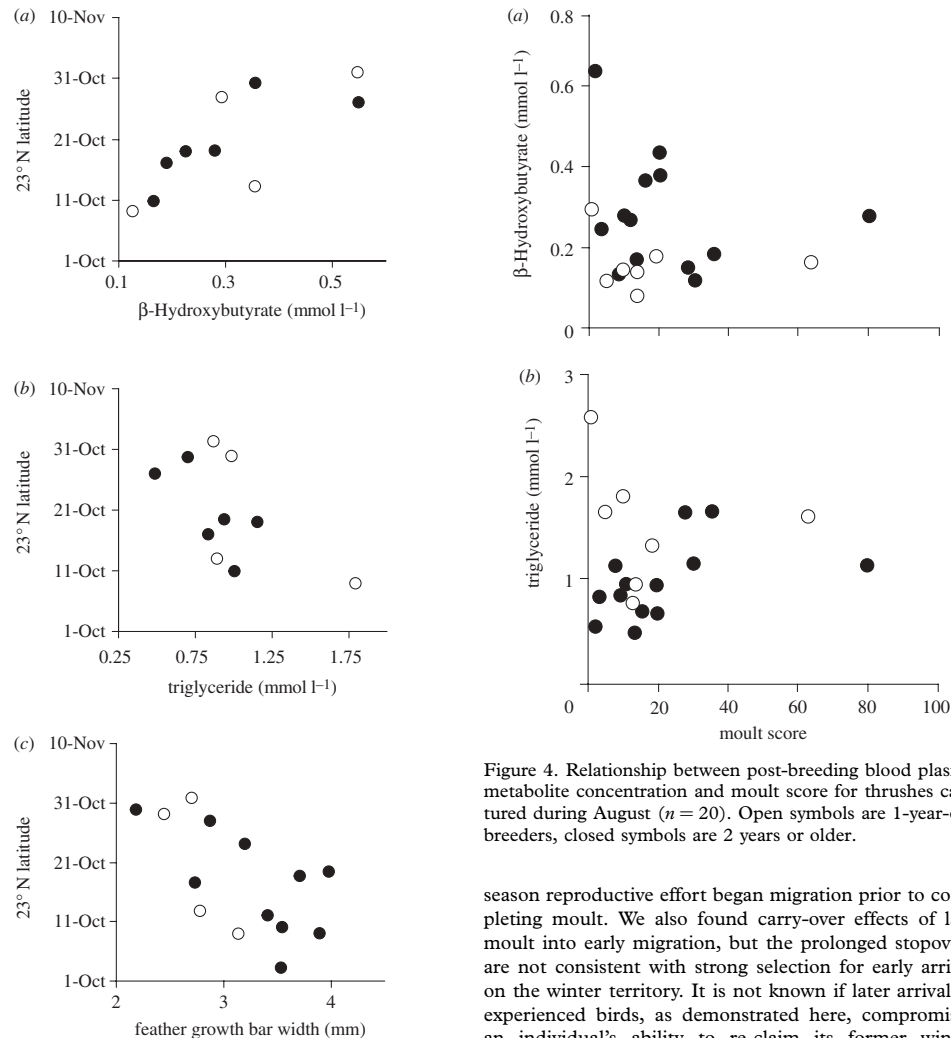


Figure 3. Timing of migration into the tropics was correlated with an individual's post-breeding energetic condition in August as measured by (a) plasma  $\beta$ -hydroxybutyrate, (b) triglyceride and (c) growth bar width of the rectrix. Open symbols are 1-year-old breeders, closed symbols are 2 years or older. (a)  $r_{\text{ss}} = -0.78$ ;  $p = 0.008$ . (b)  $r_{\text{ss}} = -0.64$ ;  $p = 0.05$ . (c)  $r_{\text{ss}} = -0.62$ ;  $p = 0.02$ .

Strong seasonal carry-over effects occur during spring migration in American redstarts (*Setophaga ruticilla*) because poor winter territory quality delays spring migration [1] and late arrival on the breeding grounds reduces reproductive success [2,4]. For species that defend individual winter territories, rapid autumn migration and early arrival at the destination could be under strong selection if early-arriving individuals can better compete for high-quality winter territories that, in turn, enhance survival and body condition [3,16,26]. Norris *et al.* [5] found that male redstarts with high late

Figure 4. Relationship between post-breeding blood plasma metabolite concentration and moult score for thrushes captured during August ( $n = 20$ ). Open symbols are 1-year-old breeders, closed symbols are 2 years or older.

season reproductive effort began migration prior to completing moult. We also found carry-over effects of late moult into early migration, but the prolonged stopovers are not consistent with strong selection for early arrival on the winter territory. It is not known if later arrival of experienced birds, as demonstrated here, compromises an individual's ability to re-claim its former winter territory or reduces survival [26].

Post-breeding energetic condition, while still on the breeding grounds, was strongly correlated with the timing of entry into the tropics two months later, and 2000 km away. Energetic condition affects day-to-day decisions of migrants to stay versus fly once the trip is underway [21–23] but why would post-breeding condition be related to a bird's position two months later?  $\beta$ -Hydroxybutyrate typically decreases, and triglyceride increases, as moult progresses and onset of migration approaches [17]. Thus individuals with low  $\beta$ -hydroxybutyrate and high triglyceride in mid-August could simply be those who finished nesting early and were in a more advanced state of moult. However, variation in post-breeding plasma metabolite concentration in wood thrushes was not significantly related to nest completion date or stage of moult. Although parental effort can have energetic carry-over effects on the moult period [18,19], post-breeding energetic condition was also not related to prior number of young fledged. Consumption

of high-protein food is an important determinant of energetic condition and fat storage in birds with a flexible diet [35] so habitat quality at the moulting site may have an important influence on post-breeding energetic condition and hence timing of migration.

However, given the prolonged autumn migration of wood thrush it is difficult to view their overall autumn migration speed, and timing of entry into the tropics, as constrained by post-breeding energetic condition two months earlier. We suggest the possibility that some individuals prepare to migrate more rapidly than others by investing more heavily in fat storage during the early stages of moult. Geolocator tracking now allows carry-over effect hypotheses for autumn migration to be tested with experimental manipulation of timing of breeding, reproductive effort and energetic condition. The selective advantages of fast versus slow migration remain to be determined and will require monitoring physiological condition, territory acquisition and survival of geolocator birds after they arrive on the wintering grounds.

We thank C. Guglielmo for use of his physiology laboratory at the University of Western Ontario. Field assistance was provided by M. Brady, R. Kresnik, T. Piraino, and C. Stanley and many volunteers. Funding was from the Natural Sciences and Engineering Research Council of Canada, the National Geographic Society, Molson Foundation and proceeds from *Silence of the Songbirds* (Stutchbury 2007, Walker & Co.).

## REFERENCES

- Marra, P. P., Hobson, K. A. & Holmes, R. T. 1998 Linking winter and summer events in a migratory bird by using stable carbon isotopes. *Science* **282**, 1884–1886. (doi:10.1126/science.282.5395.1884)
- Norris, D. R., Marra, P. P., Kyser, T. K., Sherry, T. W. & Ratcliffe, L. M. 2004a Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proc. R. Soc. Lond. B* **271**, 59–64. (doi:10.1098/rspb.2003.2569)
- Angelier, F., Holberton, R. L. & Marra, P. P. 2009 Does stress response predict return rate in a migratory bird species? A study of American redstarts and their non-breeding habitat. *Proc. R. Soc. B* **276**, 3545–3551. (doi:10.1098/rspb.2009.0868)
- Reudink, M. W., Marra, P. P., Kyser, T. K., Boag, P. T., Langin, K. M. & Ratcliffe, L. M. 2009 Non-breeding season events influence sexual selection in a long-distance migratory bird. *Proc. R. Soc. B* **276**, 1619–1626. (doi:10.1098/rspb.2008.1452)
- Norris, D. R., Marra, P. P., Mongomerie, R., Kyser, T. K. & Ratcliffe, L. M. 2004b Reproductive effort, moulting latitude, and feather color in a migratory songbird. *Science* **306**, 2249–2250. (doi:10.1126/science.1103542)
- Stutchbury, B. J. M., Tarof, S. A., Done, T., Gow, E., Kramer, P., Tautin, J., Fox, J. W. & Afanasyev, V. 2009 Tracking long-distance songbird migration using geolocators. *Science* **323**, 896. (doi:10.1126/science.1166664)
- Siikamäki, P., Hovi, M. & Ratti, O. 1994 A trade-off between current reproduction and moult in the pied flycatcher—an experiment. *Funct. Ecol.* **8**, 587–593. (doi:10.2307/2389919)
- Evans Ogden, L. J. & Stutchbury, B. J. M. 1996 Constraints on double brooding in a neotropical migrant, the hooded warbler. *Condor* **98**, 736–744. (doi:10.2307/1369855)
- Mulvihill, R. S., Latta, S. C. & Newell, F. L. 2009 Temporal constraints on the incidence of double brooding in the Louisiana waterthrush. *Condor* **111**, 341–348. (doi:10.1525/cond.2009.080037)
- Cyr, N. E., Wikelski, M. & Romero, L. M. 2008 Increased energy expenditure but decreased stress responsiveness during moult. *Physiol. Biochem. Zool.* **81**, 452–462. (doi:10.1086/589547)
- Bonier, F., Martin, P. R., Jensen, J. P., Butler, L. K., Ramenofsky, M. & Wingfield, J. C. 2007 Pre-migratory life history stages of juvenile arctic birds: costs, constraints, and trade-offs. *Ecology* **88**, 2729–2735. (doi:10.1890/07-0696.1)
- Holmgren, N. & Hedenström, A. 1995 The scheduling of molt in migratory birds. *Evol. Ecol.* **9**, 354–368. (doi:10.1007/BF01237759)
- Vega Rivera, J. H., McShea, W. J., Rappole, J. H. & Haas, C. 1998 Pattern and chronology of prebasic molt for the wood thrush and its relation to reproduction and migration departure. *Wilson Bull.* **110**, 384–392.
- Marra, P. P. 2000 The role of behavioral dominance in structuring patterns of habitat occupancy in a migrant bird during the nonbreeding season. *Behav. Ecol.* **11**, 299–308. (doi:10.1093/beheco/11.3.299)
- Johnson, M. D., Sherry, T. W., Holmes, R. T. & Marra, P. P. 2006 Assessing habitat quality for a migratory songbird wintering in natural and agricultural habitats. *Conserv. Biol.* **20**, 1433–1444. (doi:10.1111/j.1523-1739.2006.00490.X)
- Brown, D. R. & Sherry, T. W. 2008 Alternative strategies of space use and response to resource change in a wintering migrant songbird. *Behav. Ecol.* **19**, 1314–1325. (doi:10.1093/beheco/arn073)
- Jenni-Eiermann, S. & Jenni, L. 1996 Metabolic differences between the post-breeding, molting and migratory periods in feeding and fasting passerine birds. *Funct. Ecol.* **10**, 62–72. (doi:10.2307/2390263)
- Hörak, P., Jenni-Eiermann, S. & Ots, I. 1999 Do great tits (*Parus major*) starve to reproduce? *Oecologia* **119**, 293–299.
- Kern, M., Bacon, W., Long, D. & Cowie, R. J. 2005 Blood metabolite and corticosterone levels in breeding adult pied flycatchers. *Condor* **107**, 665–677. (doi:10.1650/0010-5422(2005)107[0665:BMACLI]2.0.CO;2)
- Yong, W. & Moore, F. 1997 Spring stopover of intercontinental migratory thrushes along the northern coast of the Gulf of Mexico. *Auk* **114**, 263–278.
- Goymann, W., Spina, F., Ferri, A. & Fusani, L. 2010 Body fat influences departure from stopover sites in migratory birds: evidence from whole-island telemetry. *Biol. Lett.* (doi:10.1098/rsbl.2009.1028)
- Guglielmo, C. G., Cerasale, D. J. & Eldermire, C. 2005 A field validation of plasma metabolite profiling to assess refueling performance of migratory birds. *Physiol. Biochem. Zool.* **78**, 116–125. (doi:10.1086/425198)
- Fusani, L., Cardinale, M., Carere, C. & Goymann, W. 2009 Stopover decision during migration: physiological conditions predict nocturnal restlessness in wild passerines. *Biol. Lett.* **5**, 302–305. (doi:10.1098/rsbl.2008.0755)
- Hedenström, A. 2008 Adaptations to migration in birds: behavioural strategies, morphology and scaling effects. *Phil. Trans. R. Soc. B* **363**, 287–299. (doi:10.1098/rstb.2007.2140)
- Pyle, P., Howell, S. N. G., DeSante, R. P., Yunick, R. P. & Gustafson, M. 1997 *Identification guide to North American passerines*. Bolinas, CA: Slate Creek Press.
- Rappole, J. H., Ramos, M. A. & Winker, K. 1989 Wintering wood thrush movements and mortality in southern Veracruz. *Auk* **106**, 402–410.



- 27 Jenni-Eiermann, S. & Jenni, L. 1994 Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the garden warbler. *Auk* **112**, 888–899.
- 28 Guglielmo, C. G., O'Hara, P. D. & Williams, T. D. 2002 Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living western sandpipers (*Calidris mauri*). *Auk* **119**, 437–445. (doi:10.1642/0004-8038(2002)119[0437:EAISOV]2.0.CO;2)
- 29 Grubb Jr, T. C. 1989 Ptilochronology: feather growth bars as indicators of nutritional status. *Auk* **106**, 314–320.
- 30 Shawkey, M. D., Beck, M. L. & Hill, G. E. 2003 Use of a gel documentation system to measure feather growth bars. *J. Field Orn.* **74**, 125–128.
- 31 Schaub, M. & Jenni, L. 2001 Variation of fuelling rates among sites, days and individuals in migrating passerine birds. *Funct. Ecol.* **15**, 584–594. (doi:10.1046/j.0269-8463.2001.00568.x)
- 32 Sillett, T. S. & Holmes, R. T. 2002 Variation in survivorship of a migratory songbird throughout its annual cycle. *J. Anim. Ecol.* **71**, 296–308. (doi:10.1046/j.1365-2656.2002.00599.x)
- 33 Schwilch, R., Grattarola, A., Spina, F. & Jenni, L. 2002 Protein loss during long-distance migratory flight in passerine birds: adaptation and constraint. *J. Exp. Biol.* **205**, 687–695.
- 34 Owen, J. C. & Moore, F. R. 2006 Seasonal differences in immunological condition of three species of thrushes. *Condor* **108**, 389–398. (doi:10.1650/0010-5422(2006)108[389:SDIICO]2.0.CO;2)
- 35 Smith, S. B., McWilliams, S. R. & Guglielmo, C. G. 2007 Effect of diet composition on plasma metabolite profiles in a migratory songbird. *Condor* **109**, 48–58. (doi:10.1650/0010-5422(2007)109[48:EODCOP]2.0.CO;2)

# Repeat Tracking of Individual Songbirds Reveals Consistent Migration Timing but Flexibility in Route

Calandra Q. Stanley<sup>1</sup>, Maggie MacPherson<sup>1</sup>, Kevin C. Fraser<sup>\*</sup>, Emily A. McKinnon, Bridget J. M. Stutchbury

Department of Biology, York University, Toronto, Ontario, Canada

## Abstract

Tracking repeat migratory journeys of individual animals is required to assess phenotypic plasticity of individual migration behaviour in space and time. We used light-level geolocators to track the long-distance journeys of migratory songbirds (wood thrush, *Hylocichla mustelina*), and, for the first time, repeat journeys of individuals. We compare between- and within-individual variation in migration to examine flexibility of timing and route in spring and autumn. Date of departure from wintering sites in Central America, along with sex and age factors, explained most of the variation (71%) in arrival date at North American breeding sites. Spring migration showed high within-individual repeatability in timing, but not in route. In particular, spring departure dates of individuals were highly repeatable, with a mean difference between years of just 3 days. Autumn migration timing and routes were not repeatable. Our results provide novel evidence of low phenotypic plasticity in timing of spring migration, which may limit the ability of individuals to adjust migration schedules in response to climate change.

**Citation:** Stanley CQ, MacPherson M, Fraser KC, McKinnon EA, Stutchbury BJM (2012) Repeat Tracking of Individual Songbirds Reveals Consistent Migration Timing but Flexibility in Route. PLoS ONE 7(7): e40688. doi:10.1371/journal.pone.0040688

**Editor:** Claudia Mettke-Hofmann, Liverpool John Moores University, United Kingdom

**Received:** February 13, 2012; **Accepted:** June 13, 2012; **Published:** July 25, 2012

**Copyright:** © 2012 Stanley et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was funded by the Natural Sciences and Engineering Research Council of Canada, National Geographic Society, U.S. Fish & Wildlife Service Neotropical Migratory Bird Grant, Kenneth G. Molson Foundation, Schad Foundation and proceeds from Silence of the Songbirds (2007, Walker & Co.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: fraserkev@gmail.com

These authors contributed equally to this work.

## Introduction

The degree to which long-distance migration is flexible in time and space is much debated [1]. Endogenous programs may control most of the variation in migration schedules [2,3] or these may be largely flexible at the individual level in response to environmental conditions before departure and en route [4,5]. Determining the degree of plasticity in migration schedules and routes is important for predicting responses to climate change [5]. Recent studies suggest that declines of long-distance migratory birds are a result of endogenous, relatively inflexible departure schedules from wintering sites in the tropics [6].

Inferences regarding the flexibility of migration schedules and routes have been largely restricted to observations at single breeding, winter, or stopover areas, providing only a snapshot of individual migratory behaviour. Observations of repeat migratory journeys of individual birds may yield important insights into the degree to which migratory programs are flexible, but data are rare due to the difficulty in continuous tracking of birds over large distances. Recent examples from birds large enough to carry satellite tags are illuminating; both osprey and marsh harriers showed relatively consistent migration timing, particularly in spring, but low route fidelity, suggesting strong endogenous control of schedules but relative flexibility to local conditions along migratory routes [7,8]. New developments in direct-tracking technologies [9] now allow small birds to be tracked over an annual cycle [10,11,12,13,14,15]. Using data from light-level geolocators, we compared between- and within-individual variation in migration timing and route of 45 individual songbirds

(wood thrush *Hylocichla mustelina*) and examined repeat autumn and spring journeys of 10 individuals. If migration schedules are relatively fixed at the individual level, then departure date should strongly predict arrival date [2], and individuals should exhibit high repeatability in departure date from year to year [8]. We expected high repeatability in spring compared to fall migration schedules owing to stronger stabilizing selection on arrival date in temperate spring environments [16] and carry-over effects of breeding events on autumn migration schedules [12].

## Methods

### Ethics Statement

This study was conducted in accordance with the recommendations of the Ornithological Council 'Guidelines to the Use of Wild Birds in Research' and was approved by the York University Animal Care Committee (Animal Care Protocol Number: 2009-2 W (R1)). Governmental scientific permits for capture, handling and geolocator attachment were obtained in the U.S., Belize, and Costa Rica.

We used data from light-level geolocators (MK14S, 1.6 g, British Antarctic Survey) retrieved between 2008 and 2011 at a breeding site in Pennsylvania, USA ('PA', 41.8°N, 79.9°W, n = 30), and wintering sites in Costa Rica ('CR', 10.4°N, 84.0°W, n = 19) and Belize ('BZ', 16.6°N, 88.7°W, n = 7). Geolocators were attached to birds using a leg-loop harness [17] made of 2.5 mm Teflon ribbon [9]. Total weight of the geolocator and harness was 1.9 g or less which is ca. 4% of the weight (45.9 g ± 4.3, n = 241) of



wood thrush. Ten individual birds were tracked for at least 2 years (PA,  $n=6$ ; CR  $n=4$ ). Sex of each individual was determined by breeding characters or genetically; age class was determined by plumage characteristics. Our total data set consisted of 56 fall and spring migration tracks, including 9 individuals (1 female, 8 males) tracked twice and one individual tracked three times (female). Eleven birds (7 CR and 4 BZ) tagged at wintering sites were tracked on their first spring migration, but none of these were tracked in subsequent years. Mean breeding latitude of all birds was  $41.5^{\circ}\text{N}$  (range  $33.2\text{--}46.9^{\circ}\text{N}$ ); 82% of birds bred within  $\pm 2.5^{\circ}$  of the mean.

**Geolocator analyses.** Light data were analyzed using BASTrak software package (British Antarctic Survey). Raw light data were adjusted for any clock drift (typically  $<3$  min.). Sunrise and sunset were defined as light transitions where the light levels crossed a threshold of 16 (2008 model) or 5 (2009–2010 model). These thresholds represent similar light intensities, based on static calibration of geolocators in known locations. Light transitions were then visually inspected and edited using the program TransEdit to delete false sunrises and sunsets (e.g. transitions during daytime caused by shading) and to score the quality of true sunrise and sunset transitions. The slope of the light data at dawn or dusk was visually compared to transition slopes from static geolocators with a full sun exposure. Very shallow slopes were marked as low confidence, as were transitions that included small peaks in light intensity prior to reaching sunrise threshold, or after reaching sunset threshold. In these cases, the marked transition was unlikely to be within 10 minutes of the actual sunrise/sunset transition and so was excluded from subsequent analysis. Only the transitions with a high confidence score were used in further analyses. After each light data file was edited, we used the program Locator (BAS) to transform light data into latitudinal and longitudinal positions and used a sun elevation angle calculated using season-specific data [18] gathered from birds carrying geolocators at known breeding and wintering sites.

**Movement analyses.** We relied primarily on longitude to determine timing of movements, since error in longitude is much smaller than error in latitude and longitude is not affected by the equinoxes, whereas latitude cannot be determined near the equinox (day length is the same everywhere). Position estimates may be influenced by topography, weather, seasonal changes in behaviour, and vegetation structure [19]. The influence of these factors on longitudinal position error is expected to be low as compared to latitude, but has not been quantified. Our study is unique, in that we deployed geolocators at both temperate breeding and tropical wintering locations enabling ground-truthing of position estimates. We used data retrieved from birds carrying geolocators at a winter site in Costa Rica ( $n=15$ ) and at a Pennsylvania breeding site ( $n=23$  birds) to calculate sun elevation angles for determining unknown breeding and winter sites. Using season- and location-specific sun elevations resulted in average error in longitude of  $55\pm 18$  km (mean  $\pm 95\%$  CI) at tropical winter sites, and  $105\pm 29$  km at temperate breeding sites. In a temperate, non-migratory thrush, longitude error using geolocators was  $50\pm 34$  km [18]. It is impossible to ground-truth position estimates during migration, but we assume similar error in longitudinal position during migration.

Movements away from breeding or wintering sites were defined as shifts in longitude greater than  $2^{\circ}$  in a direction consistent with migration; such shifts were typically accompanied by strong shifts in latitude consistent with migration direction. Arrival dates at breeding and wintering sites were determined when longitudinal values no longer shifted in a direction consistent with migration, varied less than  $2^{\circ}$ , and remained similar throughout the breeding

or wintering period. Autumn departure date was unobtainable for many birds because migration was due south (i.e. primarily shifting in latitude) and thus position was masked by the autumnal equinox. Therefore, we used the date birds crossed  $23.4^{\circ}\text{N}$  (entry to Tropics) as a measure of timing of migration as it occurred well after the equinox period [12]. We calculated autumn migration distance for the final leg of the trip, between crossing of  $23.4^{\circ}\text{N}$  and wintering sites. To test for spatial repeatability of migration routes, we used longitude crossing  $23.4^{\circ}\text{N}$  (Tropic of Cancer) in both spring and autumn. The Tropic of Cancer coincides with a large migration barrier for wood thrushes, the Gulf of Mexico, and therefore is the most biologically important point on the route. Preliminary examination of our migration data suggested that birds could cross this barrier by several routes which also had a strong effect on subsequent final route to the breeding site. Route repeatability of satellite-tracked harriers was estimated at three latitudes along migratory routes [8]. Geolocator error in latitude is about twice that for longitude, and latitude cannot be determined within two weeks of the autumn and spring equinox. Thus, to retain the highest accuracy in route assignment, we choose a latitude that represents a migration barrier (Gulf of Mexico;  $23.4^{\circ}\text{N}$ ) where stopovers are not possible, thus timing of crossing is usually discernible, and where the range of possible routes was maximized (i.e. routes differ by at least 100 km in longitude). Measuring repeatability at a more southerly latitude, within the tropics and prior to crossing the Gulf, would not be informative because wood thrush are naturally funneled by a narrow land mass (Yucatan Peninsula) as they pass between the Gulf of Mexico and wintering sites.

We examined three migration variables (date and longitude at cross of  $23.4^{\circ}\text{N}$  and arrival date) that are directly comparable between autumn and spring migration. The autumn equinox made it impossible to obtain departure dates for birds that did not substantially shift longitude on departure. Migration pace and duration is therefore not directly comparable between seasons. In autumn we measured pace and duration beginning at  $23.4^{\circ}\text{N}$  (i.e. the last leg of the trip) whereas in spring the pace and duration reflected the entire journey. However, timing of crossing  $23.4^{\circ}\text{N}$  in autumn is influenced by events at breeding sites [12] and in the subset of birds for which data were available, departure date was significantly correlated with date of crossing into tropics ( $F_{1,19}=7.65$ ,  $R^2=0.25$ ,  $p<0.01$ ).

**Variation in migration timing and repeatability analyses.** To explore factors influencing variation in spring and winter arrival date of all birds ( $n=56$ ), we fit general linear models with departure date, breeding latitude, sex, and age (spring only, 1<sup>st</sup> spring migration or not) as factors. We used tools in R that use a backwards step procedure (“step” function, R Development Core Team 2011) to drop individual explanatory variables one by one, refit the model each time, and then used Akaike’s Information Criterion (AIC) to measure model fit and complexity and select the optimal model [20]. With individual as a factor in an ANOVA, we compared between- and within-individual variation in migration timing and route [8]. We also determined the repeatability of time and space factors of 10 birds tracked in more than one year [21]. The same temporal and spatial variables (Table 1) were used in both analyses. Some migration variables were not available for all birds due to equinox, missing days, and battery failure. All analyses were conducted using R (R Development Core Team 2011).

## Results

The timing of spring departure from Central America explained much of the variation (71%) in arrival dates at breeding sites, along

**Table 1.** *p*-values of one-way ANOVA testing the effects of individual on migration variables of wood thrushes.

Variable	df	f	p-value
<b>autumn migration</b>			
date cross 23.4°N	38,8	3.13	0.77
longitude crossing 23.4°N	39,8	1.63	0.28
winter arrival date	43,8	1.60	0.25
Duration	42,8	1.58	0.25
<b>spring migration</b>			
departure date	42,9	4.27	0.01*
date cross 23.4°N	44,10	3.13	0.03*
longitude crossing 23.4°N	43,10	0.70	0.80
breeding arrival	43,9	4.69	0.009**
duration	51,8	2.74	0.07

Total of 56 individual fall and spring migrations tracked, including 9 individuals tracked twice and one individual tracked three times. Significance level indicated by asterisks: \* $p < 0.05$ ; \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

doi:10.1371/journal.pone.0040688.t001

with sex and age factors ( $F_{4,45} = 31.47$ ,  $R^2 = 0.71$ ,  $p < 0.001$ ) (figure 1a). Spring departure and breeding arrival were positively correlated (model estimate  $0.42 \pm 0.09$  SE,  $t = 4.5$ ,  $p < 0.0001$ ). As expected, males arrived earlier than females (estimate of  $-8.09 \pm 1.74$  SE days,  $t = -4.56$ ,  $p < 0.0001$ ) and birds on their first spring migration arrived later than birds that had migrated at least once before being tracked (estimate  $12.48 \pm 2.24$  SE days,  $t = 5.57$ ,  $p < 0.0001$ ).

Autumn date crossing 23.4°N, was the only factor retained in the minimum adequate model but explained only 25% of the variation in winter arrival date ( $F_{1,45} = 16.34$ ,  $R^2 = 0.25$ ,  $p < 0.001$ ). Individual had a significant effect in all spring migration timing variables (spring departure date, date crossing 23.4°N, breeding arrival date) except for spring migration duration (Table 1 and electronic supplementary material, Table S1). There was no effect of individual on autumn migration timing, or longitude at 23.4°N in spring or autumn (Table 1). Within-individual repeatability tests showed similar results: spring migration timing was more repeatable than longitude at 23.4°N, and spring variables were more repeatable than autumn variables (Table 2). The timing of winter arrival, spring departure, and breeding arrival were highly repeatable ( $r$ -values 0.62–0.71) (figure 1b, c, d) and spring departure dates ( $r = 0.71$ ) differed between years by an average of  $\pm 3$  d. Longitudes of migratory routes had low repeatability in both spring and autumn.

## Discussion

Our results, based on comparisons among and within individuals, suggest that the timing of songbird migration in spring is under strong endogenous control and highly repeatable from year to year. For all birds, spring departure date was a significant predictor of breeding arrival date; differing departure dates and spring routes did not uncouple the relationship between departure and arrival. Considering the 27-day range of departure dates from wintering sites, and that comparisons were made over multiple years and presumably variable environmental conditions, it is surprising that departure dates of individuals tracked in multiple years were highly consistent between years. The high repeatability in spring departure date suggests a stronger influence of

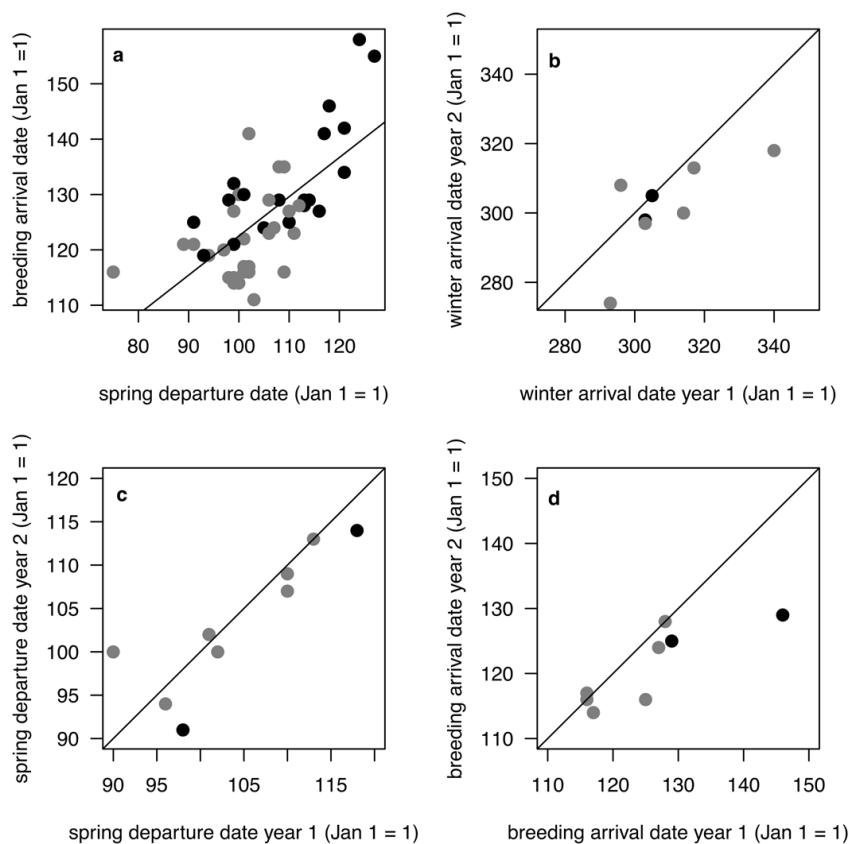
endogenous schedules than local environmental conditions, in contrast to a recent study of a Neotropical migrant warbler with strong social dominance that influences individual access to food, where departure dates were only 38% repeatable [4]. Spring migration in wood thrush is thus more similar to that of a long-distance migratory shorebird (bar-tailed godwit, *Limosa lapponica baueri*) where spring departure date from wintering sites in New Zealand was a strong predictor of arrival at Alaskan breeding sites [2] and spring departure date was highly repeatable between years [22]. However, the stronger relationship between spring departure date and breeding arrival date in wood thrush, than in godwit [2], may be explained by flexibility in the latter species in response to favourable wind conditions that mediate initial spring departure date [23].

Timing was less repeatable at date of crossing 23.4°N, which implies flexibility in migration timing en route to breeding sites [5], particularly around the period when birds cross a major open-water migration barrier, the Gulf of Mexico. These results are compatible with recent ringing studies suggesting some en route flexibility of songbird migration pace [24,25] likely in response to weather and quality of stopover sites. In wood thrushes, breeding arrival dates, although repeatable, were on average 4 days earlier in the second year of tracking than the first (6 of 8 birds came back earlier in year 2). Earlier arrival at breeding sites by older individuals is well established by mark-recapture studies and is driven by sexual selection [26]. Age and experience likely improve the fine-tuning of migration schedules and [27] reduce the fitness costs of coping with inclement early weather during early spring. Direct-tracking studies comparing repeat migratory journeys of adult and juvenile songbirds have not been performed but would be invaluable for understanding the mechanisms driving differences in migration schedules between age classes.

Winter arrival date was also consistent for individuals and en route timing (date crossing 23.4°N) explained 25% of the variation in arrival date. Field and laboratory studies indicate that autumn departure date is heritable in songbirds and largely under endogenous control [3,16]. In wood thrushes, timing of crossing 23.4°N in autumn was correlated with departure date from breeding sites but is also dependent on individual timing of molt and physiological condition prior to migration [12]. Timing and pace of autumn migration is flexible because late-breeding birds tended to moult and migrate later, though did not arrive later at the wintering territory owing to long stopovers by many birds en route [12,28]. Migration timing was generally more repeatable in spring than fall migration. While it would be ideal to compare repeatability of spring versus autumn migration over the entire journey, due to overlap of migration with the fall equinox we could only examine autumn migration during the latter portion of the trip.

In contrast to migration timing, migration route (as measured by longitude after crossing the 23.4°N), had relatively low repeatability in spring and autumn. This suggests that route is not under strong endogenous control, and may be influenced by individual energetic condition and weather patterns. A similar flexibility in migratory routes, but not timing, was found in migratory raptors [7–8]. As with harriers [8], the variation we observed in longitude may reflect a fine-tuning of migration in response to local conditions, within the constraints of timing cued by winter photoperiod and selection for optimal arrival at breeding areas.

Our low repeatability estimates for routes compared with timing of migration may occur in part if there are large differences in measurement error between these two aspects of migration behaviour. The precision of light-level based geolocation data in



**Figure 1. Migration schedules of wood thrush.** (a) Spring departure date versus breeding arrival date of 56 migration tracks of 45 different individual wood thrushes (line shows least-squares regression). Black data points indicate female, grey male. Migration timing for individuals tracked in two consecutive years: (b) winter arrival date, (c) spring departure date, (d) breeding arrival date. For b–d, lines show 1:1 relationship.  
doi:10.1371/journal.pone.0040688.g001

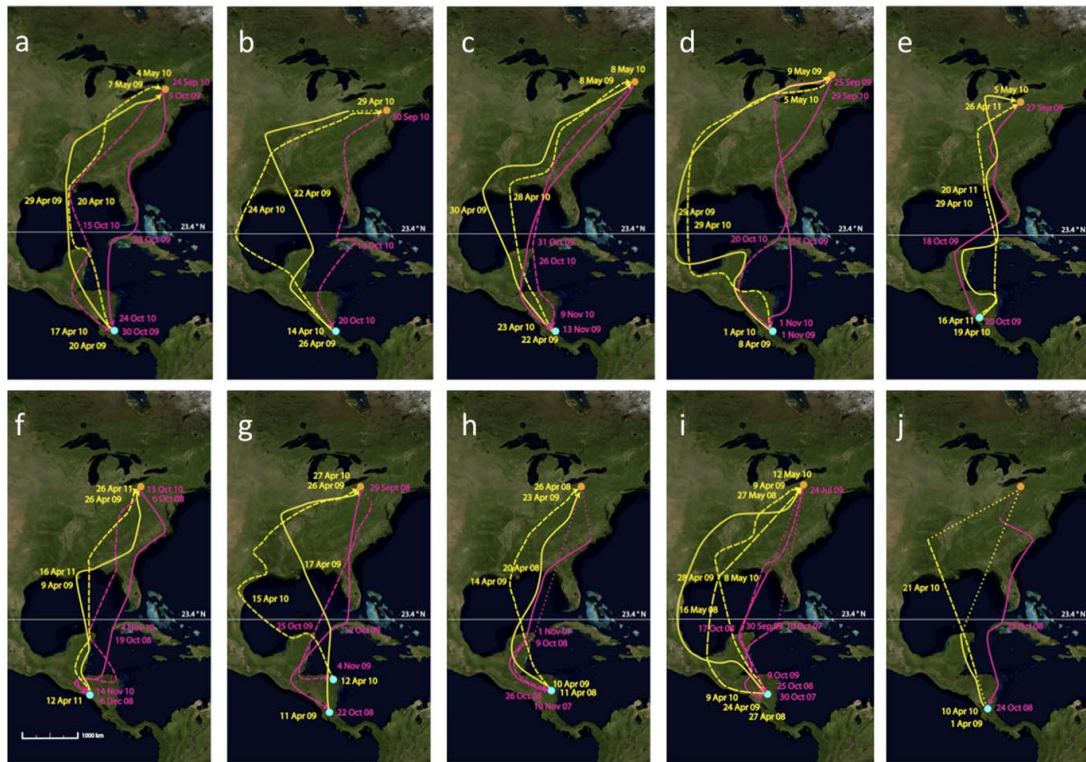
**Table 2. Repeatability ( $r$ ) of migration timing and route variables for wood thrushes tracked in two consecutive years** ( $*p < 0.05$ ;  $**p < 0.01$ ,  $***p < 0.001$ ).

variable	df	f	r	p-values
<b>autumn migration</b>				
date cross 23.4°N	7, 8	0.44	0.05	0.44
longitude crossing 23.4°N	9, 10	1.28	0.12	0.43
winter arrival date	8, 9	4.20	0.62	0.02*
autumn migration duration	6, 7	2.82	0.48	0.10
<b>spring migration</b>				
spring departure date	9, 10	5.94	0.71	0.005**
date cross 23.4°N	9, 10	2.90	0.49	0.07
longitude crossing 23.4°N	9, 10	1.28	0.12	0.35
breeding arrival date	8, 9	4.96	0.66	0.01*
spring migration duration	8, 9	2.37	0.41	0.11

doi:10.1371/journal.pone.0040688.t002

estimating location, even for longitude, is likely low compared with timing of major migration movements. We quantified spatial error in longitude ( $55 \pm 18$  km, mean  $\pm 95\%$  CI) based on data obtained from wood thrushes carrying geolocators at known wintering sites (McKinnon et al. in prep.), although error estimates during migration may be higher because there are fewer days on which to base locations. Migration timing was also based largely on longitudinal shifts, defined using the same longitudinal error estimates from ground-truthing. Since both spatial and temporal measures of migration depend on longitude, error may be comparable. Unfortunately, it is not possible to ground truth timing of migration and estimate error since a bird's movements can only be determined from the geolocators themselves. However, within-individual route differences in longitude from year to year typically deviated by more than several hundred km (see Fig. 2 g), which is greater than the measurement uncertainty. Start-to-finish spring route of many individuals were substantially different between years (Fig. 2) which contrasts dramatically with the low within-individual variation in timing of spring departure (Fig. 1).

Repeatability is a measure of individual consistency relative to other individuals in the population. Spring departure differed by



**Figure 2. Repeat migration routes of individuals tracked in consecutive years by geolocator deployment.** Birds were tracked from (a) Pennsylvania (b) Costa Rica. Yellow = spring migration, pink, pink = fall migration. Orange circle = breeding site, blue = winter site. Short-dashed lines indicate migration tracks in the second year and long-dashed lines a third year. Dotted lines indicate where migration route was unknown due to poor-quality light data, or geolocator battery failure.  
doi:10.1371/journal.pone.0040688.g002

only 3 days, on average, for individuals from one year to the next which is surprising considering that departure dates in the population spanned 30 days. In contrast, longitude of spring migration route at 23.4°N was highly flexible for some individuals (8–10° difference between years) and nearly spanned the population-level range in spring route (12° longitude). Within-individual differences in spring or fall route (Fig. 2) may reflect flexibility to inter-annual variation in local environmental conditions en route such as wind, availability of suitable stopover habitat and potential interactions with variation in the physical condition of the bird itself [26,29]. Low route fidelity suggests that birds may employ a complex interaction of compass mechanisms [30] to navigate to goal areas, such as breeding or wintering sites, using different routes [7]. More birds crossed the Gulf of Mexico in spring than in autumn, which may reflect a time-minimization strategy, consistent with selection for early breeding arrival, or may be driven by seasonal variation in wind conditions and fueling opportunities [10]. Investigation of temporal variation in environmental factors at key stopover sites and barriers (Gulf of Mexico) can allow tests of hypotheses for the remarkable within-individual and inter-seasonal differences in route we observed in wood thrushes.

Overall, our results show that migration schedules are more consistent among individuals and more repeatable within individ-

uals than migratory routes, particularly in spring. Consistent schedules, based on tracking of individual osprey [7], godwits [2], harriers [8], and songbirds (this study) may reflect strong stabilizing selection on the timing of migration in long-distance migrants [16]. We found stronger repeatability, and coupling of departure and arrival dates, for spring migration than autumn migration. Strong endogenous control of spring migration is expected because early arrival may increase mortality during cold periods [31] and late arrival reduces reproductive success [16,32]. Inflexible response of migration schedules to climate change has been implicated in population declines of long-distance migratory birds [33]. Understanding how inflexible spring migration schedules affect fitness of forest songbirds like the wood thrush is important for interpreting population declines.

## Supporting Information

**Table S1 Results of ANOVA testing the effects of individual on migration variables of wood thrushes.** Total of 56 individual fall and spring migrations tracked, including 9 individuals tracked twice and one individual tracked three times. Significance indicated in brackets (n.s., \* $p < 0.05$ ; \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). (DOC)

## Acknowledgments

We thank E. Gow, T. Done, and many field assistants and volunteers. We thank Thomas Alerstam and two anonymous reviewers for their comments on an earlier draft of this manuscript.

## References

- Knudsen E, Linden A, Both C, Jonzen N, Pulido F, et al. (2011) Challenging claims in the study of migratory birds and climate change. *Biological Reviews* 86: 928–946.
- Conklin JR, Battley PF, Potter MA, Fox JW (2010) Breeding latitude drives individual schedules in a trans-hemispheric migrant bird. *Nature Communications* 1.
- Berthold P (1996) *Control of Bird Migration*. London: Chapman & Hall.
- Studds CE, Marra PP (2011) Rainfall-induced changes in food availability modify the spring departure programme of a migratory bird. *Proceedings of the Royal Society B-Biological Sciences* 278: 3437–3443.
- Both C (2010) Flexibility of timing of avian migration to climate change masked by environmental constraints en route. *Current Biology* 20: 243–248.
- Both C, Bouwuis S, Lessells CM, Visser ME (2006) Climate change and population declines in a long-distance migratory bird. *Nature* 441: 81–83.
- Alerstam T, Hake M, Kjellen N (2006) Temporal and spatial patterns of repeated migratory journeys by ospreys. *Animal Behaviour* 71: 555–566.
- Vardanis Y, Klaassen RHG, Strandberg R, Alerstam T (2011) Individuality in bird migration: routes and timing. *Biology Letters* 7: 502–505.
- Stutchbury BJM, Tarof SA, Done T, Gow E, Kramer PM, et al. (2009) Tracking long-distance songbird migration by using geolocators. *Science* 323: 896–896.
- Tottrup AP, Klaassen RH, Strandberg R, Thorup K, Kristensen MW, et al. (2012) The annual cycle of a trans-equatorial Eurasian-African passerine migrant: different spatio-temporal strategies for autumn and spring migration. *Proc Biol Sci* 279: 1008–1016.
- Bairlein F, Norris DR, Rolf N, Bulte M, Voigt CC, et al. (2012) Cross-hemisphere migration of a 25 g songbird. *Biology Letters*.
- Stutchbury BJM, Gow EA, Done T, MacPherson M, Fox JW, et al. (2011) Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics. *Proceedings of the Royal Society B-Biological Sciences* 278: 131–137.
- Heckscher CM, Taylor SM, Fox JW, Afanasyev V (2011) Veery (*Catharus fuscescens*) wintering locations, migratory connectivity, and a revision of its winter range using geolocator technology. *Auk* 128: 531–542.
- Ryder TB, Fox JW, Marra PP (2011) Estimating migratory connectivity of Gray Catbirds (*Dumetella carolinensis*) using geolocator and mark-recapture data. *Auk* 128: 448.
- Schmaljohann H, Buchmann M, Fox JW, Bairlein F (2012) Tracking migration routes and the annual cycle of a trans-Saharan songbird migrant. *Behavioural Ecology and Sociobiology*.
- Pulido F, Widmer M (2005) Are long-distance migrants constrained in their evolutionary response to environmental change? Causes of variation in the timing of autumn migration in a blackcap (*Sylvia atricapilla*) and two garden warblers (*S. borin*) populations. In: Bauchinger U, Goymann W, Jenni-Eiermann S, editors. *Bird Hormones and Bird Migrations: Analyzing Hormones in Droppings and Egg Yolks and Assessing Adaptations in Long-Distance Migration*. pp. 228–241.

## Author Contributions

Conceived and designed the experiments: CS MM KF EM BS. Performed the experiments: CS MM KF EM BS. Analyzed the data: CS MM KF EM BS. Wrote the paper: CS MM KF EM BS.

- Rappole JH, Tipton AR (1991) New harness design for attachment of radio transmitters to small passerines. *Journal of Field Ornithology* 62: 335–337.
- Fudickar AM, Wikelski M, Partecke J (2010) Tracking migratory songbirds: accuracy of light-level loggers (geolocators) in forest habitats. *Methods in Ecology and Evolution* 3: 47–52.
- Lisovski S, Hewson CM, Klassen RHG, Korner-Nievergelt F, Kristensen MW, et al. (2012) Geolocation by light: accuracy and precision affected by environmental factors. *Methods in Ecology and Evolution*.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. New York: Springer.
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities - a common mistake. *Auk* 104: 116–121.
- Battley PF (2006) Consistent annual schedules in a migratory shorebird. *Biology Letters* 2: 517–520.
- Conklin JR, Battley PF (2011) Impacts of wind on individual migration schedules of New Zealand bar-tailed godwits. *Behavioral Ecology* 22: 854–861.
- Marra PP, Francis CM, Mulvihill RS, Moore FR (2005) The influence of climate on the timing and rate of spring bird migration. *Oecologia* 142: 307–315.
- Tottrup AP, Thorup K, Rainio K, Yosef R, Lehtikoinen E, et al. (2008) Avian migrants adjust migration in response to environmental conditions en route. *Biology Letters* 4: 685–688.
- Newton I (2008) *The Migration Ecology of Birds*. London: Academic Press.
- Thorup K, Bisson I-A, Bowlin MS, Holland RA, Wingfield JC, et al. (2007) Evidence for a navigational map stretching across the continental US in a migratory songbird. *Proceedings of the National Academy of Sciences of the United States of America* 104: 18115–18119.
- Stutchbury BJM, Gow EA, Done T, MacPherson M, Fox JW, et al. (2011) Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics. *Proceedings of the Royal Society B-Biological Sciences* 278: 131–137.
- Sandberg R, Moore FR (1996) Migratory orientation of red-eyed vireos, *Vireo olivaceus*, in relation to energetic condition and ecological context. *Behavioral Ecology and Sociobiology* 39: 1–10.
- Alerstam T (2006) Conflicting evidence about long-distance animal navigation. *Science* 313: 791–794.
- Brown CR, Brown MB (2000) Weather-mediated natural selection on arrival time in cliff swallows (*Petrochelidon pyrrhonota*). *Behavioral Ecology and Sociobiology* 47: 339–345.
- Norris DR, Marra PP, Kyser TK, Sherry TW, Ratcliffe LM (2004) Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proceedings of the Royal Society of London Series B-Biological Sciences* 271: 59–64.
- Both C, Van Turnhout CAM, Bijlsma RG, Siepel H, Van Strien AJ, et al. (2010) Avian population consequences of climate change are most severe for long-distance migrants in seasonal habitats. *Proceedings of the Royal Society B-Biological Sciences* 277: 1259–1266.

## Estimating geolocator accuracy for a migratory songbird using live ground-truthing in tropical forest

### Abstract

Miniaturized light-level geolocators allow year-round tracking of small migratory birds, but most studies use calibration only at breeding sites to estimate geographic positions. Ground-truthing of positions in tropical habitat is needed to determine how accurate breeding site calibrations (i.e. sun elevations) are for estimating location of winter sites. We tested the accuracy of geographic assignments using geolocator data collected from Wood Thrushes (*Hylocichla mustelina*) in Central America. For a given light threshold, sun elevation angle was higher in the tropics than at breeding sites and also varied significantly at tropical winter sites between wet (Oct-Dec) and dry (Jan-Mar) seasons. However, estimation of Wood Thrush territory latitude did not differ significantly when using breeding or tropical dry season sun elevation. Average error in assignment to tropical sites was  $365 \pm 97$  km ( $0.2\text{--}4.4^\circ$ ) in latitude. To obtain the best latitude estimates in the tropics with geolocators, we recommend using locations during the dry season where sun elevations are closer to those measured at breeding sites. We emphasize the importance of longitude in assigning forest birds to unknown sites; longitude estimates for Wood Thrushes in the tropics were, on average, within  $66 \pm 13$  km ( $0\text{--}0.6^\circ$ ) of actual longitude. Latitude estimates were more accurate ( $180 \pm 48$  km) when assigning birds to breeding sites using deployments of geolocators in the tropics. Studies of species that are territorial in winter could collect more accurate migratory connectivity data by deploying geolocators at tropical wintering sites.

### Keywords

Migration • Geo-loggers • Tracking • Sun elevation • Central America

© Versita Sp. z o.o.

Emily A. McKinnon<sup>1</sup>,  
Calandra Q. Stanley<sup>1</sup>,  
Kevin C. Fraser<sup>1</sup>,  
Maggie M. MacPherson<sup>1</sup>,  
Garth Casbourn<sup>1</sup>,

Peter P. Marra<sup>2</sup>,  
Colin E. Studds<sup>2</sup>,  
Nora Diggs<sup>2</sup>,  
Bridget J. M. Stutchbury<sup>1</sup>

<sup>1</sup>Dept. of Biology, York University,  
4700 Keele St., Toronto, Ontario,  
Canada, M3J 1P3

<sup>2</sup>Smithsonian Conservation Biology Institute,  
Migratory Bird Center, National Zoological  
Park, Washington, D.C., USA, 20008

<sup>3</sup>ARC Centre of Excellence for Environmental  
Decisions, the NERP Environmental  
Decisions Hub, Centre for Biodiversity  
& Conservation Science,  
University of Queensland,  
Brisbane, Queensland 4072

Received 03 November 2012

Accepted 28 December 2012

### Introduction

Tracking migration of small (<80g) songbirds has been limited to following small-scale movements using radio-telemetry [e.g., 1] or rare recoveries of banded birds [e.g., 2]. The recent surge in the use of miniaturized light-level geolocators has provided novel information on year-round movements of small, long-distance migratory birds [3–13]. Geolocators detect and archive light levels continually relative to an internal clock; thus, day length and noon/midnight times can be estimated upon retrieval to determine approximate latitude and longitude. Geographic assignments from geolocators are invaluable for determining patterns of migratory connectivity between breeding and wintering populations thousands of kilometres apart [14], identifying stopover regions [15], and informing effective conservation and management across broad spatial and temporal scales [16–18].

Differences in vegetation type, topography, and daily and seasonal weather cause variance in the ambient light

levels detected by geolocators, producing error in location estimates [19,20]. Geolocators have been used successfully on birds that inhabit open environments, such as seabirds [21,22], open-country raptors [23], and songbirds [7,12,24]. Increasingly, researchers are deploying geolocators on forest-dwelling songbirds [5,6] and ground-truthing is essential to validate the usefulness of this method. Developing methods for improving geolocator accuracy for forest-dwelling species is important because many of these species are experiencing long-term population declines and geolocators have the potential to be an important tool to inform conservation priorities [25].

The most commonly used method for analysing light data from geolocators is the ‘threshold method’, in which geolocators must be calibrated at known locations so that the light threshold chosen to define sunrise and sunset can be associated with an accurate sun angle relative to the horizon (‘sun elevation’). Latitude assignments using geolocator data are sensitive to

\* E-mail: emilymck@yorku.ca



sun elevation because they are based on total day length (i.e. lower sun elevations = relatively longer day length). In contrast, longitude assignments are not sensitive to sun elevation changes. Longitude assignments are determined by the time midway between the sunrise and sunset, relative to an internal clock. As long as the sunrise and sunset are determined by the same light level (threshold), longitude assignments using geolocators are inherently more accurate than latitude assignments.

To date, tests of geocator accuracy in terrestrial environments have been performed using (1) live deployments on a non-migratory species and assessing accuracy across the year [19], (2) static deployments (i.e., not on live birds) to assess geographic differences in accuracy across a range of sites in Europe [19], (3) static deployments across habitat types on the breeding grounds [20], and (4) comparisons of geocator accuracy at the breeding grounds using live deployments on species that differ greatly in ecology and behaviour [20]. Most geocator studies aim to estimate the position of a bird when it is hundreds or, more typically, thousands of kilometres away from the deployment site. A remaining critical gap in our knowledge of geocator accuracy is the extent to which sun elevation measured at a known breeding site provides a good estimate of location for a bird at its unknown wintering site and vice versa.

Methods for geocator tracking of long-distance migratory songbirds have been based on calibrations from breeding sites in North America [3,5,6] or Europe [4,19]. Using a breeding-site calibration for sun elevation assumes similar sun elevation angles are encountered during the non-breeding period (during migration and at winter sites). This assumption is typically made for logistical reasons; however, given that bird behaviour, climate, and habitat differ greatly between breeding and non-breeding sites, the sun elevation angles at which a given light threshold is achieved are expected to differ for individuals occupying temperate versus tropical forests.

We calculated sun elevation angles from light data downloaded from geolocators deployed on Wood Thrush (*Hylocichla mustelina*), a long-distance migratory bird that breeds in temperate deciduous forest in eastern North America and migrates to tropical wet forest in Central America. To mimic the assignment of a bird to an unknown location, we estimated latitude for birds during stationary breeding and wintering periods using both the breeding- and winter-derived sun elevations. We then calculated the accuracy of each sun elevation by comparing latitude estimates to the actual latitude. We predicted that tropical forests, which are more heavily shaded than temperate forests, would have significantly higher sun elevation values (i.e. the sun must be higher on the horizon to achieve the same ambient light level) and thus season-specific sun elevations would provide the best accuracy (lowest error) in mapping locations of migratory birds. This study is the first to report ground-truthing of sun elevations for both breeding and wintering sites and to quantify how the use of breeding-ground sun elevations affects accuracy of wintering site latitude estimates.

## Methods

We analyzed light data from 59 geolocators (50 model MK14S, and 9 MK10S, British Antarctic Survey - BAS) deployed on birds at five breeding sites (Pennsylvania, North Carolina, Virginia, and Vermont, USA; and in Ontario, Canada) and two winter sites (Belize and Costa Rica) from 2007 to 2011 (see Table 1 for coordinates of sites). We collected light data from geolocators retrieved after bi-annual migrations ( $n = 43$ ) (i.e., ~ 1 year post-deployment) and from birds that were recaptured within the same summer or winter prior to migration ( $n = 16$ ) by downloading data without removing the geocator ('live downloads'). At our main study sites in Pennsylvania, Belize, and Costa Rica, return rates for geocator-tagged birds (14-38%, depending on site and sex) were higher or equal to return rates for birds that were banded only (BJM Stutchbury, CQ Stanley, and EA McKinnon, *unpubl. data*).

We calculated sun elevations using light data from all individuals captured, including some birds captured in more than one year (Pennsylvania  $n = 5$ , Costa Rica  $n = 2$ ) and 2 birds that had 'live downloads' and were recaptured the following season (Belize  $n = 2$ ). Samples from the same individual were always from different years (e.g. winter 2010/11 and winter 2011/12), and we therefore treated each as independent.

We examined how average sun elevation changed seasonally within a site due to predictable differences in foliage emergence, bird behaviour, and rainfall. At breeding sites, sun elevation angles were calculated separately for arrival (1-15 May), breeding (Jun-Jul), and moult (15 Aug – 14 Sept) periods, which correspond with vegetation changes as deciduous forests experience leaf-out and as birds become reclusive during moult in late summer and early fall [15,26]. Sun elevation angles were determined for winter sites in Central America from both the

**Table 1.** Sun elevation average  $\pm$  95% confidence interval from geolocators on birds at breeding sites in North America in July-Aug, and winter sites in Central America during the dry season from Jan-Mar.

Deployment Site	Lat	Long	n	Live calibration sun elevation
Vermont, USA	44.4	72.9	2	-2.65
Ontario, Canada	43.3	80.5	4	-1.39
Pennsylvania, USA	41.8	79.9	24	-2.10 $\pm$ 0.31
Virginia, USA	38.7	77.1	1	-1.90
North Carolina, USA	35.4	83.1	5	-1.98
Breeding site mean			5	-2.01
Belize	16.5	88.7	17	-1.05 $\pm$ 0.43
Costa Rica	10.4	84.0	25	-0.04 $\pm$ 0.83
Winter site mean			2	-0.55

rainy season (Oct-Dec) and dry season (Jan-Mar) to account for vegetation and weather changes between these two periods. Sample size for each seasonal period varied owing to the deployment date of the geolocator. For example, birds captured and tagged with a geolocator in late March and recaptured in November may not have had any useable light data for the dry season (Jan-Mar); but individuals captured in March with a geolocator from the previous winter had data from both wet season (Oct-Dec) and dry season (Jan-Mar).

All light data were adjusted for clock drift if necessary (1-3 minutes) using the program Decompressor (British Antarctic Survey, BAS). False sunrises and sunsets were deleted (see Supplemental Material Figure S1) and remaining light transitions were scored based on comparisons with light data from open habitat static calibrations (both at breeding and winter sites) using the programs TransEdit (BAS) or TransEdit2 (BAS) (Supplemental Material Figure S2). In open habitat, light data from static calibrations show smooth curves at sunset and sunrise; data from birds that showed a similar smooth curve were scored as high-quality transitions (Figure S2). Data with light peaks prior to the threshold for sunrise or after the threshold for sunset has passed were scored as poor-quality and removed from all analyses.

Changes in geolocator technology since the original deployments in 2007 resulted in our data being collected from a variety of geolocator light-sensitivities and stalk-lengths. In 2010, geolocators were manufactured with a higher light sensitivity and we determined from open habitat calibration that a light threshold of 5 corresponded to a threshold of 16 with the original MK14S geolocators. We tested for an effect of geolocator sensitivity and stalk-length on sun elevations at known sites for these light thresholds and found no significant effect on breeding and winter sun elevations across sites (one-way ANOVA, breeding  $F_{1,34} = 2.41$ ,  $P = 0.13$ , winter  $F_{1,26} = 0.25$ ,  $P = 0.61$ ) or within a single site (Pennsylvania) where four geolocator types were used (one-way ANOVA,  $F_{1,22} = 2.17$ ,  $P = 0.15$ ). Thus all geolocators were grouped for subsequent analysis.

We calculated sun elevations using the average of all sites rather than the average of all individuals because sample sizes varied across sites and our goal was to obtain a sun elevation that best represented conditions across the breeding or wintering range for Wood Thrush. We did not weight our average using sample size because a single site could have a bias due to topography or habitat that would skew sun elevation in a particular direction [20]. We did not use an individual bird's sun elevation to calculate its later locations because as soon as an individual departs on migration (or its behaviour or habitat changes) sun elevation angle will likely also change. Instead, we calculated average sun elevation for each site using multiple individuals.

To test the accuracy of latitude estimation of known-site birds, we used the program Locator (BAS) to determine latitude for each bird using a given sun elevation. In all analyses, we included both midnight and noon locations to determine average locations since Wood Thrushes are territorial and stationary during

breeding and wintering periods. We calculated latitude and error for each individual's known breeding or wintering site using both breeding- and winter-derived sun elevations. To assign birds to breeding sites, we used locations from the months of June and July since all Wood Thrushes at our Pennsylvania breeding site were stationary at this time (assessed using geolocator data, radio-tracking data, and observation/captures at the sites). We used January and February locations to assign birds to winter sites because all Wood Thrushes from Belize and Costa Rica were stationary during this period (assessed using geolocator data, radio-tracking data, and observation/captures at the sites) and fewer days during this season (dry season) were omitted due to poor quality light transitions.

Relatively close to the equator, geolocators record many latitude outliers because of shorter dawn and dusk twilight periods as well as greater shading in dense tropical forests. We excluded unrealistic latitudes in our winter assignments by filtering latitudes that fell outside the possible winter range for Wood Thrushes (7.2°N to 21.0°N). This filter was not necessary for breeding assignments since all latitudes fell within the Wood Thrush breeding range.

To determine if sun elevation varied within a site, we compared sun elevations using one-way ANOVA (pre-breeding, breeding, or moulting period) or a Student's *t*-test (rainy vs. dry season in winter). The accuracy (measured in km of error) of assigning birds to known breeding and winter sites using season-specific sun elevations was compared using Student's *t*-test with sun elevation as the dependent variable. All statistical analyses were conducted using the software R (version 2.14.0, R Development Core Team, R Foundation for Statistical Computing, <http://www.r-project.org/>) and means are reported  $\pm$  standard error unless otherwise specified.

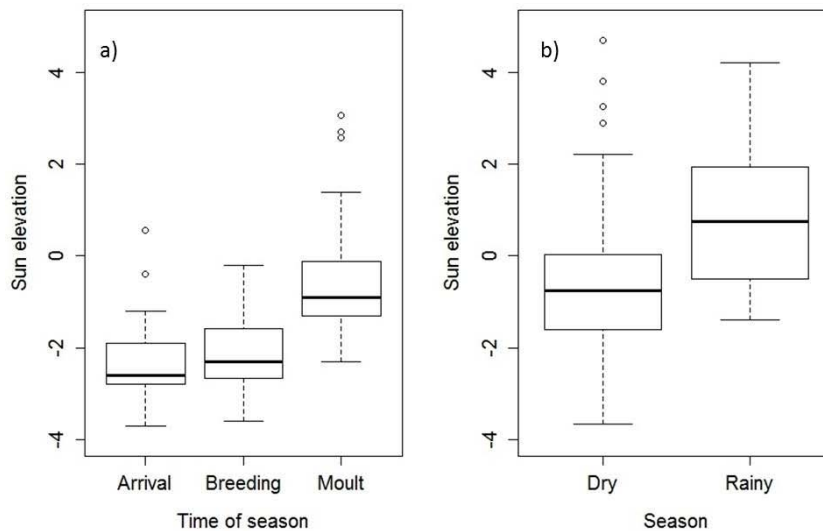
## Results

*Within-season sun elevation differences.*—Sun elevations during spring arrival at breeding sites in North America (early May;  $n = 25$ , mean  $-2.31 \pm 0.19$ ) and peak breeding (June-July;  $n = 36$ , mean  $-2.03 \pm 0.14$ ) periods were significantly different from sun elevation during the moult period (Aug-Sept;  $n = 37$ , mean  $-0.51 \pm 0.22$ ) (one-way ANOVA,  $F_{2,91} = 27.31$ ,  $P < 0.0001$ ) (Figure 1a). At Central American tropical sites, sun elevations during the rainy season ( $n = 23$ , mean  $0.81 \pm 0.33$ ) were significantly higher than during the dry season ( $n = 42$ , mean  $-0.45 \pm 0.28$ ) (*t*-test,  $t = -2.94$ ,  $df = 50.73$ ,  $P = 0.005$ ) (Figure 1b).

*Between-season sun elevation differences.*—As expected, sun elevations were higher at tropical forest 'wintering' sites in Central America than at deciduous forest breeding sites in North America (Table 1). Within the tropics, sun elevation angle in secondary rain forest in Belize (~11 year old forest regrowth after hurricane damage) was significantly lower (i.e. less shady environment) than in primary rain forest in Costa Rica ( $t = -2.12$ ,  $df = 33.99$ ,  $P = 0.04$ ; Table 1).

*Sun elevation and geolocator error.*—When estimating winter locations of birds that received geolocators in Belize and Costa





**Figure 1.** Within-season variation in sun elevation angles at known locations in a) eastern North America (see Table 1 for location coordinates) and b) Central America (Costa Rica and Belize). Arrival = 1–15 May ( $n = 25$  individuals), breeding = 1 Jun – 31 Jul ( $n = 36$ ), moulting = 15 Aug – 14 Sept ( $n = 37$ ). Dry season = Jan–Mar ( $n = 42$ ), rainy season = Oct–Dec ( $n = 23$ ). Mean is indicated by a dark line, and boxes extend to 25<sup>th</sup> and 75<sup>th</sup> quartiles, with whiskers extending to maximum and minimum values, and outliers indicated by open circles.

Rica, geolocator error in latitude was similar using breeding (Jun–Jul) and winter dry season sun elevations ( $t = 0.55$ ,  $df = 46.6$ ,  $P = 0.58$ ) (Figure 2a, Figure 3) (Table 2). The breeding sun elevation averaged across sites ( $-2.01$ ) resulted in latitude estimates in the tropics within  $0.3^\circ$  and  $4.4^\circ$  of actual latitude for Belize and Costa Rica, respectively (Table 2). Winter sun elevation calculated from the dry season ( $-0.55$ ) resulted in latitude estimates that differed on average from actual locations by  $2.7^\circ$  in Belize and  $1.5^\circ$  in Costa Rica. Longitude estimates were highly accurate during the dry season for both wintering sites (average distance error 66 km,  $0.6^\circ$ ; Table 2, Figure 3).

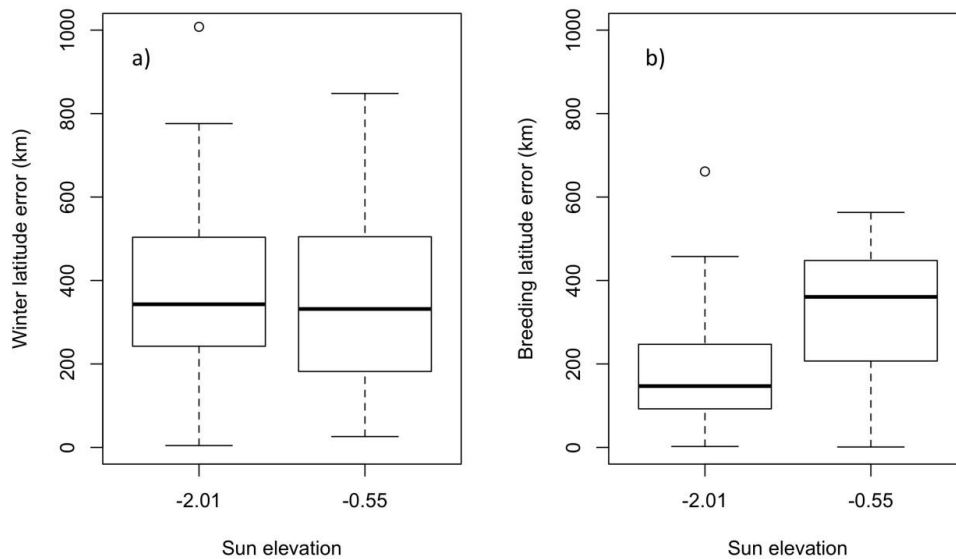
Migratory connectivity mapping can also be accomplished by deploying geolocators on the wintering grounds and estimating breeding location. Geolocators were more accurate for estimating breeding site location when the breeding sun elevation, rather than the winter dry season sun elevation, was used. For the Pennsylvania population, the breeding sun elevation ( $-2.01$ ) resulted in an average latitude  $1.2^\circ$  north of the actual breeding site (Table 2, Figure 3). However, use of winter dry season sun elevation from Belize and Costa Rica doubled the latitude error and over-estimated breeding latitude for the Pennsylvania population by an average of  $2.6^\circ$  (Figure 3) and resulted in a significantly larger average distance error ( $t = -3.3$ ,  $df = 43.8$ ,  $P = 0.002$ ) (Figure 2b).

Regardless of sun elevation angle used, latitude error was higher at Central American sites relative to North American sites. In contrast to latitude, longitude had lower error at Central American sites (Table 2).

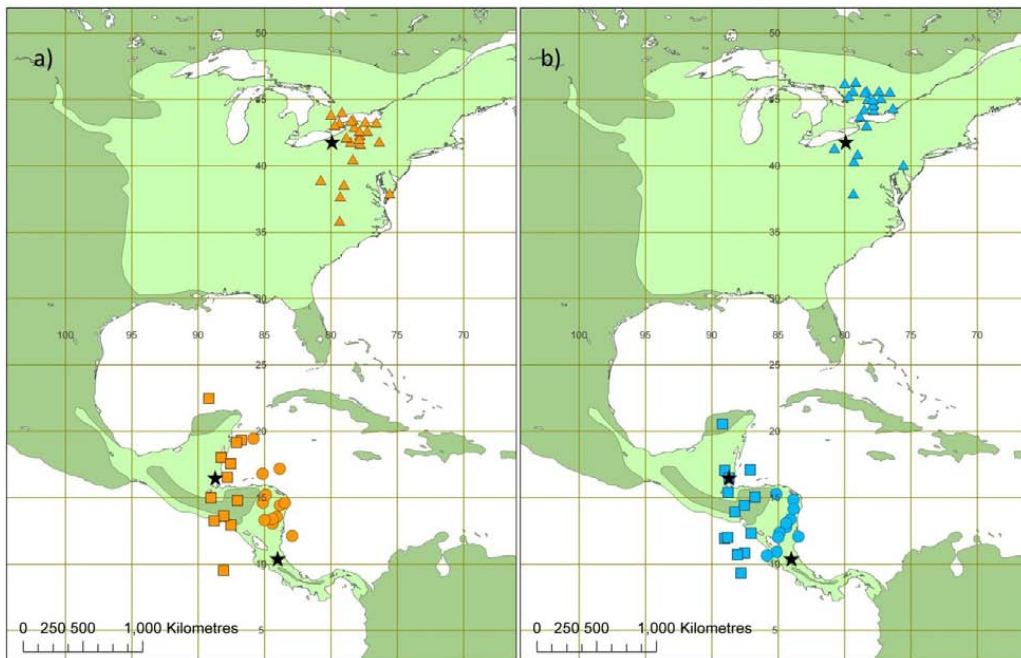
## Discussion

The use of miniaturized geolocators is providing remarkable new insights into migratory connectivity, migration routes, and within-season movements of songbirds [3–12,15]. Most studies to date have used breeding-site calibrated sun elevations at deployment sites to estimate latitude at unknown locations during the non-breeding season. Our results show that data from geolocators could be used with breeding-ground sun elevation calibration to assign a forest songbird to winter sites in Central America with good accuracy (for Wood Thrushes, within  $0.2$ – $4.4^\circ$  of actual latitude, and within  $0$ – $0.6^\circ$  of actual longitude, on average).

Obtaining ground-truthed geolocator data in the tropics is logistically impractical for many species, particularly those that are not territorial in winter or those inhabiting relatively remote regions. Another proposed method for calibrating sun elevation angles without having ground-truthing data is the Hill-Ekstrom calibration [20]. This method relies on the fact that latitude estimates deviate more from true latitude as the equinoxes approach, and by plotting latitude over time using several different sun elevation angles, the sun elevation that causes the smallest deviation in latitude is apparent. However, a major assumption of this method, outlined by [20], is that shading must be equal throughout the stationary period of the bird. Our results show that sun elevation angles differ within stationary periods at both breeding and wintering sites (Figure 1). Sun elevation was significantly higher in the wet season than the dry season in the Neotropics (Figure 1b). Heavy and frequent rainfall during



**Figure 2.** Latitude error in km from sun elevations derived from breeding habitat (-2.01,  $n = 5$  sites) and dry season wintering habitat (-0.55) for Wood Thrushes at known locations in a) Central American tropical lowland forest ( $n = 23$ ), and b) eastern North American deciduous forest ( $n = 35$ ). Mean is indicated by a dark line, and boxes extend to 25<sup>th</sup> and 75<sup>th</sup> quartiles, with whiskers extending to maximum and minimum values, and outliers indicated by open circles.



**Figure 3.** Geolocator-derived mean locations for breeding ( $n = 23$ ) and wintering birds ( $n = 11$  Costa Rica,  $n = 14$  Belize) stationary at deployment sites (indicated by black stars) in Pennsylvania, USA (triangles), Belize (squares) and Costa Rica (circles), using two different sun elevation angles: a) breeding sun elevation angle (-2.01), b) winter sun elevation angle (-0.55). Light green shading indicates the breeding and winter range for Wood Thrushes. Maps were generated using ArcGIS 10 (ERSI).

**Table 2.** Estimated locations and error using the breeding season (Jun-Jul; -2.01) versus winter dry season (Jan-Mar; -0.55) sun elevations for Wood Thrushes breeding in Pennsylvania ( $n = 23$ ) and wintering in Costa Rica ( $n = 13$ ) and Belize ( $n = 15$ ). Note that longitude is not affected by sun elevation; thus, only one longitude value is shown per site. Error is calculated by comparing the estimated location from the geolocator with the known location of the bird. Values shown are means and 95% confidence intervals. Blank boxes indicate where values were not applicable.

Site	Breeding sun elevation (-2.01)		Winter sun elevation (-0.55)		Longitude estimate (°W)	Longitude error (km)
	Latitude Estimate (°N)	Error (km)	Latitude Estimate (°N)	Error (km)		
Belize 16.5°N 88.7°W	16.0±2.0	315±125	13.8±4.1	400±135	88.1±0.4	64±24
Costa Rica 10.4°N 84.0°W	14.8±1.2	491±131	11.95±2.0	327±141	84.0±1.0	67±46
Both winter sites		403±96		365±97		66±13
Pennsylvania 41.8°N 79.9°W	41.6±0.9	188±67	44.3±0.9	344±64	78.3±0.5	105±29

the wet season likely caused higher sun elevation angles owing to cloud cover during dawn and dusk, such that the sun would have to be higher on the horizon to produce the same light levels. Furthermore, vegetation is fuller during the rainy season as plants grow in response to precipitation. Because of these seasonal differences, the Hill-Ekstrom calibration may be more appropriately applied within season to obtain accurate results.

We also found that sun elevation angles varied within the breeding season between early arrival, breeding, and moulting periods. As expected, sun elevation angles were lowest in early May, when fewer leaves on the trees would result in less shading from habitat, and highest in Aug-Sept, when Wood Thrushes begin moult [26]. Many forest-dwelling birds use structurally complex and dense habitats during the post-fledging/moulting period [27], and exhibit cryptic behaviour. Thus, higher sun elevations in Aug-Sept are probably due to both micro-scale habitat selection and behaviour. Data collected from geolocators deployed on European Blackbirds (*Turdus merula*) had larger latitudinal error during the birds' moulting period; this likely reflects similar habitat selection and or cryptic behaviour for this species [19]. For connectivity maps, using only locations from periods matching the calibration period (i.e., June and July) would improve accuracy.

Most studies deploy geolocators on the breeding grounds, and our study suggests that mapping wintering sites during the dry season, when sun elevation is most similar to breeding site calibrations, will give the most accurate winter site latitude. For species that undertake intra-tropical migrations [5,9], mapping locations from the entire non-breeding period is important. Latitude estimates for birds are likely to differ to some extent due to sun elevation over the non-breeding period if the birds experience strong seasonality (wet and dry season) or if they change habitat type. Birds can be confirmed as sedentary during both the wet and dry season using longitude estimates. It is useful to have multiple ways to confirm that birds are stationary versus

migrating; a function in the R package GeoLight ('changelight') can also be used to confirm that birds are stationary [28], although to our knowledge, the analyses in this package have not been ground-truthed.

During both spring and fall migration, longitude can indicate movements and stopovers regions with a high degree of accuracy relative to latitude [7,12,19]. For species that winter in Central America, like the Wood Thrush, longitude alone can distinguish wintering locations of individuals because of the east-west orientation of the land bridge (Figure 3), and relatively high accuracy of longitude estimates in Central America (Table 2). Longitude estimates for birds wintering in Belize versus Costa Rica did not overlap (Figure 3), although latitude estimates did overlap extensively (Table 2).

Although longitude estimates had very low error compared with latitude, micro-scale habitat use differences between sunrise and sunset or local topography can result in bias in longitude estimates. The eastward bias in estimated locations of the Pennsylvania birds (Figure 3) are likely a result of the local topography of the field site- a west-facing slope. A similar eastward bias (although much smaller) can be seen in birds from Belize (Figure 3), and is likely an effect of a slight rise in elevation (west-facing) at the site relative to a local river. Topography can also affect latitude estimates. Individuals with unusually southerly latitude estimates from Pennsylvania or northerly latitude estimates from tropical sites (Figure 3) may have occupied territories on high ridges or in stream valleys where apparent day length would be longer or shorter than day length recorded at ground level.

Relatively few studies deploy geolocators on the wintering grounds, but it also must be determined whether sun elevation at deployment sites is similar to sun elevation in the opposite season. Overall error in latitude assignment for geolocators was much higher at Central American sites ( $365 \pm 97$  km 95% CI) than at North American breeding sites ( $180 \pm 48$  km). This is due

in part to latitude estimation becoming inherently less accurate toward the equator. In contrast, longitude assignment was more accurate in Central America ( $66 \pm 13$  km) than in North America ( $113 \pm 31$  km). For long-distance migratory forest birds that shift from temperate to tropical forest, deployment of geolocators in the tropics, when possible, is expected to result in more accurate migratory connectivity mapping for latitude than deployment of geolocators on the breeding grounds. However, return rates of birds in the tropics may be lower than at breeding sites (15-25% for Wood Thrushes, versus 30-60% return rate at breeding sites), because of high mortality rates of first-year birds and lower site fidelity, thus requiring more geolocator deployments to achieve a sufficient sample size in returns.

Geolocators currently provide the most accurate method to remotely assign small (<80g) forest-dwelling landbirds to non-breeding sites in the tropics. Using live calibrations from birds at tropical sites, we show for the first time that sun elevation angles calibrated at breeding sites provided accurate assignment of birds to winter sites. Longitude estimates from geolocators were

more accurate than latitude estimates, and therefore geolocators will provide more accurate migratory connectivity information for forest-dwelling birds with an east-west winter distribution. Our results also indicate that for territorial species with good winter return rates, deployment of geolocators in the tropics would result in accurate assignments to breeding sites in temperate areas using a breeding-site calibrated sun elevation.

## Acknowledgements

We thank the following sources of funding: Natural Sciences and Engineering Research Council of Canada, National Geographic Society, Schad Foundation, Kenneth G. Molson Foundation, U.S. Fish and Wildlife Service Neotropical Migratory Bird Grant, Environment Canada, York University, proceeds from *Silence of the Songbirds* (2007, Walker & Co.). We thank the many dedicated field assistants and volunteers for help with deployment and retrieval of geolocators at multiple sites across North and Central America.

## References

- [1] Cochran W.W., Wikelski M., Individual migratory tactics of New World Catharus thrushes, In: Greenberg R. and Marra P.P. (Eds.), *Birds of Two Worlds*, The Johns Hopkins University Press, Baltimore, MA, 2005
- [2] Rimmer C.C., McFarland K.P., Known breeding and wintering sites of a Bicknell's Thrush, *Wilson Bull.*, 2001, 113, 234-236.
- [3] Stutchbury B.J.M., Tarof S.A., Done T., Gow E., Kramer P.M., Tautin J., et al., Tracking long-distance songbird migration by using geolocators, *Science*, 2009, 323, 896, doi:10.1126/science.1166664.
- [4] Bächler E., Hahn S., Schaub M., Arlettaz R., Jenni L., Fox J.W., et al., Year-round tracking of small trans-saharan migrants using light-level geolocators, *PLOS ONE*, 2010, 5, 4, doi:e9566 10.1371/journal.pone.0009566.
- [5] Heckscher C.M., Taylor S.M., Fox J.W., Afanasyev V., Veery (*Catharus fuscens*) wintering locations, migratory connectivity, and a revision of its winter range using geolocator technology, *Auk*, 2011, 128, 531-542, doi:10.1525/auk.2011.10280.
- [6] Ryder T.B., Fox J.W., Marra P.P., Estimating migratory connectivity of Gray Catbirds (*Dumetella carolinensis*) using geolocator and mark-recapture data *Auk*, 2011, 128, 448-453, doi:10.1525/auk.2011.11091.
- [7] Schmaljohann H., Buchmann M., Fox J.W., Bairlein F., Tracking migration routes and the annual cycle of a trans-Saharan songbird migrant, *Behav. Ecol. Sociobiol.*, 2012, 66, 915-922, doi:10.1007/s00265-012-1340-5.
- [8] Seavy N.E., Humple D.L., Cormier R.L., Gardali T., Establishing the breeding provenance of a temperate-wintering North American passerine, the Golden-Crowned Sparrow, using light-level geolocation, *PLOS ONE*, 2012, 7, doi:e34886 10.1371/journal.pone.0034886.
- [9] Stach R., Jakobsson S., Kullberg C., Fransson T., Geolocators reveal three consecutive wintering areas in the thrush nightingale, *Animal Migration*, 2012, 1, 1-7, doi:10.2478/ami-2012-0001.
- [10] Beason J.P., Gunn C., Potter K.M., Sparks R.A., Fox J.W., The northern Black Swift: Migration path and wintering area revealed Wilson J. *Ornithol.*, 2012, 124, 1-8, doi:http://dx.doi.org/10.1676/11-146.1.
- [11] Åkesson S., Klaassen R., Holmgren J., Fox J.W., Hedenström A., Migration routes and strategies in a highly aerial migrant, the Common Swift *Apus apus*, revealed by light-level geolocators, *PLOS ONE*, 2012, 7, e41195, doi:e41195 10.1371/journal.pone.0041195.
- [12] Bairlein F., Norris D.R., Nagel R., Bulte M., Voight C.C., Fox J.W., et al., Cross-hemisphere migration of a 25g songbird, *Biol. Lett.*, 2012, 2012, 505-507, doi:10.1098/rsbl.2011.1223.
- [13] Delmore K.E., Fox J.W., Irwin D.E., Dramatic intraspecific differences in migratory routes, stopover sites and wintering areas, revealed using light-level geolocators, *Proc. R. Soc. B-Biol. Sci.*, 2012, Published Online 26 Sept, doi:10.1098/rspb.2012.1229.
- [14] Fraser K.C., Stutchbury B.J.M., Silverio C., Kramer P.M., Barrow J., Newstead D., et al., Continent-wide tracking to determine migratory connectivity and tropical habitat associations of a declining aerial insectivore, *Proc. R. Soc. B-Biol. Sci.*, 2013, 279, 4901-4906.
- [15] Stutchbury B.J.M., Gow E.A., Done T., MacPherson M., Fox J.W., Afanasyev V., Effects of post-breeding moult and energetic condition on timing of songbird migration into the

- tropics, *Proc. R. Soc. B-Biol. Sci.*, 2011, 278, 131-137, doi:10.1098/rspb.2010.1220.
- [16] Martin T.G., Chades I., Arcese P., Marra P.P., Possingham H.P., Norris D.R., Optimal conservation of migratory species, *PLOS ONE*, 2007, 2, e751, doi:e751 10.1371/journal.pone.0000751.
- [17] Sheehy J., Taylor C.M., McCann K.S., Norris D.R., Optimal conservation planning for migratory animals: integrating demographic information across seasons, *Conserv Lett*, 2010, 3, 192-202, doi:Doi 10.1111/J.1755-263x.2010.00100.X.
- [18] Marra P.P., Hunter D., Perrault A.M., Migratory connectivity and the conservation of migratory animals, *Environmental Law. Lewis and Clark.*, 2011, 41, 317-354.
- [19] Fudickar A.M., Wikelski M., Partecke J., Tracking migratory songbirds: accuracy of light-level loggers (geolocators) in forest habitats, *Methods in Ecology and Evolution*, 2012, 3, 47-52, doi:10.1111/J.2041-210x.2011.00136.X.
- [20] Lisovski S., Hewson C.M., Klaassen R.H.G., Korner-Nievergelt F., Kristensen M.W., Hahn S., Geolocation by light: accuracy and precision affected by environmental factors, *Methods in Ecology and Evolution*, 2012, 3, 603-612, doi:10.1111/j.2041-210X.2012.00185.x.
- [21] Harris M.P., Daunt F., Newell M., Phillips R.A., Wanless S., Wintering areas of adult Atlantic puffins *Fratercula arctica* from a North Sea colony as revealed by geolocation technology, *Mar Biol*, 2010, 157, 827-836, doi:10.1007/s00227-009-1365-0.
- [22] Nisbet I.C.T., Mostello C.S., Veit R.R., Fox J.W., Afanasyev V., Migrations and winter quarters of five Common Terns tracked using geolocators, *Waterbirds*, 2011, 34, 32-39.
- [23] Rodriguez A., Negro J.J., Bustamante J., Fox J.W., Afanasyev V., Geolocators map the wintering grounds of threatened Lesser Kestrels in Africa, *Divers Distrib*, 2009, 15, 1010-1016.
- [24] Tøttrup A.P., Klaassen R.H.G., Strandberg R., Thorup K., Kristensen M.W., Jørgensen P.S., et al., The annual cycle of a trans-equatorial Eurasian-African passerine migrant: different spatio-temporal strategies for autumn and spring migration, *Proc. R. Soc. B-Biol. Sci.*, 2012, 279, 1008-1016, doi:Doi 10.1098/Rspb.2011.1323.
- [25] Faaborg J., Holmes R.T., Anders A.D., Bildstein K.L., Dugger K.M., Gauthreaux S.A., et al., Conserving migratory land birds in the New World: Do we know enough?, *Ecological Applications*, 2010, 20, 398-418.
- [26] Vega Rivera J.H., McShea W.J., Rappole J.H., Haas C.A., Pattern and chronology of prebasic molt for the Wood Thrush and its relation to reproduction and migration departure, *Wilson Bull.*, 1998, 110, 384-392.
- [27] Chandler C.C., King D.I., Chandler R.B., Do mature forest birds prefer early-successional habitat during the post-fledging period?, *Forest Ecol Manag*, 2012, 264, 1-9, doi:10.1016/j.foreco.2011.09.018.
- [28] Lisovski S., Bauer S., Emmenegger T., Analysis of light based geolocator data, 1.02, 2012, CRAN, cran.r-project.org/web/packages/GeoLight/GeoLight.pdf

1    **Connecting breeding, wintering, and migration sites by range-wide tracking of a declining**  
2    **migratory songbird**

5    **RUNNING HEAD:** Range-wide tracking of a declining bird

6    **KEY WORDS:** migratory connectivity, geocator, tropical deforestation, migration route,  
7    Central America, wood thrush, *Hylocichla mustelina*

8    **WORD COUNT:** 6052

9    **AUTHORS:** Calandra Q. Stanley<sup>a,b,c</sup>, Emily A. McKinnon<sup>a,b,g</sup>, Kevin C. Fraser<sup>b</sup>, Maggie P.  
10    MacPherson<sup>b,d</sup>, Garth Casbourn<sup>b</sup>, Lyle Friesen<sup>e</sup>, Peter P. Marra<sup>c</sup>, Colin Studds<sup>c,f</sup>, T. Brandt  
11    Ryder<sup>c</sup>, Nora Diggs<sup>c</sup> and Bridget J. M. Stutchbury<sup>b</sup>

13    <sup>a</sup> joint first authors

14    <sup>b</sup> Dept. of Biology, York University, Toronto, Canada M3J 1P3

15    <sup>c</sup> Smithsonian Conservation Biology Institute, Migratory Bird Center, National Zoological Park,  
16    Washington DC 20013

17    <sup>d</sup> Current address: Dept. of Ecology and Evolutionary Biology, Tulane University, New Orleans,  
18    LA, 70118

19    <sup>e</sup> Canadian Wildlife Service, Ontario Region, 867 Lakeshore Rd, Burlington, Ontario, Canada

20    <sup>f</sup> Current address: Dept. of Geography & Environmental Systems, 221 Sondheim Hall,  
21    University of Maryland, Baltimore County

22    <sup>g</sup>Corresponding Author, [emilymck@yorku.ca](mailto:emilymck@yorku.ca), 416-736-2100 ext. 66637

## 24   **Abstract**

25   Many migratory animals are experiencing rapid population declines but migration data with the  
26   geographic scope and resolution to quantify the complex network of movements between  
27   breeding and non-breeding regions are often lacking. Determining the most frequently used  
28   migration routes and non-breeding regions for different breeding populations, and the species as  
29   a whole, is critical for understanding population dynamics and making effective conservation  
30   decisions. We tracked the migration of individual songbirds (wood thrushes; *Hylocichla*  
31   *mustelina*;  $n = 102$ ) from across their range by using light-level geolocators, and for the first  
32   time, quantified migration routes and wintering regions for distinct breeding populations. We  
33   developed regional and species-level migratory connectivity networks for this declining songbird  
34   by combining our tracking results with range-wide breeding abundance estimates and forest  
35   cover data. Our species-level migratory connectivity network estimated that over 50% of the  
36   species occupies the eastern wintering range (Honduras to Costa Rica), a region that includes  
37   only one third of all wintering habitat and which is undergoing intensive deforestation. We  
38   estimated that half of all wood thrushes in North America migrate south through Florida in fall,  
39   while in spring approximately 73% funnel northwards through a narrow span along the central  
40   U.S. Gulf Coast (88-93°W). Migratory networks are an important new tool for songbird  
41   conservation and we illustrate with wood thrushes how they can identify conservation hotspots  
42   for regional populations as well as the species as a whole.

43

44

45

46 Conserving migratory species is challenging because threats often occur at unknown locations,  
47 thousands of kilometers away from breeding sites (Martin et al. 2007). Dozens of species of  
48 migratory land birds have experienced steep population declines driven by multiple stressors at  
49 temperate breeding sites, along migration routes and on their tropical non-breeding (hereafter  
50 “wintering”) grounds (Faaborg et al. 2010). One of the biggest challenges in migratory songbird  
51 conservation has been determining the link between well-studied temperate breeding populations  
52 and threats elsewhere in their life cycle (Sherry & Holmes 1996; Sillett et al. 2000), so that  
53 conservation actions can occur where they are most needed and throughout the range of at-risk  
54 populations. Until recently there was little or no information on where specific breeding  
55 populations migrate to, or how they get there, i.e., migratory connectivity (Marra et al. 2006;  
56 Webster et al. 2002). The miniaturization of light-level geolocators small enough for songbirds  
57 has provided a breakthrough for studies of migratory connectivity (Stutchbury et al. 2009).  
58 Geolocators currently provide the most precise (within ~300km in latitude, ~150km longitude)  
59 means to track small birds throughout their annual cycle, offering an unprecedented opportunity  
60 to quantify patterns of connectivity for declining migratory songbirds (McKinnon et al. 2013a).

61       Patterns of migratory connectivity between breeding and wintering sites of migratory  
62 animals can be described qualitatively on a gradient from ‘strong’ to ‘weak’ (Webster et al.  
63 2002). If breeding populations segregate on the wintering grounds, then migratory connectivity is  
64 strong (Fig. 1a) and habitat loss at specific wintering regions should have a large impact on  
65 corresponding breeding regions (Rubenstein et al. 2002). In contrast, if breeding populations mix  
66 extensively in the non-breeding season (Fig. 1b), then migratory connectivity is weak and habitat  
67 loss in one non-breeding region is expected to have a diffuse effect on breeding populations  
68 (Webster et al. 2002).



69            Migratory connectivity studies using band returns and intrinsic markers (e.g. genetic  
70 structure) have documented moderate to strong connectivity at broad continental (Ambrosini et  
71 al. 2009; Irwin et al. 2011; Norris et al. 2006) and sub-continental (Rubenstein et al. 2002)  
72 scales. Two recent studies that tracked individual Purple Martins (*Progne subis*) and Bobolinks  
73 (*Dolichonyx oryzivorus*) found breeding-wintering migratory connectivity was very weak, with  
74 extensive mixing of North American breeding populations on the wintering grounds in South  
75 America (Fraser et al. 2012; Renfrew et al. 2013). Therefore, for these species, threats to  
76 wintering habitat will be similar for all breeding populations. Connectivity of migratory routes  
77 has rarely been studied, since indirect methods, such as stable isotope or genetic analyses, cannot  
78 provide information on start-to-finish migratory routes of songbirds. Many species of songbirds  
79 exhibit loop migration, where spring and fall routes occur along different longitudes (McKinnon  
80 et al. 2013a), but for most species it is unknown to what extent migration routes vary among  
81 breeding populations (but see Fraser et al. 2013).

82            A pattern of strong connectivity can be maintained by segregation of migration routes by  
83 breeding populations due to major geographical barriers (Delmore et al. 2012; Fraser et al. 2012)  
84 or differences in ancestral migration routes (Bairlein et al. 2012; Delmore et al. 2012). A  
85 ‘leapfrog’ connectivity pattern, where birds breeding further north winter further south (Newton  
86 2008), can be a result of a latitudinal gradient in winter habitat quality, if the costs of migrating  
87 farther are mitigated by higher quality habitat (Bell 1997). Strong connectivity can also result  
88 from parallel migration, in which breeding longitude and winter longitude of individuals are  
89 positively correlated (Newton 2008). Segregation of migration routes, regardless of the  
90 mechanism, means that threats along these routes could differ among breeding populations.

91            Migratory connectivity can be summarized using a network, which identifies and  
92        quantifies the connections between breeding, migration, and wintering sites (Taylor & Norris  
93        2010). This network can then be used to develop spatially realistic population models (Marra et  
94        al. 2006; Taylor & Norris 2010) to predict effects of threats in one part of the migratory network  
95        on the species as a whole. These models show that habitat loss in one ‘node’ can have a ripple  
96        effect throughout the network, and can affect populations that are not even directly connected to  
97        the area of habitat loss. Networks can also be used to identify habitat critical for conservation,  
98        by highlighting regions used by a large proportion of the species overall (Martin et al. 2007).  
99        There are diverse theoretical approaches for understanding spatial networks (e. g. Marra et al.  
100        2006; Runge & Marra 2005; Taylor & Norris 2010), but empirical data to quantify migratory  
101        networks for songbirds are still lacking.

102            Migratory networks can be useful for conservation at multiple scales. A migratory  
103        network based on a single regional population is informative for conservation organizations and  
104        government environmental agencies, which are often responsible for developing policies to  
105        safeguard specific populations (e.g. at the level of the state, province, ecoregion). Certain  
106        breeding populations may be also important to conserve because of local adaptations (Lesica &  
107        Allendorf 1995). Species-level conservation requires identification of the habitat that is most  
108        likely benefit the species overall, which requires a species-level migratory network (Martin et al.  
109        2007). This large-scale network quantifies which migratory routes and wintering sites are used  
110        by the greatest proportion of individuals. A species-level network is also a prerequisite for  
111        testing hypotheses regarding the overall mechanisms for population declines and for predictive  
112        modelling (Taylor & Norris 2010).

In this study, we tracked the round-trip migrations of individual wood thrushes (*Hylocichla mustelina*) by deploying geolocators at multiple breeding and wintering sites. Like many other forest songbirds, wood thrush populations have declined (-1.8%/year) over the past ~50 years (Sauer et al. 2012). Our objective was to provide a model of how migration data can be used to develop migratory networks and inform conservation measures. We created the first regional and species-level migratory networks for a songbird, and we quantified forest loss in its Neotropical wintering range. Threats at temperate breeding sites have been well-studied (e. g. Hames et al. 2002), however with information on migratory connectivity, threats from winter habitat loss can now be quantified for specific breeding populations. Our study provides an operational framework to assess habitat loss and protection on the wintering grounds for both specific breeding populations and for a species as a whole.

## Methods

**Geocator Deployment.** We deployed light-level geolocators ( $n = 733$ ) on wood thrushes at 7 breeding sites and 4 wintering sites from 2007-2013 (Table S1) and obtained migration movement data for 102 different individuals. Geolocators were manufactured by British Antarctic Survey: MK14S [renamed MK5790 in 2013 and manufactured by BioTrack], 1.6 g,  $n = 78$ ; MK10S 1.1g  $n = 9$ , MK20S + radiotransmitter, 1.6g,  $n = 3$ ; and Lotek: LightBugs, 2g,  $n = 12$ . We captured birds with mist nets, and geolocators were attached by using a leg-loop backpack harness (Rappole & Tipton 1991) made of 2-mm-wide Teflon ribbon (Stutchbury et al. 2009). The geocator and harness weighed 2.2 g or less, or approximately 4.8% of the body mass ( $45.9 \text{ g} \pm 4.3$ ,  $n = 241$ ) of the average wood thrush. Geocator recovery rate varied by site (Table S1) due to differences in search effort and return rate of birds. The percentage of

geolocator birds that returned carrying geolocators ranged from 23-38% but was not significantly lower than return rates of non-geolocator birds (Table S2). Two individual birds in Belize were captured 2 years after the geolocators were deployed and were no longer wearing their geolocator and harness. There were no cases of harness failure for birds captured within one year of geolocator deployment. We determined the sex of birds by breeding behavior and morphology, or by amplifying sex-linked alleles from blood or feather samples with a modified P8/P2 primer set following Griffiths et al. (1998). We used plumage characteristics to assign birds to age classes (first year versus older) (Pyle 1997).

#### **Geolocator analysis**

Geolocators record light levels every 2 or 10 min in relation to an internal timer, depending on model and year of production. This allows the determination of sunrise and sunset times, and thus day length and solar midday and midnight, which were then used to estimate latitude and longitude with BASTrak software package (British Antarctic Survey). We estimated location by using the ‘threshold method’, where at the light threshold chosen to define sunrise and sunset, the average angle of the sun on the horizon is determined from birds at known locations (geolocator analysis details in *Supplemental Information*). Average location error at breeding sites was 153 km in latitude and 121 km in longitude, and at tropical wintering sites was 291 km and 83 km, respectively (Table S3) (McKinnon et al. 2013b). Sample sizes differ among analyses due to missing data owing to geolocator battery failure, migration during equinoxes (when geolocators cannot determine latitude), or habitat/behavioural shading of the geolocator.

Breeding locations were defined by the average latitude and longitude for June and July, and winter sites were defined by the average values from January and February. To extract a

conservative measure of migration route from our data, we examined the point when birds crossed the Gulf of Mexico (see Fig. 1 for a map showing the Gulf of Mexico relative to the wood thrush range). Since longitude measurements from geolocators are more accurate than latitudes (McKinnon et al. 2013b) (Table S3), we used longitude of fall exit from and spring re-entry to the U.S. at the Gulf of Mexico (at  $\sim 30^{\circ}\text{N}$ ) to represent the overall migration route. Therefore in all of our subsequent analyses, each bird has a single longitude value associated with its fall and spring migratory route.

**Migratory connectivity networks.** We broadly defined breeding regions as Northeast (north of Pennsylvania, USA;  $\sim 40\text{--}50^{\circ}\text{N}$ ,  $77\text{--}83^{\circ}\text{W}$ ), Central-east (North Carolina to Pennsylvania, USA;  $\sim 38\text{--}43^{\circ}\text{N}$ ,  $67\text{--}78^{\circ}\text{W}$ ), Midwest (Indiana south to Alabama, USA and westward;  $\sim 36\text{--}40^{\circ}\text{N}$ ,  $85\text{--}94^{\circ}\text{W}$ ) and Southeast (South Carolina to Florida, USA;  $\sim 30\text{--}38^{\circ}\text{N}$ ,  $79\text{--}85^{\circ}\text{W}$ ) (Fig. 1). We divided the wintering range into three regions according to longitude: Western (Mexico excluding Yucatan state;  $>92^{\circ}\text{W}$ ), Central (Yucatan, MX, Belize, Guatemala, El Salvador and western Honduras;  $87.5\text{--}92^{\circ}\text{W}$ ), and Eastern (eastern Honduras, Nicaragua, and Costa Rica;  $<87.5^{\circ}\text{W}$ ) (Fig. 1). We divided migration routes into categories spanning  $5^{\circ}$  longitude along the northern Gulf of Mexico coast. For the regional-level network, we used the number of birds tracked from each breeding and wintering site to quantify the strength of connectivity. For the species-level network, we used an estimate of relative abundance of wood thrushes in each region to quantify the connectivity of wood thrush overall between breeding, migration and winter sites.

Relative wood thrush abundance was determined using the number of individuals counted along standardized survey routes (Breeding Bird Survey, see *Supplemental Information*

for details) conducted in each breeding region (Sauer et al. 2012), averaged from 2000-2010. We then used the global forest monitoring data (Hansen et al. 2008, 2010) to estimate total forest area within each breeding region (Table 1). The percentage of the entire wood thrush population occupying each breeding region was calculated as relative abundance multiplied by relative forest area in that region, and then converted to a percentage of the total (Table 1). The strength of connections between each node determined by geolocator-tracking (i.e., the regional network) was then adjusted according to the relative population size in each breeding region to create the species-level network.

**Habitat Mapping.** We used ArcMap10 (ESRI) to analyze forest cover and loss within the wood thrush breeding and wintering range by using data from a global forest monitoring database (<http://globalmonitoring.sdstate.edu/projects/gfm>) (Hansen et al. 2010; Hansen et al. 2008). These data were generated by Hansen et al. (2008, 2010) by integrating both moderate spatial resolution (250 m, 500 m, and 1 km) data from the MODerate Resolution Imaging Spectroradiometer (MODIS) and high-spatial-resolution Landsat Enhanced Thematic Mapper Plus (ETM+) data (28.5m) to estimate biome-scale forest change from 2000-2005. MODIS data were used to identify regions of low, medium, and high probability of forest clearing. A stratified random sample of 18.5 km x18.5 km blocks taken within these regions was interpreted for forest cover and forest clearing with high-spatial-resolution Landsat imagery from 2000 and 2005. To assess potential threats to wood thrushes from tropical deforestation, we quantified lowland humid tropical forest cover and extent of forest loss from data retrieved from the global forest monitoring database (Hansen et al. 2010; Hansen et al. 2008) within three wintering regions defined for wood thrush (Fig. 1, Table 2).

**Statistical analyses.** We quantified the strength of connectivity between breeding and wintering locations by calculating the Mantel correlation coefficient ( $r_M$ ) (Ambrosini et al. 2009; Cormier et al. 2013) using XLStat 2014 (Microsoft Corp.). We calculated a breeding and a wintering distance matrix (the distance between each individual and every other individual) by using the geographical coordinates with the Geographic Distance Matrix Generator v. 1.2.3 (American Museum of Natural History 2006). If individuals that breed close together also winter close together (i.e. the population shows strong connectivity) then a strong positive correlation is expected between the two matrices. The program uses a randomization procedure to estimate the expected distribution of  $r(AB)$ , where A and B are the distance matrices for the breeding and wintering locations. We set the number of random permutations to 10,000 to estimate the  $p$ -value of the observed correlation coefficient  $r_M$ .

To determine if breeding and wintering sites for the same individuals followed a spatial pattern (e.g. leap-frog migration, where birds wintering further north breed further south, and the reverse) (Newton 2008) and if there was spatial structure in migration routes (e.g. birds breeding further east migrated along a more easterly route), we used generalized linear models (GLMs) in the program R (R Development Core Team 2011). We used a GLM to incorporate potential sex effects on breeding-wintering connectivity and migration route patterns, and potential age effects on spring migration route (since some birds were juveniles and thus naïve spring migrants). We ran four models with the following dependent variables: winter latitude, winter longitude, fall route (longitude at gulf crossing) and spring route (longitude at gulf crossing). Independent factors included in the model were breeding location (both latitude and longitude), sex, and age (spring route only). We used a backwards step procedure to drop factors from the full model one by one and evaluated the model fit with Akaike's Information Criterion.

## Results

**Breeding-Wintering Migratory Connectivity.** Mapping migration destination ( $n = 103$ , includes one band return) revealed moderate breeding-wintering connectivity (Fig. 2). Almost all (91%; 32 of 35) wood thrushes tracked from northeastern and central-eastern breeding populations wintered in a narrow region in the eastern part of the winter range (Fig. 2b) (82.5 to 87.5 °W). In contrast, most (65%; 11 of 17) birds tracked from southeastern or mid-western U.S. breeding populations travelled to the central winter range (87.5-92 °W) (Fig. 2c). Only 2 of 52 birds tracked from breeding sites wintered in the western wintering range in southern Mexico (both bred in the Midwest). Tracking via wintering deployments showed a similar pattern; none of the birds tracked from the central wintering region ( $n = 24$ ) bred in the northeast and birds tracked from the eastern winter region rarely (1 of 26) bred in the southeast or mid-western breeding range (Fig. 2d-f).

The Mantel correlation coefficient (Ambrosini et al. 2009) indicated significant breeding-wintering connectivity in wood thrushes ( $r_M = 0.33$ ,  $n = 103$ ,  $p < 0.0001$ ). Birds tracked from our main breeding study sites (Pennsylvania, Indiana) were on average,  $319 \pm 10.7$  km (range from 15-1196 km) apart from other individuals from the same breeding population during the winter. For Belize and Costa Rica deployments, individuals from the same winter population averaged  $532 \pm 22.1$  km (range from 11-1611 km) apart in the breeding season. GLMs indicated that winter latitude was predicted by breeding latitude ( $\beta = -0.20 \pm 0.07$ ,  $t = -2.74$ ,  $p = 0.007$ ) and breeding longitude ( $\beta = 0.33 \pm 0.06$ ,  $t = 5.60$ ,  $p < 0.001$ ), in that birds wintering further south bred further north and eastwards. Winter longitude was also predicted by breeding latitude ( $\beta = -0.21 \pm 0.08$ ,  $t = -2.78$ ,  $p = 0.006$ ) and longitude ( $\beta = 0.25 \pm 0.06$ ,  $t = 4.06$ ,  $p < 0.001$ ), in that birds wintering further west bred further south and west. These results correspond to an overall broad



pattern of parallel, leap-frog migration (Fig. 2). Sex was not retained as a factor in the model for wintering longitude ( $p = 0.78$ ) or latitude ( $p = 0.71$ ). Full model results are reported in Table S4. Overall, breeding location (latitude + longitude) explained 31% of the variation in winter longitude ( $r^2 = 0.31$ ,  $F_{2,99} = 22.61$ ,  $p < 0.001$ ), and 41% of the variation in winter latitude ( $r^2 = 0.41$ ,  $F_{2,99} = 34.62$ ,  $p < 0.001$ ).

**Migration Route Connectivity.** In fall, migration route varied depending on breeding region; most individuals (83%) from northeastern breeding populations migrated in fall through southern Florida and Cuba ( $<83^\circ\text{W}$ ) (Fig. 3a), whereas only one quarter of mid-western and southeast breeding populations used this route (Fig. 3c). In contrast, during spring migration, most wood thrushes from all breeding regions entered the U.S. along a relatively narrow stretch of the Gulf Coast ( $88\text{-}93^\circ\text{W}$ ) (Fig 3d-f). Breeding longitude was a significant factor predicting fall migration route ( $\beta = 0.26 \pm 0.10$ ,  $t = 2.48$ ,  $p < 0.01$ ), in that birds breeding further east also left the U.S. further east. Breeding latitude ( $\beta = -0.12 \pm 0.12$ ,  $t = -1.05$ ,  $p = 0.30$ ) and sex ( $\beta = -0.42 \pm 0.57$ ,  $t = -0.73$ ,  $p = 0.47$ ) were not significant predictors of fall migration route. The best model for spring migration route included breeding longitude and sex, but not age (first spring migration) ( $\beta = -0.64 \pm 0.82$ ,  $t = -0.78$ ,  $p = 0.44$ ) or breeding latitude ( $\beta = 0.03 \pm 0.11$ ,  $t = 0.23$ ,  $p = 0.77$ ). Despite the fact that most birds traveled through a relatively narrow range of longitudes ( $88\text{-}93^\circ\text{W}$ ) in spring, longitude of breeding destination was still a significant predictor of longitude at the US Gulf Coast during spring migration ( $\beta = 0.23 \pm 0.10$ ,  $t = 2.30$ ,  $p = 0.02$ ), with birds breeding further east crossing the Gulf of Mexico further eastward. Controlling for breeding longitude, there was a tendency for females to travel farther west than males ( $\beta = 1.07 \pm 0.56$ ,  $t = 1.90$ ,  $p = 0.06$ ).

**Migratory Connectivity Network.** Spatial structure in connectivity and migratory routes was also evident in the regional and species-level migratory networks (Fig. 4). For instance, for the northeastern breeding region, 83% of individuals migrated through southern Florida ( $<83^{\circ}\text{W}$ ) in fall, 96% occupied the eastern winter range, and 74% returned in spring via the central U.S. Gulf coast ( $88\text{--}93^{\circ}\text{W}$ ). In contrast, birds from the southeast breeding region travelled primarily through the eastern Gulf (50%;  $83\text{--}88^{\circ}\text{W}$ ) and south Florida (36%) fall migration routes, and most (87%) wintered in the Central winter range. Most southeastern breeding birds (60%) also used the central route across the Gulf of Mexico in spring.

The species-level migratory network highlights several nodes of conservation importance for wood thrushes. The eastern wintering region is estimated to support 56% of the species, primarily (91%) those from the north- and central east breeding regions (Fig. 4d). The central wintering region was used by 41% of all wood thrushes, which originated from breeding sites in the Midwest (9%), south (16%) and central east (14%), with few birds (1%) from the northeast (Fig. 4d). Only a small proportion of all wood thrushes (3%) was estimated to winter in the western non-breeding range, and all of these birds also bred in the Midwest (Fig. 4d). Fall and spring migration showed a loop migration pattern; no birds used the most westerly route in fall ( $> 93^{\circ}\text{W}$ ), and none used the most easterly route in spring ( $<83^{\circ}\text{W}$ ) (Fig. 4e-f). In fall, the southern Florida route ( $<83^{\circ}\text{W}$ ) was used by 52% of the species, and when the adjacent eastern U.S. Gulf coast ( $83\text{--}88^{\circ}\text{W}$ ) is considered, 83% of all wood thrushes used a fall migration route east of  $88^{\circ}\text{W}$  (Fig. 4e). In spring, wood thrushes were even more concentrated, with 73% of the species migrating northwards through the central U.S. Gulf coast (Fig. 4f), a span of  $5^{\circ}$  in longitude ( $\sim 480\text{km}$ ).

**Tropical Deforestation.** One of the key benefits of determining migratory connectivity is the ability to assess how potential threats on the wintering grounds vary among breeding regions. Wood thrushes have declined significantly throughout most of their breeding range (Fig 5a) and most steeply in the northeast and southeast breeding regions (Table 1). The eastern winter range supports about half of the species, and almost all northeastern breeders, yet this region contains only one third of all available wood thrush wintering habitat (Table 2, Fig 5b) and has recently experienced deforestation at a rate of -0.4% forest cover lost per year (Fig. 5c). This represents a cumulative loss of ~ 2% of forest area in only five years, and some sites in eastern Nicaragua are deforestation hotspots with a very high cumulative forest loss of 15-35% (Fig. 5c). The central wintering region supports most southeastern breeders and contained the most extensive wood thrush habitat (50.6%, Table 2, Fig. 5b) but also had a high deforestation rate (-0.5%/yr; Fig. 5c).

## **Discussion**

A major challenge for conservation of migratory songbirds is determining the geographic connections between declining breeding populations, their migratory routes, and their non-breeding sites (Faaborg et al. 2010). For the first time, our study quantified geographic linkages across the entire annual cycle of a migratory songbird, including population-specific migration routes and breeding-wintering connectivity. We found significant migratory connectivity structure for wood thrushes, where northeastern breeding populations migrated along more easterly routes in fall, and wintered furthest east (parallel migration) and south (leapfrog migration). In spring, however, there was weak connectivity as geographically distant breeding populations mixed extensively along a similar migration route. These results emphasize the importance of measuring migratory connectivity throughout the annual cycle, as breeding

populations with distinct wintering regions may nevertheless share similar threats during migration when mortality risk is thought to be highest (Sillett & Holmes 2002).

The migratory network we developed provides a quantitative framework for prioritizing conservation efforts for wood thrushes along the entire 5,000-10,000 km migration journey. For example, habitat protection focussed on the Mississippi River delta along the U.S. Gulf Coast (83-88°W) would benefit wood thrushes primarily during spring migration, and affect 73% of the total population of this declining species. In contrast, habitat protection in Florida or western Cuba (<83°W) would affect wood thrushes almost exclusively during fall migration and affect primarily central-eastern and northeastern breeding populations. This example illustrates the importance of migratory networks in identifying conservation ‘hotspots’ where limited conservation funds can be directed to conserve the greatest number of individuals.

The migratory network also highlights how decisions as to where to best invest resources can differ depending on whether the goal is regional conservation of specific breeding populations or the species as whole. The species-level network suggests that the eastern wintering region should be a top conservation priority for wood thrush because it supports over half of the species (Fig. 4d). This relatively small region still has extensive forest cover but the rate of tropical deforestation is high (Fig. 5b-c); for instance Nicaragua, in the center of this region, lost 6.9% of forest cover from 2000 to 2012 (Hansen et al. 2013). Conservation of forest via protected parks and promotion of rustic shade coffee (Bakermans et al. 2009; Philpott & Bichier 2012) in this region would benefit the species overall, and is critical for the northeastern breeding populations. The central wintering region was occupied by birds from a broader range of breeding populations (Fig. 4d and 5b) and therefore habitat loss in this region is expected to have an important but diffuse effect on breeding populations. Guatemala, a major portion of the

central winter range, has lost 8.2% of its tropical forest cover since 2000 and is considered a tropical deforestation hotspot (Hansen et al. 2013). Although few birds were tracked to the western wintering range, this forest is nevertheless important at a regional level for conservation of midwestern breeding populations of wood thrushes.

A key conservation question is which fall and spring stopover regions have the greatest impact on fitness of migrating birds. In wood thrushes, annual adult survival is higher for central-eastern and northeastern populations than for southeastern populations (Saracco et al. 2010), a pattern that could be due to differences in migratory routes. Most adults from the central and northeast did not cross the Gulf of Mexico in fall, but travelled through Florida (Fig. 3e). It is not clear which is a riskier strategy, traversing an urbanized landscape in Florida or, as is the case for most southeastern birds, an open-water crossing of 800-1000km during hurricane season. Field studies could assess relative habitat quality along migration routes identified here, via measuring individual birds' refueling rates (e. g. Guglielmo et al. 2005; Seewagen et al. 2011) or radio-tracking to quantify survival or foraging behaviour (e. g. Ktitorov et al. 2010). It is not yet possible to measure mortality en route since satellite transmitters are not small enough to be deployed on songbirds.

Quantitative estimates of migratory connectivity are important for comparative studies of the ecological mechanisms that drive connectivity patterns, and to test predictions about the consequences of migratory connectivity for population stability. Measured using the Mantel test, breeding-wintering connectivity for wood thrushes was higher ( $r_M = 0.35$ ) than that reported for European barn swallows (*Hirundo rustica*) ( $r_M = 0.22$ ) (Ambrosini et al. 2009), but less than that reported for coastal populations of Swainson's thrush (*Catharus ustulatus*) ( $r_M = 0.72$ ) (Cormier et al. 2013). As more studies quantify connections between breeding and wintering sites of

migratory songbirds, it will be possible to test if the strength of connectivity is an important predictor of population declines.

For the first time, conservation action for wood thrushes can be focussed on the specific breeding, stopover, and wintering regions that are used by a given population, breeding region or the species as a whole. According to Breeding Bird Survey data, wood thrush populations have declined by an average of -1.8%/year (95% CI: -1.7 to -2.1) across the breeding range since 1966 (Fig. 5a) and by -3.2%/year from 2001-2011 (95% CI: -3.8 to -2.7) (Sauer et al. 2012).

Population modelling by using migratory networks parameterized with demographic estimates for each region, once available, can be used to explore different scenarios of habitat loss and protection to predict impacts on population trends (Faaborg et al. 2010) and prioritize conservation actions (Mattson et al. 2012). Migratory connectivity networks have the potential to revolutionize conservation strategies for migratory songbirds because we can finally see into, and measure, the contents of the so-called “black box” of the migration period (Taylor & Norris 2010). A quantitative understanding of migratory connectivity within a migratory network framework can help us better anticipate and respond to species-level consequences of environmental change at locations throughout the annual cycle.

## Acknowledgments

We thank J. Sauer for analysis of Breeding Bird Survey data and S. Barretto for GIS analysis..

We thank the following funding sources: Natural Sciences and Engineering Research Council of Canada, National Geographic Society, U.S. Fish and Wildlife Service Neotropical Migratory Bird Grant, Environment Canada, Schad Foundation, Kenneth M. Molson Foundation, York University, and proceeds from *Silence of the Songbirds* (Walker & Co.). P.P.M., T.B.R., and C. E. S. received support from the Department of Defence's Legacy Program and the Strategic Environmental Research Development Program. The National Audubon Society provided funding and Pro-Aves Veracruz provided assistance for fieldwork in Mexico. Tropical fieldwork was conducted at the Belize Foundation for Research and Environmental Education, La Selva in Costa Rica, and Reserva Nebliselva El Jaguar in Nicaragua. We thank J. Marlin, J. Rotenberg, L. Duriaux-Chavarria, and G.Duriaux for field support in Central America.

## Supporting Information

Appendix S1 is available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

## Literature Cited

- Ambrosini, R., A. P. Moller, and N. Saino. 2009. A quantitative measure of migratory connectivity. *Journal of Theoretical Biology* **257**:203-211.
- Bairlein, F., D. R. Norris, R. Nagel, M. Bulte, C. C. Voight, J. W. Fox, D. J. T. Hussell, and H. Schmaljohann. 2012. Cross-hemisphere migration of a 25g songbird. *Biology Letters* **2012**:505-507.
- Bakermans, M. H., A. C. Vitz, A. D. Rodewald, and C. G. Rengifo. 2009. Migratory songbird use of shade coffee in the Venezuelan Andes with implications for conservation of Cerulean Warbler. *Biological Conservation* **142**:2476-2483.
- Bell, C. P. 1997. Leap-frog migration in the fox sparrow: Minimizing the cost of spring migration. *Condor* **99**:470-477.
- Cormier, R. L., D. L. Humple, T. Gardali, and N. E. Seavy. 2013. Light-level geologgers reveal strong migratory connectivity and within-winter movements for a coastal California Swainson's Thrush (*Catharus ustulatus*) population. *Auk* **130**:283-290.
- Delmore, K. E., J. W. Fox, and D. E. Irwin. 2012. Dramatic intraspecific differences in migratory routes, stopover sites and wintering areas, revealed using light-level geolocators. *Proceedings of the Royal Society B-Biological Sciences* **279**:4582-4589.
- Faaborg, J., et al. 2010. Conserving migratory land birds in the New World: Do we know enough? *Ecological Applications* **20**:398-418.
- Fraser, K. C., et al. 2013. Consistent range-wide pattern in fall migration strategy of Purple Martin (*Progne subis*), despite different migration routes at the Gulf of Mexico. *Auk* **130**:291-296.



423 Fraser, K. C., et al. 2012. Continent-wide tracking to determine migratory connectivity and  
 424 tropical habitat associations of a declining aerial insectivore. *Proceedings of the Royal*  
 425 *Society B-Biological Sciences* **279**:4901-4906.

426 Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds.  
 427 *Molecular Ecology* **7**:1071-1075.

428 Guglielmo, C. G., D. J. Cerasale, and C. Eldermire. 2005. A field validation of plasma  
 429 metabolite profiling to assess refueling performance of migratory birds. *Physiological*  
 430 *and Biochemical Zoology* **78**:116-125.

431 Hames, R. S., K. V. Rosenberg, J. D. Lowe, S. E. Barker, and A. A. Dhondt. 2002. Adverse  
 432 effects of acid rain on the distribution of the Wood Thrush *Hylocichla mustelina* in North  
 433 America. *Proceedings of the National Academy of Sciences of the United States of*  
 434 *America* **99**:11235-11240.

435 Hansen, M. C., et al. 2013. High-Resolution Global Maps of 21st-Century Forest Cover Change.  
 436 *Science* **342**:850-853.

437 Hansen, M. C., S. V. Stehman, and P. V. Potapov. 2010. Quantification of global gross forest  
 438 cover loss. *Proceedings of the National Academy of Sciences of the United States of*  
 439 *America* **107**:8650-8655.

440 Hansen, M. C., et al. 2008. Humid tropical forest clearing from 2000 to 2005 quantified by using  
 441 multitemporal and multiresolution remotely sensed data. *Proceedings of the National*  
 442 *Academy of Sciences of the United States of America* **105**:9439-9444.

443 Irwin, D. E., J. H. Irwin, and T. B. Smith. 2011. Genetic variation and seasonal migratory  
 444 connectivity in Wilson's warblers (*Wilsonia pusilla*): species-level differences in nuclear  
 445 DNA between western and eastern populations. *Molecular Ecology* **20**:3102-3115.

446 Ktitorov, P., A. Tsvey, and A. Mukhin. 2010. The good and the bad stopover: behaviours of  
 447 migrant reed warblers at two contrasting sites. *Behavioral Ecology and Sociobiology*  
 448 **64**:1135-1143.

449 Lesica, P., and F. W. Allendorf. 1995. When are peripheral-populations valuable for  
 450 conservation. *Conservation Biology* **9**:753-760.

451 Marra, P. P., D. R. Norris, S. M. Haig, M. S. Webster, and J. A. Royle 2006. Migratory  
 452 connectivity. Cambridge University Press, New York.

453 Martin, T. G., I. Chades, P. Arcese, P. P. Marra, H. P. Possingham, and D. R. Norris. 2007.  
 454 Optimal conservation of migratory species. *PLOS ONE* **2**:e751.

455 Mattson, B. J., M. C. Runge, J. H. Devries, G. S. Boomer, J. M. Eadie, D. A. Haukos, J. P.  
 456 Fleskes, D. N. Koons, W. E. Thogmartin, and R. G. Clark. 2012. A modeling framework  
 457 for integrated harvest and habitat management of North American waterfowl: Case-study  
 458 of northern pintail metapopulation dynamics. *Ecological Modelling* **225**:146-158.

459 McKinnon, E. A., K. C. Fraser, and B. J. M. Stutchbury. 2013a. New discoveries in landbird  
 460 migration using geolocators and a flight plan for the future. *Auk* **130**:1-12.

461 McKinnon, E. A., C. Q. Stanley, K. C. Fraser, M. MacPherson, G. Casbourn, P. P. Marra, C. E.  
 462 Studds, N. Diggs, and B. J. M. Stutchbury. 2013b. Estimating geocator accuracy for a  
 463 migratory songbird using live ground-truthing in tropical forest. *Animal Migration* **1**:31-  
 464 38.

465 Newton, I. 2008. *The Migration Ecology of Birds*. Academic Press, London.

466 Norris, D. R., P. P. Marra, G. J. Bowen, L. M. Ratcliffe, J. A. Royle, and T. K. Kyser. 2006.  
 467 Migratory connectivity of a widely distributed songbird, the American Redstart  
 468 (*Setophaga ruticilla*). *Ornithological Monographs* **61**:14-28.

469 Philpott, S. M., and P. Bichier. 2012. Effects of shade tree removal on birds in coffee  
 470 agroecosystems in Chiapas, Mexico. *Agriculture Ecosystems & Environment* **149**:171-  
 471 180.

472 Pyle, P. 1997. Identification guide to North American birds. Volume I. Slate Creek Press,  
 473 Bolinas, CA.

474 R Development Core Team. 2011. R: a language and environment for statistical computing. R  
 475 Foundation for Statistical Computing, Vienna, Austria.

476 Rappole, J. H., and A. R. Tipton. 1991. New harness design for attachment of radio transmitters  
 477 to small passerines. *Condor* **62**:335-337.

478 Renfrew, R. B., D. Kim, N. Perlut, J. A. Smith, J. Fox, and P. P. Marra. 2013. Phenological  
 479 matching across hemispheres in a long-distance migratory bird. *Diversity and*  
 480 *Distributions* **19**:1-12.

481 Rubenstein, D. R., C. P. Chamberlain, R. T. Holmes, M. P. Ayres, J. R. Waldbauer, G. R.  
 482 Graves, and N. C. Tuross. 2002. Linking breeding and wintering ranges of a migratory  
 483 songbird using stable isotopes. *Science* **295**:1062-1065.

484 Runge, M., and P. P. Marra. 2005. Modeling seasonal interactions in the annual cycle of  
 485 migratory birds. Pages 375-389 in R. Greenberg, and P. P. Marra, editors. *Birds of Two*  
 486 *Worlds: The Ecology and Evolution of Migration*. Johns Hopkins University Press,  
 487 Baltimore.

488 Saracco, J. F., J. A. Royle, D. F. Desante, and B. Gardner. 2010. Modeling spatial variation in  
 489 avian survival and residency probabilities. *Ecology* **91**:1885-1891.

490 Sauer, J. R., J. E. Hines, J. E. Fallon, K. L. Pardieck, D. J. Ziolkowski Jr., and W. A. Link. 2012.  
 491 The North American Breeding Bird Survey, Results and Analysis 1966-2010. Version  
 492 12.07.2012. USGS Patuxent Wildlife Research Center, Laurel, MD.

493 Seewagen, C. L., C. D. Sheppard, E. J. Slayton, and C. G. Guglielmo. 2011. Plasma metabolites  
 494 and mass changes of migratory landbirds indicate adequate stopover refueling in a  
 495 heavily urbanized landscape. *Condor* **113**:284-297.

496 Sherry, T. W., and R. T. Holmes. 1996. Winter habitat quality, population limitation, and  
 497 conservation of neotropical nearctic migrant birds. *Ecology* **77**:36-48.

498 Sillett, T. S., and R. T. Holmes. 2002. Variation in survivorship of a migratory songbird  
 499 throughout its annual cycle. *Journal of Animal Ecology* **71**:296-308.

500 Sillett, T. S., R. T. Holmes, and T. W. Sherry. 2000. Impacts of a global climate cycle on  
 501 population dynamics of a migratory songbird. *Science* **288**:2040-2042.

502 Stutchbury, B. J. M., S. A. Tarof, T. Done, E. Gow, P. M. Kramer, J. Tautin, J. W. Fox, and V.  
 503 Afanasyev. 2009. Tracking long-distance songbird migration by using geolocators.  
 504 *Science* **323**:896.

505 Taylor, C. M., and D. R. Norris. 2010. Population dynamics in migratory networks. *Theoretical*  
 506 *Ecology* **3**:65-73.

507 Webster, M. S., P. P. Marra, S. M. Haig, S. Bensch, and R. T. Holmes. 2002. Links between  
 508 worlds: unraveling migratory connectivity. *Trends in Ecology & Evolution* **17**:76-83.

509

510

Table 1. Forest habitat area, wood thrush abundance, and estimated proportion of the global population of wood thrushes occupying each region, and population trends (see Fig. 1 for map of regions).

Region	% forest cover	% forest cover relative to species range <sup>a</sup>	Forest area (ha) (2005)	Wood thrush abundance (birds/route $\pm$ SE) <sup>b</sup>	Estimated % of global population	Population trend 1966-2010 (% per year, with 95% Bayesian confidence intervals) <sup>d</sup>
Northeast	63.2	28.4	33,783,766	$3.8 \pm 0.25$	18	-2.76 (-3.06, -2.46)
Central-east	51.3	18.8	22,369,415	$15.2 \pm 0.21$	48	-1.36 (-1.62, -1.09)
Southeast	47.4	19.3	22,962,751	$5.9 \pm 0.17$	19	-2.53 (-2.89, -2.02)
Midwest	33.3	33.5	39,790,677	$2.7 \pm 0.05$	15	-0.94 (-1.25, -0.63)
Total	-	-	118,906,609	-	-	-1.8 (-2.1, -1.7)

<sup>a</sup>Percent of the total forest area in each region divided by the total forest area within the entire range.

<sup>b</sup> Average abundance per year based on Breeding Bird Surveys from 2000-2010, shown as a mean value  $\pm$  standard error (SE).

<sup>c</sup>Percent of global population was calculated by multiplying the average abundance of wood thrushes per region by the relative proportion of forest area in that region.

<sup>d</sup>Population trends within each region ( $n = 369, 418, 377$ , and  $782$  routes, respectively)

calculated with hierarchical Bayesian analysis of Breeding Bird Survey data.

Table 2. Forest area and forest loss within different regions of the wood thrush wintering range (see Fig. 1 for map of regions).

Region	% forest cover	% forest cover relative to species range (%) <sup>*</sup>	Forest area (2005)	Forest area lost (ha) (2000-2005)	Deforestation rate (%/year) (2000-2005)
Western	36.6	17.1	3,746,794	28,244	-0.1
Central	60.7	50.6	11,120,383	264,457	-0.5
Eastern	51.0	32.3	7,093,537	135,487	-0.4
Total	-	-	22,457,902	428,188	-0.4

<sup>\*</sup>Percent of the total forest area in each region divided by the total forest area within the entire winter range.

## Figure Legends

**Fig. 1.** Theoretical patterns of migratory connectivity shown relative to the wood thrush range (dark gray shading), and black outlines showing major breeding and wintering regions used in this study (areas with very low wood thrush abundance were not included in the regions). The width of arrows indicates the proportion of birds from each breeding region migrating to each wintering region. (a) Strong migratory connectivity occurs when individuals from different breeding regions segregate on their wintering grounds. In contrast, weak migratory connectivity (b) occurs when individuals from different breeding regions mix throughout the wintering range, e.g. an individual from the Northeast may winter in the same place as an individual breeding in the Midwest. Consequences of habitat loss in winter are different depending on the strength of the connectivity pattern observed.

**Fig. 2.** Migration destination of wood thrushes tracked from (a) seven breeding sites ( $n = 52$  individuals) to their wintering sites in Central America (b, c) and tracked from (d) four wintering sites ( $n = 50$ ) to their breeding sites (e, f) in North America. Origin and destination are color coded for each bird by population. Grey shading is breeding and wintering range; four levels of shading on breeding range indicate relative abundance from Breeding Bird Survey (2007-2011) (Sauer et al. 2012). Data for the Nicaragua study site (d, e) include one band recovery during the 2011 breeding season. Average error in latitude and longitude from ground-truthing at deployment sites (McKinnon et al. 2013b) (Table S3) is shown with error bars for one individual location per panel.

**Fig. 3.** Estimated fall (blue) and spring (red) migration routes for wood thrushes that bred in the northeast (a, d), central-east (b, e), southeast and midwest (c, f) breeding regions. Gray shading indicates breeding and wintering range. Solid circles indicate geolocator deployment sites and

average location error from ground-truthing shown for Pennsylvania, Belize, and Costa Rica (McKinnon et al. 2013b). In North America, geolocator accuracy is ~150km in latitude and ~130km in longitude, on average, while in Central America accuracy averages ~291km latitude and ~83km longitude (Table S3). Dashed lines are migration movements where location is uncertain due to persistent shading or during fall or spring equinox when latitude cannot be resolved. Pie charts show proportion of individuals in each breeding region making use of migration routes classified according to longitude along the Gulf coast, with sample size indicated next to the chart.

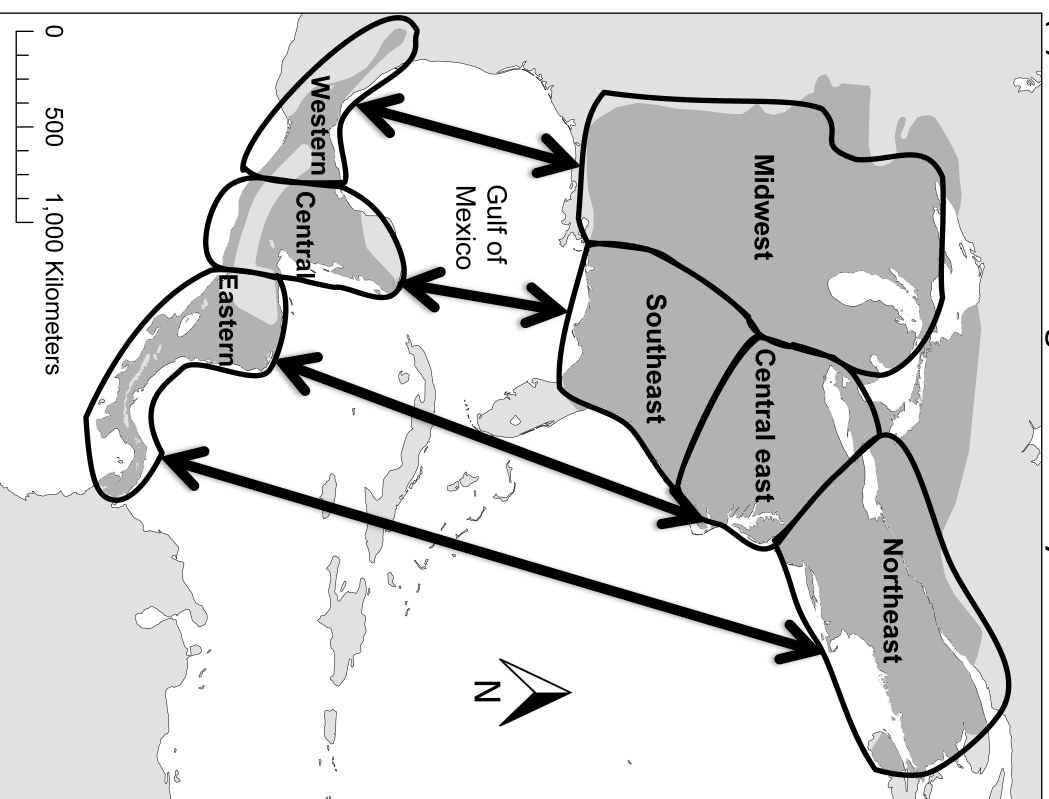
**Fig. 4.** Migratory connectivity networks for wood thrush wintering sites, and fall and spring migration routes. Regional networks (a, b, c) show for each breeding region, by color-coded lines, the proportion of individuals that occupy that site and travel along that route. The width of connecting lines is proportional to the percent of the regional population travelling to a particular winter region (e.g., MX = Mexico) or migrating along a particular migration route in fall or spring (indicated by longitude, e.g., 88-93 C. Gulf = 88-93°W, central Gulf of Mexico route). The species-level networks (d, e, f) indicated the estimated proportion of the entire wood thrush population that moves between a given breeding and non-breeding site, taking into account regional breeding abundance (Table 1). Lines colour-coded by breeding origin and width proportional to the percent of the global population indicate connections between breeding and non-breeding sites. Values inside the non-breeding site (squares and triangles) are the proportion of all wood thrush that occupy that site. For global networks, the size of circle indicates proportion of global breeding population (Table 1) and the size of wintering region boxes (d) indicates relative area of humid lowland forest cover in 2005 (Table 2).



**Fig. 5.** Geographic patterns in breeding population trends and winter habitat availability and deforestation for wood thrushes. (a) Population trends for breeding wood thrushes measured by Breed Bird Survey (1966-2010; Sauer et al. 2012) vary over the breeding range. (b) Humid lowland tropical forest cover in the year 2005 (Hansen et al. 2010) within the wood thrush winter range with pie charts showing connections to breeding regions (northeast, central-east, southeast and midwest), and numbers indicating the percent of all wood thrush using that winter region. (c) Percent forest loss in the wood thrush wintering range from 2000-2005 (Hansen et al. 2008). Details of forest area and deforestation rates per region can be found in Table 2.

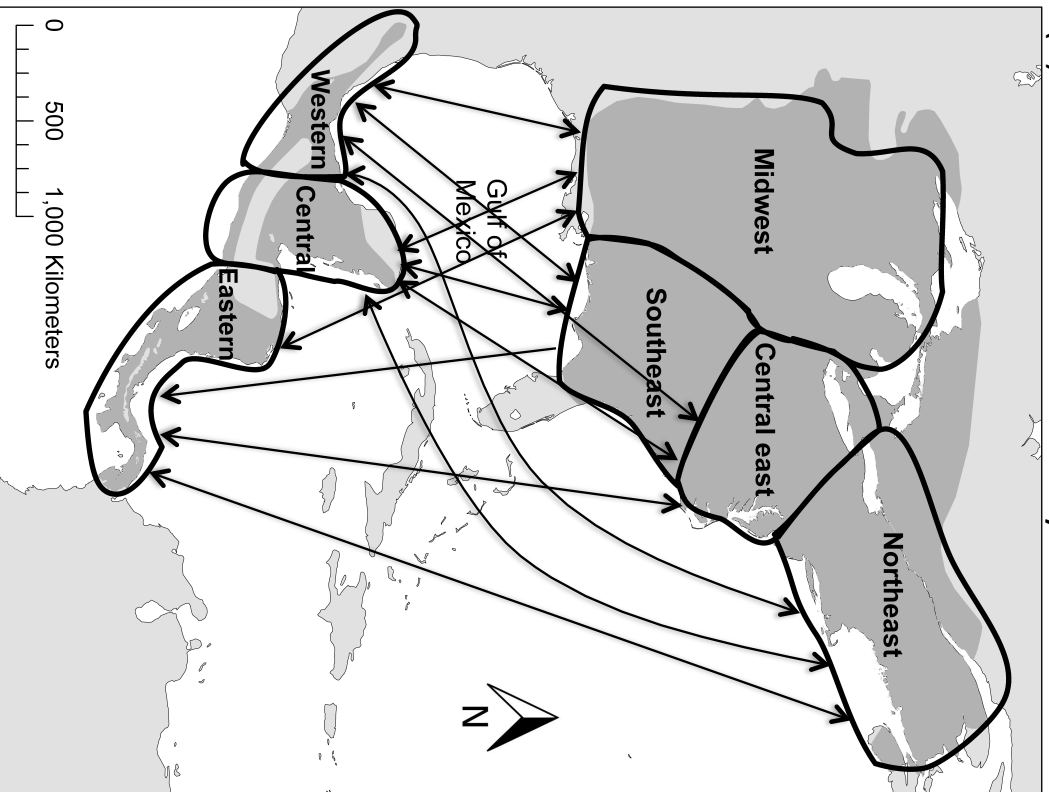
(a)

Strong connectivity

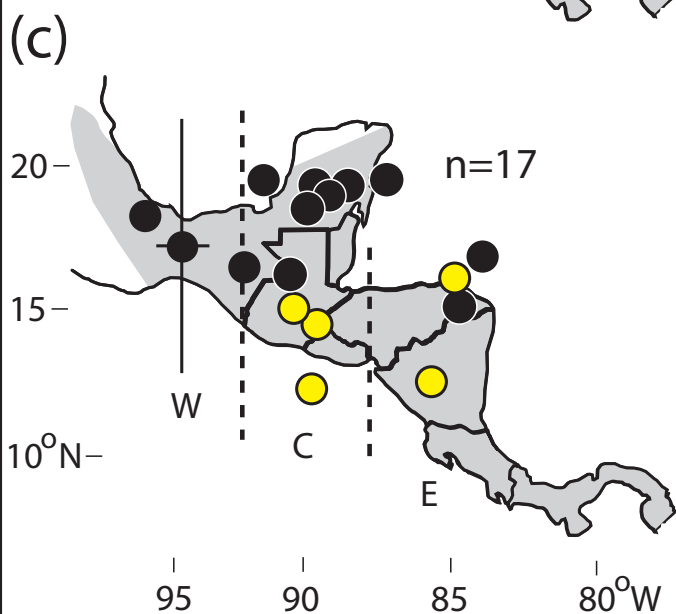
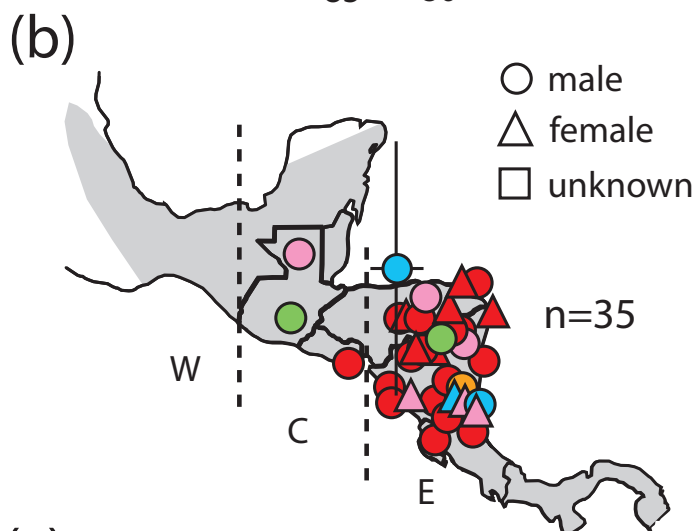
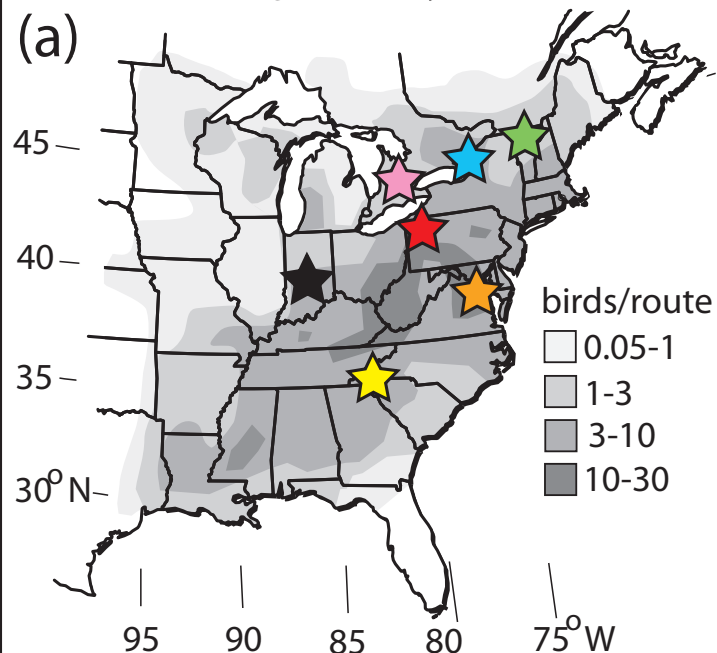


(b)

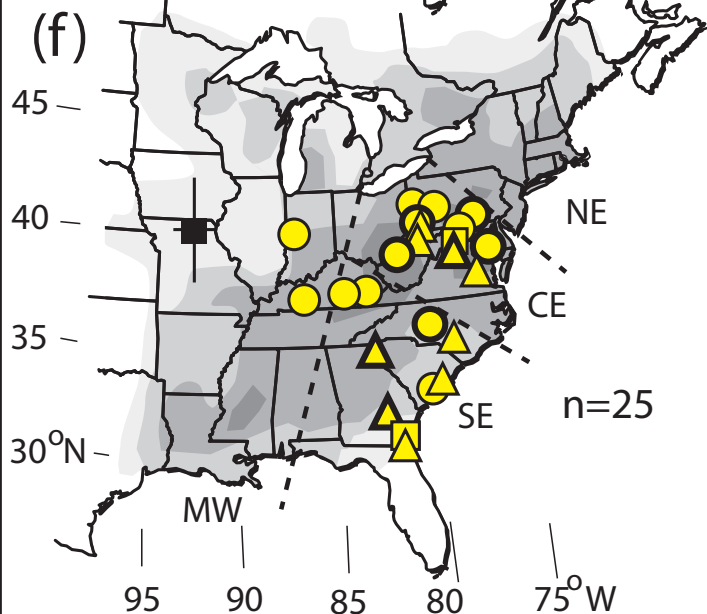
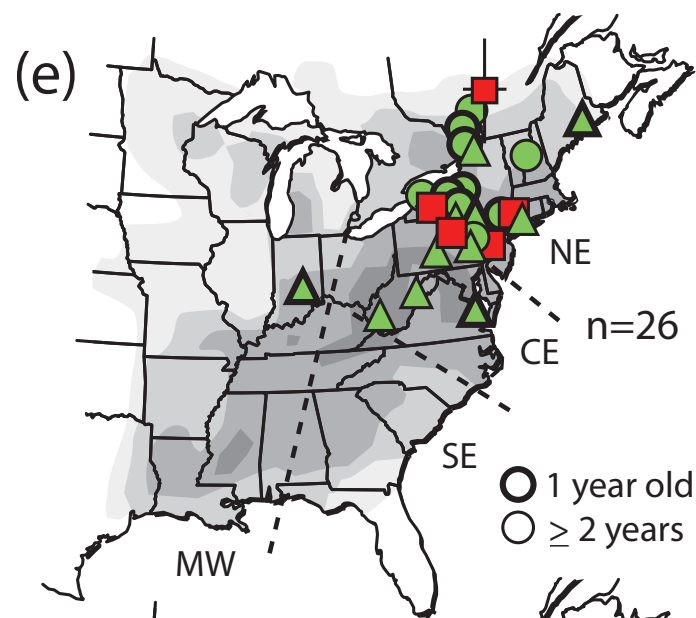
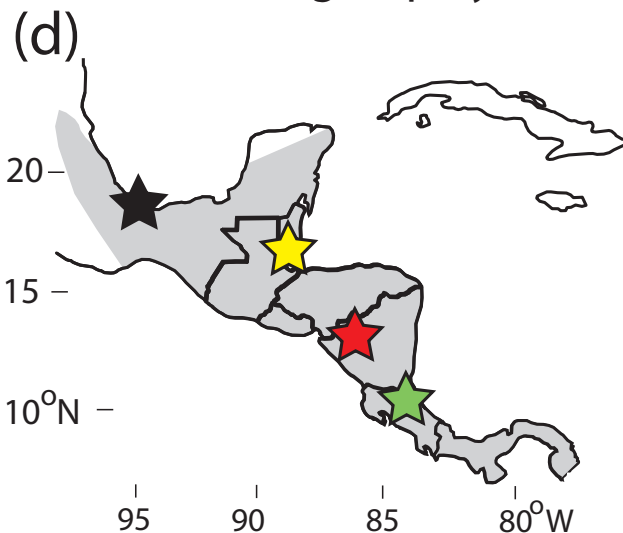
Weak connectivity

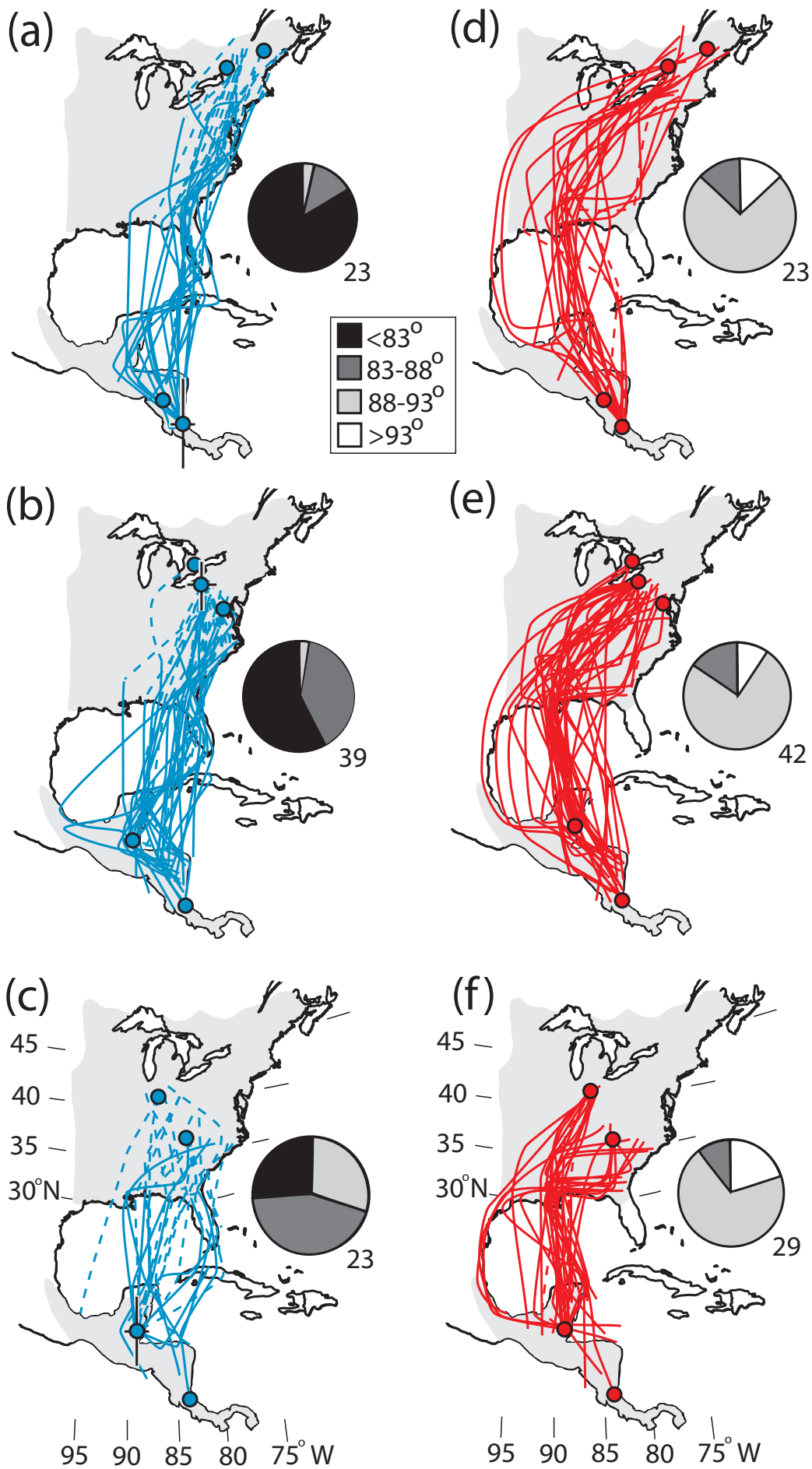


## Breeding Deployments

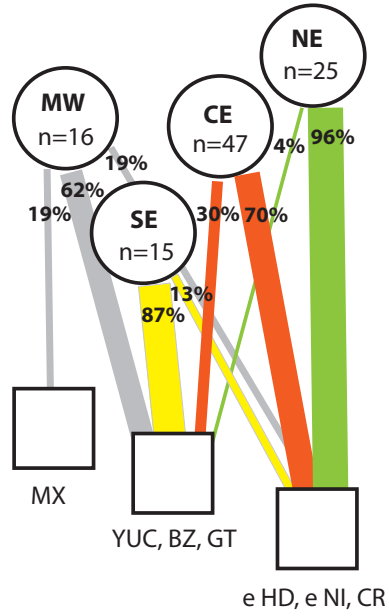


## Wintering Deployments

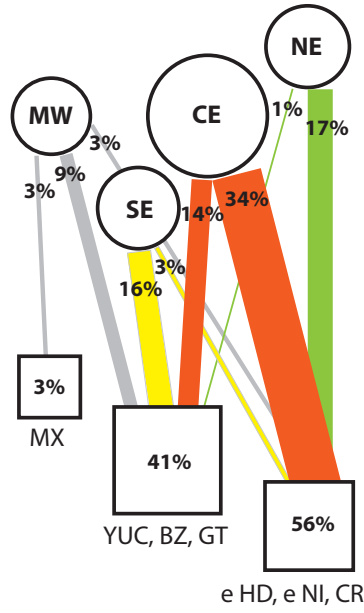




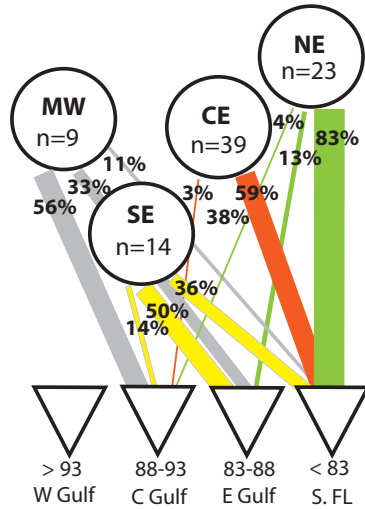
(a) Wintering (Regional)



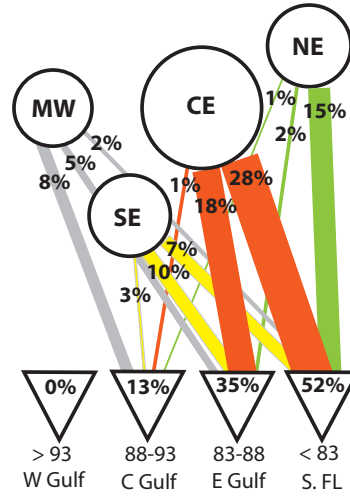
(d) Wintering (Species)



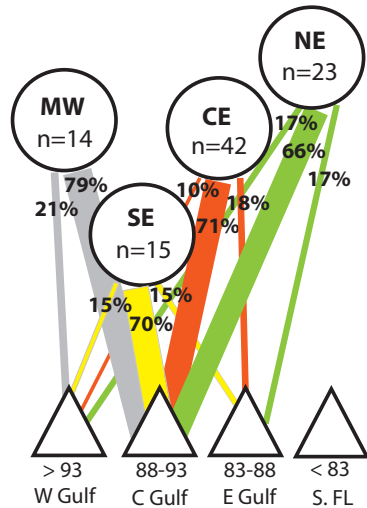
(b) Fall Route (Regional)



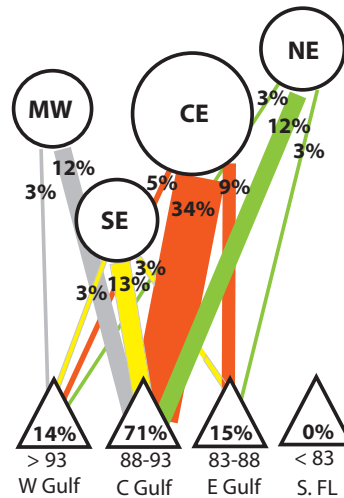
(e) Fall Route (Species)

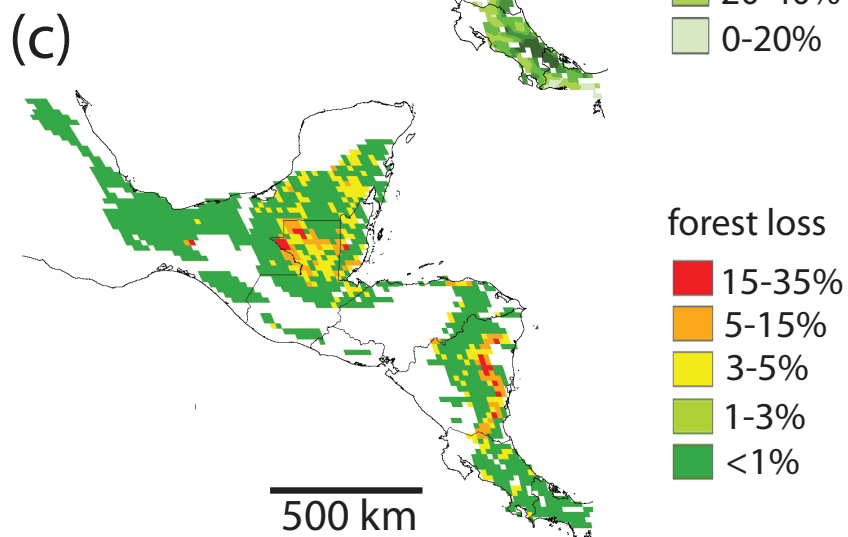
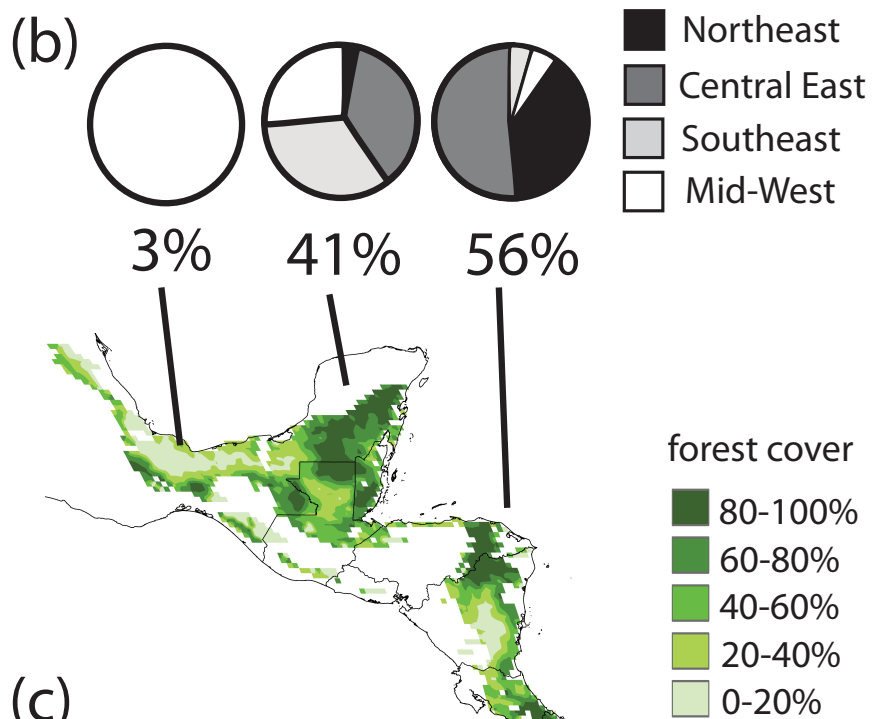
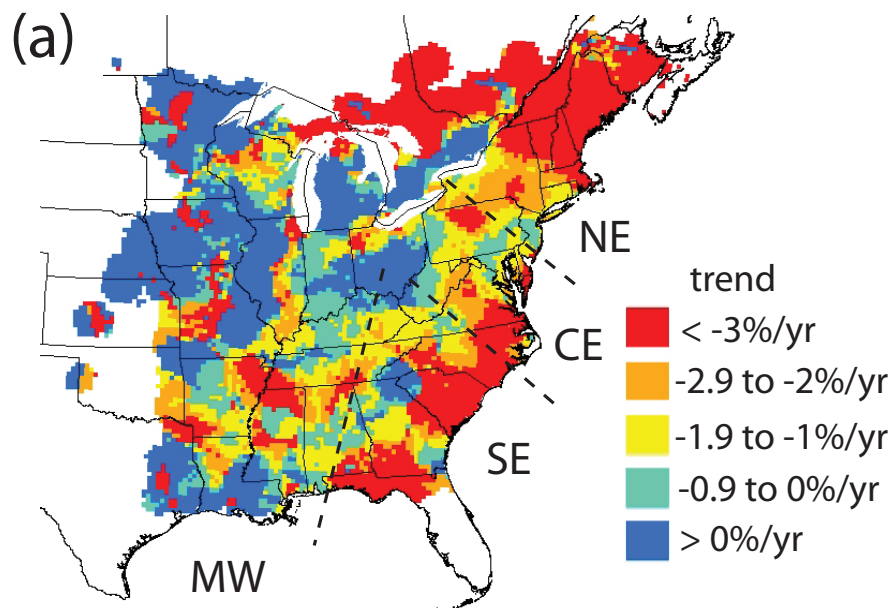


(c) Spring Route (Regional)



(f) Spring Route (Species)





## **Supplemental Information**

**Stanley et al.**

### **Supplemental Methods**

**Geolocator Analysis.** Light data were analyzed using BASTrak software package (British Antarctic Survey). Raw light data were adjusted for any clock drift >3 minutes. Sunrise and sunset were defined as light transitions where the light levels crossed a threshold of 16 (2008 model) or 5 (2009-2012 model, for which light sensitivity was higher). The thresholds we used to define sunrise and sunset in the different models represent similar light intensities based on static calibration of geolocators at known locations. Light transitions were then visually inspected and edited using the program TransEdit (British Antarctic Survey) to delete false sunrises and sunsets (e.g. transitions during daytime caused by shading) and to score the quality of true sunrise and sunset transitions (McKinnon et al. 2013). Very shallow slopes were marked as low confidence, as were transitions that included small peaks in light intensity prior to reaching sunrise threshold, or after reaching sunset threshold (see examples in McKinnon et al. 2013). In these cases, the marked transition was unlikely to be within 10 minutes of the actual sunrise/sunset transition and so was excluded from subsequent analysis. Only the transitions with a high confidence score were used in further analyses.

We then used the average sun elevation angle calculated using season-specific data gathered from birds carrying geolocators at known breeding and wintering sites (McKinnon et al. 2013) to transform light data into latitudinal and longitudinal positions using the program Locator (British Antarctic Survey). For breeding ground deployments we used a sun elevation of -0.5 (obtained from wood thrushes at known tropical sites) to estimate winter location (dry season, Jan-Mar), and for winter ground deployments we used a sun elevation of -2 (obtained from wood thrushes at known temperate deciduous forest sites) to estimate breeding locations (June-July). Both noon and midnight locations were used to estimate location during stationary periods, and noon locations only were used to estimate migration locations (wood thrush are nocturnal migrants).

For breeding ground deployments, winter location (Jan-Feb; March was used to estimate longitude but not latitude) was based on average of  $39.4 \pm 3.4$  high confidence noon or midnight location estimates per bird for latitude and  $77.6 \pm 6.0$  for longitude. Locations for winter sites that yielded latitudes outside the known winter range ( $< 7^\circ\text{N}$  and  $> 21^\circ\text{N}$ ) were excluded as outliers (McKinnon et al. 2013). For wintering ground deployment, breeding location (June-July) was based on average of  $67.5 \pm 2.6$  high confidence noon or midnight location estimates per bird (equal for latitude and longitude). During these time periods (Jun-Jul, Jan-Mar) wood thrush are stationary at breeding/non-breeding territories (unpubl. data), and in the tropics, the dry season coincides with this period and results in more high-quality geolocator transitions that can be used to calculate locations (McKinnon et al. 2013).

Migration routes could not be determined for all individuals, and the number of high confidence noon locations used to estimate route varied with timing of migration (i.e. overlap with the equinox), extent of shading, and the distance and duration of migration. The number of distinct locations (with a latitude and longitude estimate) along fall routes averaged  $3.0 \pm 0.1$  per individual based on  $9.5 \pm 1.29$  d ( $n = 68$  individuals). For spring routes, the number of distinct locations (latitude and longitude estimate) averaged  $3.9 \pm 0.1$  per individual based on  $9.4 \pm 0.6$  d ( $n = 81$  individuals) high confidence days. Partial migration routes were also inferred from large shifts in longitude during the equinox, when latitude could not be determined. For all route analyses, we used longitude at the Gulf of Mexico coast as a proxy for migration route. At the Gulf of Mexico, birds have a broad area over which they can travel, from Florida ( $\sim 80^\circ\text{W}$ ) to western Texas ( $\sim 97^\circ\text{W}$ ) thus we could detect major differences in migratory routes even given the error in geolocation (ca  $1^\circ$  in longitude). On either side of the Gulf crossing, birds tend to be funnelled into a narrow range of longitudes by the shape of the land, either up the Yucatan peninsula in Mexico, or along the Appalachian mountain range in eastern USA.

Ground-truthing prior to migration, for sites with at least 3 birds tracked, indicates that average error at breeding sites ( $n = 4$  sites) was 153 km for latitude and 121 km for longitude; average error at wintering sites ( $n = 3$  sites) was 291 km for latitude and 83 km for longitude (Table S3).



**Breeding Bird Survey analyses.** The Breeding Bird Survey is conducted annually during the peak breeding period for wood thrushes, and consists of a 40.2 km (24.5 miles) roadside survey transect with a three-minute point count conducted every 0.8 km for a total of 50 counts (Sauer et al. 2012). Point count protocol is to record all the individuals of any bird species seen or heard with the 3-minute duration of the count. The number of individuals for a species is summed across all 50 counts per transect to estimate abundance. To determine both relative abundance (used to create the species-level network above) and population trends for wood thrushes in each breeding region (Table 1), we used a hierarchical Bayesian method to analyze Breeding Bird Survey data (Link and Sauer 2002, Wilson et al. 2011; for methods see Sauer and Link 2011).

### **Supplemental Literature Cited**

Link, W. A., and J. R. Sauer. 2002. A hierarchical analysis of population change with application to Cerulean Warblers. *Ecology* **83**:2832-2840.

McKinnon, E. A., C. Q. Stanley, K. C. Fraser, M. MacPherson, G. Casbourn, P. P. Marra, C. E. Studds, N. Diggs, and B. J. M. Stutchbury. 2013. Estimating geolocator accuracy for a migratory songbird using live ground-truthing in tropical forest. *Animal Migration* **1**:31-38.

Sauer, J. R., and W. A. Link. 2011. Analysis of the North American Breeding Bird Survey using hierarchical models. *Auk* **128**:87-98.

Sauer, J. R., J. E. Hines, J. E. Fallon, K. L. Pardieck, D. J. Ziolkowski Jr., and W. A. Link. 2012. The North American Breeding Bird Survey, Results and Analysis 1966-2010. Version 12.07.2012. USGS Patuxent Wildlife Research Center, Laurel, MD.

Wilson, S., S. L. LaDeau, A. P. Tøttrup, and P. P. Marra. 2011. Range-wide effects of breeding- and nonbreeding-season climate on the abundance of a Neotropical migrant songbird. *Ecology* **92**:1789-1798.

Table S1. Number of geolocators deployed on wood thrush and retrieved by study site, and final number of geolocators (*n*) used to estimate migration destination (not including repeat-tracked individuals and geolocators that failed prematurely).

Study site (geolocator model)	Location	Year deployed	Total geolocators deployed	Total geolocator s retrieved	<i>n</i>
Pennsylvania (MK14S)	41.8° N 79.9°W	2007	14	5	5
		2008	33	11	9
		2009	34	11	8
		2010	15	3	1
		Pennsylvania Subtotal:		96	30 (31%)
Vermont (MK10S)	44.4° N 72.9°W	2009	28	2	2
Virginia (MK10S)	38.7° N 77.2°W	2009	23	1	1
North Carolina (MK10S)	35.4° N 83.1°W	2009	27	5	5
Ontario (MK10S and Geo-Tx MK20S)	44.3° N 76.5°W	2011	26	5	5
	43.4° N 80.6°W	2012	38	4	4
Indiana (Light Bug)	38.7°N 86.4°W	2011	57	12 (21%)	12
	38.9°N 86.9°W				
	39.0°N 85.4°W				
	39.3°N 86.6°W				
	39.3°N 86.7°W				
<b>Breeding Deployment Subtotal:</b>			<b>295</b>	<b>59 (20%)</b>	<b>52</b>
Costa Rica (MK14S)	10.4° N 84.0°W	2009	30	8	7
		2010	56	11	8
		2011	23	6	6
Costa Rica Subtotal:			109	25 (23%)	21
Belize (MK14S)	16.5° N 88.7°W	2010	40	1	1
		2011	74	12	10
		2012	52	17	11
		2013*	86	3*	2
Belize Subtotal:			166*	30 (18%)*	24
Mexico (MK 10S)	18.5° N 95.0°W	2010	20	1	1
Nicaragua	13.2° N 86.0°W	2011	63	4	4

(MK14S)

<b>Wintering Deployment Subtotal:</b>	<b>352</b>	<b>61 (17%)</b>	<b>50</b>
---------------------------------------	------------	-----------------	-----------

Total:		116 (19%)	102
--------	--	-----------	-----

---

\*Fieldwork is currently underway in Belize therefore the 2013 numbers were not included in the Belize subtotal values.

Table S2. Return rate of geolocator versus non-geolocator wood thrushes from summer deployments (Pennsylvania and Indiana) and winter deployments (Costa Rica and Belize).

Deployment site (year)	% geo re-sighted ( <i>n</i> deployed)	% non-geo re-sighted ( <i>n</i> banded, no geo)	$\chi^2$	<i>p</i>
Pennsylvania (2007-2010) <sup>a</sup>				
Male	56 % (52)	40 % (55)	2.66	0.102
Female	18 % (45)	13 % (56)	0.81	0.369
Total	38% (97)	26% (111)	2.25	0.134
Indiana (2011) <sup>b</sup>				
Male	25 % (57)	30 % (256)	0.45	0.500
Costa Rica (2008-2010) <sup>a</sup>				
Male	19 % (72)	3 % (37)	11.64	<0.001
Female	36 % (31)	19 % (31)	5.25	0.022
Unknown sex	0 % (6)	0 % (32)	-	-
Total	23 % (109)	7% (100)	8.53	0.003
Belize (2011-2013) <sup>a</sup>				
Male	30 % (54)	24 % (51)	0.67	0.414
Female	24 % (46)	20 % (51)	0.36	0.546
Unknown sex	8 % (26)	0 % (45)	-	-
Total	23 % (126)	15 % (147)	1.68	0.194

<sup>a</sup> Geolocator model MK14 S (1.6g, British Antarctic Survey/Biotrack)

<sup>b</sup> Geolocator model 'LightBugs' (2g, Lotek)

Table S3. Geolocator accuracy calculated from estimated positions at deployment sites prior to, or after the bird's migration (breeding: June-July, sun elevation -2.0; wintering: Jan-Mar, sun elevation -0.5).

Deployment Site	<i>n</i>	Average Latitude Estimate	Average Latitude Error (km)	Average Longitude Estimate	Average Longitude Error (km)
Ontario					
43.4° N 80.6°W	6	44.4 ± 0.2	117 ± 28	78.5 ± 0.5	136 ± 44
Pennsylvania					
41.8°N 79.9°W	23	41.6 ± 0.9	188 ± 67	78.3 ± 0.5	105 ± 29
Indiana					
39.0°N 85.4°W	5	40.4 ± 0.3	159 ± 26	83.4 ± 0.5	173 ± 38
North Carolina					
35.4° N 83.1°W	5	35.7 ± 1.6	148 ± 61	82.7 ± 0.9	72 ± 44
Overall Breeding	4 sites	-	153	-	121
Belize					
16.5°N 88.7°W	15	13.8 ± 4.1	400 ± 135	88.1 ± 0.4	64 ± 24
Nicaragua					
13.2° N 86.0°W	4	14.6 ± 0.9	145 ± 95	84.9 ± 0.7	119 ± 75
Costa Rica					
10.4°N 84.0°W	13	11.9 ± 2.0	327 ± 141	84.0 ± 1.0	67 ± 46
Overall Winter	3 sites	-	291	-	83

Table S4. Full model results from generalized linear models on wintering site and migration route for wood thrushes tracked using geolocators.

Dependent factor	Independent factors	Model estimate ( $\pm$ standard error)	<i>t</i>	<i>p</i>
Winter latitude	Intercept	-4.33 $\pm$ 6.70	-0.65	0.52
	Breeding latitude*	-0.20 $\pm$ 0.07	-2.74	0.007
	Breeding longitude*	0.33 $\pm$ 0.06	5.60	<0.001
	Sex (M)	-0.10 $\pm$ 0.37	-0.28	0.78
Winter longitude	Intercept	74.96 $\pm$ 7.00	10.7	<0.001
	Breeding latitude*	-0.21 $\pm$ 0.08	-2.77	0.006
	Breeding longitude*	0.25 $\pm$ 0.06	4.06	<0.001
	Sex (M)	0.15 $\pm$ 0.39	0.37	0.71
Fall route	Intercept	57.03 $\pm$ 6.6	8.62	<0.001
	Breeding latitude	-0.12 $\pm$ 0.12	1.05	0.30
	Breeding longitude*	0.34 $\pm$ 0.08	4.05	0.001
	Sex (M)	-0.42 $\pm$ 0.57	-0.73	0.47
Spring route	Intercept	70.85 $\pm$ 10.90	9.80	<0.001
	Breeding latitude	0.03 $\pm$ 0.11	0.29	0.77
	Breeding longitude*	0.23 $\pm$ 0.10	2.30	0.02
	Sex (M)*	1.07 $\pm$ 0.56	1.90	0.06
	Age (J)	-0.64 $\pm$ 0.82	-0.78	0.44

\* Significant factor retained in the top model.