

RESEARCH ARTICLE

Depleted lean body mass after crossing an ecological barrier differentially affects stopover duration and refuelling rate among species of long-distance migratory birds

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Funding information

American Ornithologists' Union; National Science Foundation, Grant/Award Number: IOS-1656726; University of Massachusetts Amherst; Wilson Ornithological Society

Handling Editor: Tony Williams

Abstract

1. During the long-distance migratory flights of birds, lean mass breakdown occurs in concert with fat catabolism and is expected to have repercussions on total stopover duration because birds require time to rebuild lean tissue before accumulating fat reserves. Despite this, little is known about the role of in-flight lean mass breakdown on stopover duration because direct measurements are restricted by the destructive nature of traditional body composition analysis and the technological limitations of tracking small birds over large expanses.
2. We used non-lethal, non-invasive Quantitative Magnetic Resonance technology and plasma metabolite profiling to measure the body composition and physiological state of free-living birds captured at a migratory stopover site after flight across the Gulf of Mexico, and an automated radiotelemetry array covering ~5000 km² to track stopover duration and regional movements. We tested whether stopover duration is prolonged in individuals arriving with lower lean mass and investigated how lean mass affects regional movements.
3. Stopover duration decreased by 22% for each additional gram of lean mass in Northern Waterthrush (*Parkesia noveboracensis*), but this relationship was not apparent in Swainson's Thrush (*Catharus ustulatus*), Grey-cheeked Thrush (*Catharus minimus*) or Yellow-billed Cuckoo (*Coccyzus americanus*), even though these species also arrived with depleted lean mass. Stopover duration increased for Swainson's Thrush with higher plasma uric acid, a marker of protein catabolism. Northern Waterthrush with higher plasma triglycerides had longer stopover duration.
4. Our findings suggest that migratory birds may compensate for substantial lean mass losses by increasing refuelling rate or moving to a different stopover site, and highlights species-level differences in lean mass breakdown and the associated impacts on physiological function. Our results highlight the strategies used by different species to recover from a trans-Gulf of Mexico flight and resume migration, which improves our understanding of the annual cycle of migratory birds.

KEYWORDS

ecological barrier, lean mass, migration, Motus, QMR, stopover

1 | INTRODUCTION

Before embarking on non-stop long-distance flights between breeding and non-breeding grounds, birds store fuel loads by increasing both fat mass (Bayly et al., 2013; Gómez et al., 2017; Marsh, 1983) and muscle mass (Hua et al., 2013; Landys-Ciannelli et al., 2003; Marsh, 1984). Lipid deposits can account for >50% of body mass prior to flight and are the primary energy stores used during continuous flight (Guglielmo, 2018; Jenni & Jenni-Eiermann, 1998). Prior to flight, lean mass may be selectively increased, as in the case of hypertrophy of flight muscles in Gray catbirds (*Dumetella carolinensis*) to increase flight capacity (DeMoranville et al., 2019; Marsh, 1984), or decreased as in the case of shorebirds with small, atrophied gizzards that may allow reduced flight costs once fuelling is complete (Piersma et al., 1999; Piersma & Gill Jr, 1998).

Birds often land with depleted fat stores after long-distance non-stop flights, especially after crossing barriers like the Sahara Desert or the Gulf of Mexico where there is no opportunity to stop and eat or drink (Kuenzi et al., 1991; Moore & Kerlinger, 1987; Yong & Moore, 1997). After long-distance flights, migratory birds may also arrive with depleted lean mass, evidenced by concave pectoral muscles and reduced organ masses (Battley et al., 2000; Bauchinger & Biebach, 2001; Gutierrez Ramirez, Kneidel, et al., 2022; Jenni-Eiermann et al., 2011; Piersma et al., 1999; Piersma & Gill Jr, 1998). The breakdown of lean mass during flight is supplemental to fat catabolism (Gerson & Guglielmo, 2013; Groom et al., 2019), and lean mass depletion can account for up to 50% of mass loss during migration, despite the persistence of unused fat stores (Salewski et al., 2009).

Because long-distance flight is fuelled primarily by fat, due to its high-energy density (Jenni & Jenni-Eiermann, 1998; Klaassen, 1996), we have a better understanding of its role in migration and stopover. For instance, stopover duration is significantly shorter for fat birds than for birds with no fat (Goymann et al., 2010), who generally stay longer at stopover sites (Bayly et al., 2012; Loria & Moore, 1990; Yong & Moore, 1997), and move further and faster to find food resources (Cohen et al., 2012). However, the consequences of significant in-flight reduction of lean mass on stopover and overall migration have not been thoroughly investigated.

Reductions in digestive organs may result in reduced nutrient assimilation (Gannes, 2002; Karasov & Pinshow, 2000) and may explain why some birds initially continue to lose body mass and have low fuel deposition rates upon first arrival to a stopover site (Schwilch & Jenni, 2001). Rebuilding lean tissue components, including those of the digestive tract, occurs slowly (Carpenter et al., 1993; Gannes, 2002) and is necessary to facilitate the deposition of lipids before the continuation of migration (Biebach, 1998; Carpenter et al., 1993; Karasov & Pinshow, 1998). Therefore, rebuilding lean mass lost during flight is expected to be a constraint to migrating birds since it is a time-consuming process, and may consequently

increase the time required for stopover (Carpenter et al., 1993; Gannes, 2002). Direct measures of the influence of lean mass on stopover duration and migration time are limited (Seewagen & Guglielmo, 2010) and have not been conducted after non-stop flights when birds are potentially at their leanest.

To understand the importance of lean body mass dynamics to stopover, we established a Motus Wildlife Tracking System automated radio telemetry array in the northern Gulf of Mexico to record spring stopover duration of migratory birds that had just completed a trans-Gulf flight and arrived on a barrier island (St. George Island, Florida, USA, 29.672679, -84.841423). We used this array to precisely determine the stopover duration of spring migratory birds at the local (island) and regional levels (~5000km²); used quantitative magnetic resonance (QMR) technology to accurately and non-destructively measure fat and lean mass (Guglielmo et al., 2011); and used plasma metabolite profiling to assess refuelling rate and lean mass breakdown (Guglielmo et al., 2005; Jenni-Eiermann & Jenni, 1991).

We test the hypothesis that lean mass breakdown during flight increases stopover duration because birds will require time to rebuild lean tissue before accumulating fat reserves needed to resume migration (Carpenter et al., 1993; Karasov & Pinshow, 1998; Wojciechowski et al., 2014). We set up four predictions. First, we predict that individuals with lower lean mass at arrival will have longer stopover durations. Second, that individuals with higher protein catabolism, measured through plasma uric acid concentration and an indication of lean mass breakdown, will have longer stopover durations. Third, that individuals with higher refuelling rates will have shorter stopover duration and resume migration sooner, because birds may compensate for the loss of lean mass during migratory flight by increasing refuelling rate during stopover (Griego et al., 2021; Muñoz-García et al., 2012). Fourth, we predict that individuals with an island-only stopover strategy will have longer stopover durations than those with an island-mainland stopover strategy. Coastal habitats and barrier islands of the northern Gulf of Mexico are regarded as poorer quality sites for refuelling migratory songbirds (Buler & Moore, 2011; Kuenzi et al., 1991; Mehlman et al., 2005) compared to inland forests (Buler et al., 2007), so birds that leave the island and relocate to the mainland could potentially access better quality habitat allowing them to resume migration sooner.

2 | MATERIALS AND METHODS

2.1 | Bird capture, sampling, and measurements

We captured birds using standard mist-netting techniques, and measured body composition, collected blood and feather samples, and radio-tagged migrating Northern Waterthrush (NOWA; *Parkesia*

noveboracensis), Swainson's Thrush (SWTH; *Catharus ustulatus*), Gray-cheeked Thrush (GCTH; *Catharus minimus*) and Yellow-billed Cuckoo (YBCU; *Coccyzus americanus*) at a constant-effort banding station in the Apalachicola National Estuarine Research Reserve on St. George Island, Florida, USA from April 1 to May 15, 2016–2018. St. George Island is a barrier island along the northeastern coast of the Gulf of Mexico (Figure 1, Supplemental Methods). We measured fat mass, lean mass and total water in grams using a QMR body composition analyser (EchoMRI-B, Echo Medical Systems) in the field, with a total scanning time of up to 180 s (Guglielmo et al., 2011; Kennedy et al., 2017; Seewagen & Guglielmo, 2011). The QMR was calibrated every morning using 1.0, 5.02 and 15.0 g of canola oil standards and was plugged into a stable power source with a signal stabilizer. Birds were scanned in duplicate or triplicate at three accumulations, to achieve a CV under 15%. We collected blood samples to determine sex genetically and to measure plasma metabolites. We measured plasma triglyceride concentrations to assess refueling rate (Guglielmo et al., 2005) and plasma uric acid to assess protein catabolism (Jenni et al., 2000). We collected feather samples to measure deuterium stable isotopes to serve as a proxy for breeding latitude (Hobson & Wassenaar, 1997; Supplemental Methods). Bird banding, sample collection, and radio-tagging were authorized through US Bird Banding Laboratory (permit 23979) and the Florida Fish and Wildlife Conservation Commission (LSSC-16-00033).

University of Massachusetts Amherst IACUC (2015-0019) approved of all methods conducted in this study. Access to the site for bird captures was permitted by the Apalachicola National Estuarine Research Reserve (state property) and by private landowners (Levi/Piotrowski, Edmiston, Ashmore, Lewis, Cummins, England, Ramont, and Pettit families).

2.2 | Radio telemetry

Coded nano-tags transmitters (Lotek Wireless, Newmarket, Ontario, Canada; NTBQ-2, NTBQ-3, NTBQ-4-2) were programmed to a frequency of 166.380 MHz, with varying pulse intervals and estimated lifespans (Table S10). Nano-tags weighed <3% of body mass and were attached using a loop harness secured around the legs (Rappole & Tipton, 1991). All birds were released at site of capture.

2.3 | Automated tracking in northern Florida

We operated an automated radio-telemetry array consisting of 8–14 towers situated along the Apalachicola floodplain and bay (Supplemental Methods, Figure 1), each with three 8-element, 5-element or stacked 6-element Yagi antennas connected to a

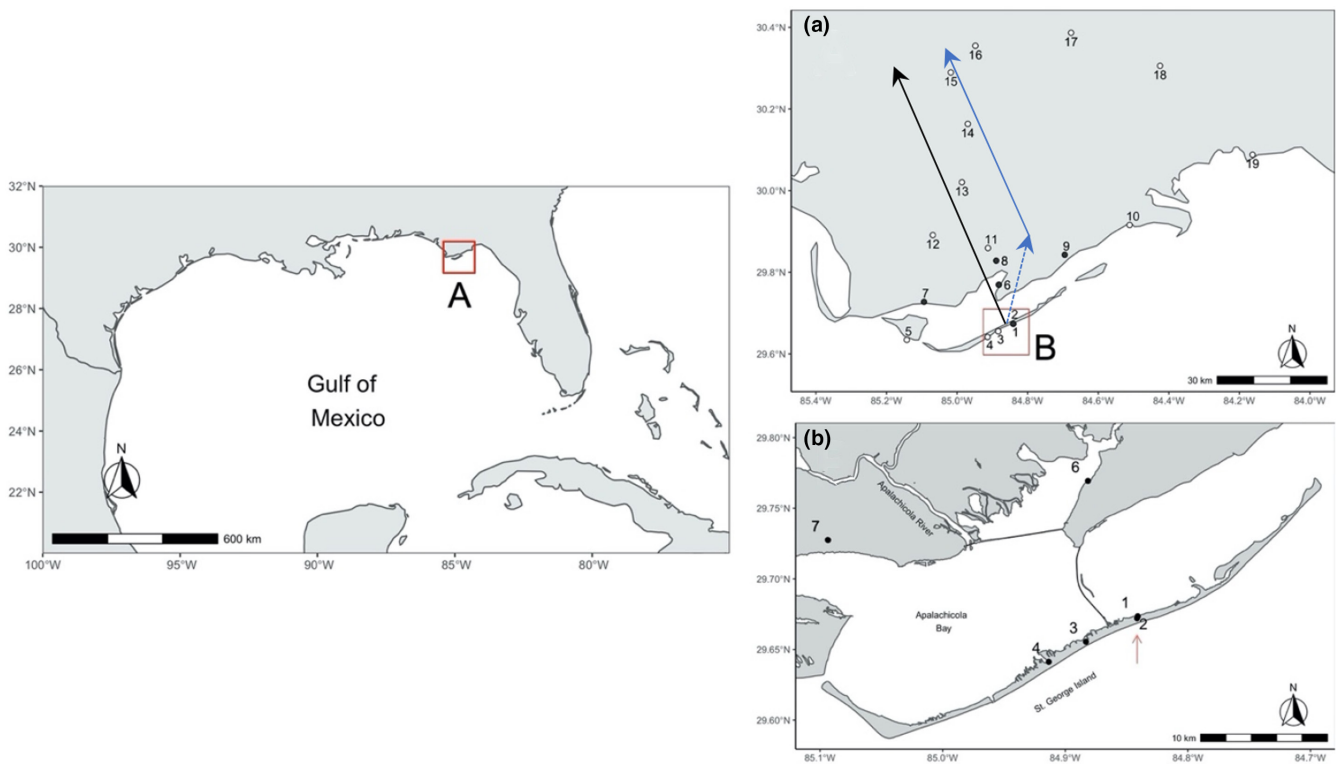


FIGURE 1 Location of the study site in the northern Gulf of Mexico. (a) Automated radio telemetry array in coastal northwest Florida. Receivers were operated between 2016 and 2018 every season (black circles) or for one or two seasons (open circles). (b) Location of our capture site (red arrow) and local receivers on St. George Island (black circles). The distance between the capture site and the northernmost receivers is approximately 75 km. The two stopover strategies are illustrated in (a). The continuous black arrow represents the island-only strategy, where birds depart on migratory flight from the island. The broken blue arrow illustrates the island-mainland strategy, where birds relocate from the island to the mainland to stopover before a true migratory departure.

receiver (SensorGnome, CompuData, Inc.). The array had a total coverage of ~1500 km² in 2016 to ~5000 km² in 2017–2018 and allowed for continuous detection of tags. We established 'telemetry fences' spanning longitudinally along the island and coast to determine departure from the island (receivers 1–6 in Figure 1a), and within the Apalachicola National Forest to determine regional departure (receivers 7–19 in Figure 1a). The local array coverage was consistent throughout the 3 years of the study, but the regional array coverage increased in 2017–2018. Telemetry receivers were located on private, state, and federal lands and were appropriately permitted (Table S11).

2.4 | Estimating local and regional stopover duration

Local stopover duration was estimated from the time of initial capture to the final detection at any local receiver. We used graphs of signal strength and time of day from the island receiver to determine departure time. A true migratory flight departing the island was confirmed as a nocturnal flight indicated by a peak in signal strength, immediately followed by subsequent detections as the bird moved north through the array until the signal vanished that same night. If a bird departed the island and was redetected days later within the array, we considered there had been a landscape relocation and the bird had not continued migration (regional stopover). However, if the signal was detected within the array and disappeared <24 h after departing the island, we considered it a transient flight across the mainland of an individual in true migratory movement (Supplemental Methods). If a clear nocturnal departure flight was not evident, the signal on the island was lost during the day and not redetected in any array receiver, or the signal was continuously detected as stationary (no increase/decrease in signal), the bird was excluded from analysis as we could not reliably determine its stopover duration. We estimated total stopover duration as the time from initial capture to the final detection from any receiver in the array. We classified birds as either having an island only or an island-mainland stopover strategy.

2.5 | Statistical analyses

We constructed species-specific general linear models to examine variables known to influence variation in stopover behaviour in spring: day (Alerstam & Lindström, 1990), sex (Seewagen et al., 2013) and breeding destination (Hays et al., 2018; Paxton & Moore, 2017). Only significant variables were included as covariates in subsequent analyses of stopover duration (sex in the case of SWTH and GCTH, ordinal day for YBCU; breeding destination was not significant).

We used generalized linear models with a Poisson distribution and log-link function to examine local and regional stopover duration for each species separately. For our prediction that birds with lower lean mass upon first capture will have longer stopover, we modelled stopover duration as a function of lean and fat masses,

with applicable covariates. We also ran the model using the lipid index (the ratio of fat/lean mass, van der Meer & Piersma, 1994), as a way to jointly describe the fat and protein storage (Supplemental Results). To evaluate the influence of protein catabolism on stopover, we fit models with stopover duration as a function of uric acid. For our prediction that birds with higher refuelling rates will have shorter stopover, we modelled stopover duration as a function of residual triglyceride, with applicable covariates. To assess the influence of habitat use on stopover, we modelled total stopover duration as the response variable, and stopover strategy (categorical: island only or island-mainland) as the predictor variable, and controlled for species. All analyses were conducted with the program R 4.0.1 (Supplemental Methods).

3 | RESULTS

3.1 | Stopover duration

We measured minimum stopover duration and body condition of 27 Swainson's Thrushes, 28 Northern Waterthrushes, 15 Gray-cheeked Thrushes and 8 Yellow-billed Cuckoos after a trans-Gulf spring migratory flight (Tables S1 and S2). The ratio of fat to lean in these individuals was below 10% (Figure S5). Across species, birds that used an island-mainland strategy had longer total stopover duration than those with an island-only strategy ($F_{1,55} = 7.8, p < 0.01$; Table 1;

TABLE 1 Average island stopover duration and average regional stopover duration (d, day since capture) ± SD for 3 species of migratory birds using either an island-only or island-mainland stopover strategy during stopover on St. George Island, Florida for springs 2016–2018. Percent of total birds in parenthesis. We were unable to estimate regional stopover duration for Yellow-billed Cuckoo (SI Methods)

	Island stopover	Regional stopover	Total stopover
Swainson's Thrush			
All	2.69 ± 2.91	3.39 ± 3.32	6.08 ± 4.07
Island only (45.83%)	3.36 ± 3.17	—	4.5 ± 4.6
Island-Mainland (54.17%)	2.08 ± 2.64	5.42 ± 0.99	7.5 ± 3.03
Northern Waterthrush			
All	4.29 ± 4.77	1.19 ± 3.06	5.48 ± 4.99
Island only (92.3%)	4.83 ± 4.95	—	4.89 ± 5.02
Island-Mainland (7.69%)	1.0 ± 0	8.0 ± 3.46	9.0 ± 3.46
Gray-cheeked Thrush			
All	3.13 ± 2.50	1.8 ± 2.60	4.9 ± 2.34
Island only (66.67%)	3.8 ± 2.09	—	4.0 ± 1.89
Island-Mainland (33.33%)	1.8 ± 2.95	5.0 ± 2.0	6.8 ± 2.17
Yellow-billed Cuckoo			
All	6.0 ± 6.80	NA	NA

FIGURE 2 Total stopover duration for birds using different stopover strategies (island only or island-mainland).

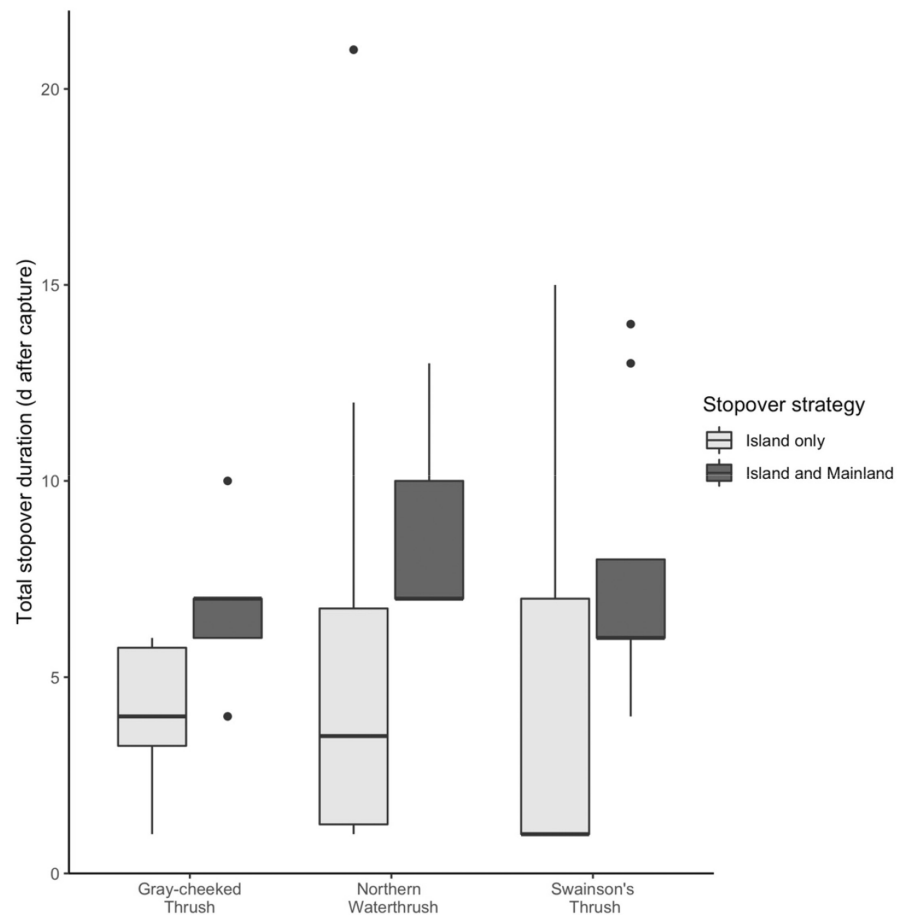


Figure S1; Figure 2), but there were no differences in stopover duration between species ($F_{2,55} = 0.38$, $p = 0.7$).

3.2 | Influence of body condition on stopover duration

Across species, fat mass had a significant negative effect on island and total stopover durations (island: $\beta = -1.3 \pm 0.3$, $p < 0.01$; total: $\beta = -1.5 \pm 0.4$, $p < 0.01$), but lean mass did not (island: $\beta = -0.03 \pm 0.08$, $p = 0.8$; total: $\beta = -0.08 \pm 0.1$, $p = 0.6$). However, examining species separately we found that both fat and lean mass significantly negatively influenced stopover duration in Northern Waterthrush (island: lean: $F_{1,18} = -3.32$, $p < 0.001$; fat: $F_{1,18} = -3.7$, $p < 0.001$; regional: lean: $F_{1,18} = -3.32$, $p < 0.001$; fat: $F_{1,18} = -3.53$, $p < 0.001$; Figure 3, Tables S5 and S6). Island stopover duration decreased by 29% for each additional gram of fat and decreased by 22% for each gram of lean body mass. Only three Northern Waterthrush (14%) relocated to the mainland to continue stopover. Regional stopover duration decreased by 26% for each gram of fat and decreased by 25% for each gram of lean.

For Swainson's Thrush, Gray-cheeked Thrush, and Yellow-billed Cuckoo, island stopover duration was significantly negatively influenced by fat mass, but not lean mass (SWTH: fat: $F_{1,22} = -2.49$, $p = 0.01$; lean: $F_{1,22} = -0.49$, $p = 0.6$; GCTH: fat: $F_{1,14} = -3.1$, $p = 0.002$;

lean: $F_{1,14} = -0.9$, $p = 0.36$; YBCU: fat: $F_{1,4} = -2.9$, $p = 0.003$; lean: $F_{1,4} = 1.4$, $p = 0.2$; Figure 3). For each additional gram of fat, island stopover duration decreased by 32% for Swainson's Thrush, 44% for Gray-cheeked Thrush, and by 62% for Yellow-billed Cuckoo. Although we did not find that arrival lean mass influenced island stopover duration, our estimates indicate an 11% decrease in stopover duration for each additional gram of lean mass in Gray-cheeked Thrush and a 5% decrease in Swainson's Thrush.

Fat mass and lean mass did not explain regional stopover duration for Swainson's Thrush (Table S4). Fat mass negatively influenced regional stopover duration for Gray-cheeked Thrush, decreasing by 26% for each additional gram of fat ($F_{1,11} = -2.27$, $p = 0.02$; Table S8). We were unable to determine regional stopover duration in Yellow-billed Cuckoo, so its relationship with body condition was not examined.

3.3 | Influence of lean mass breakdown on stopover duration

There was a positive relationship between plasma uric acid and island stopover duration in Swainson's Thrush ($F_{1,21} = 2.98$, $p = 0.003$; Figure 4); island stopover duration increased by 106% for each unit increase of log uric acid. Plasma uric acid was unrelated to island stopover duration for Gray-cheeked Thrush and Northern

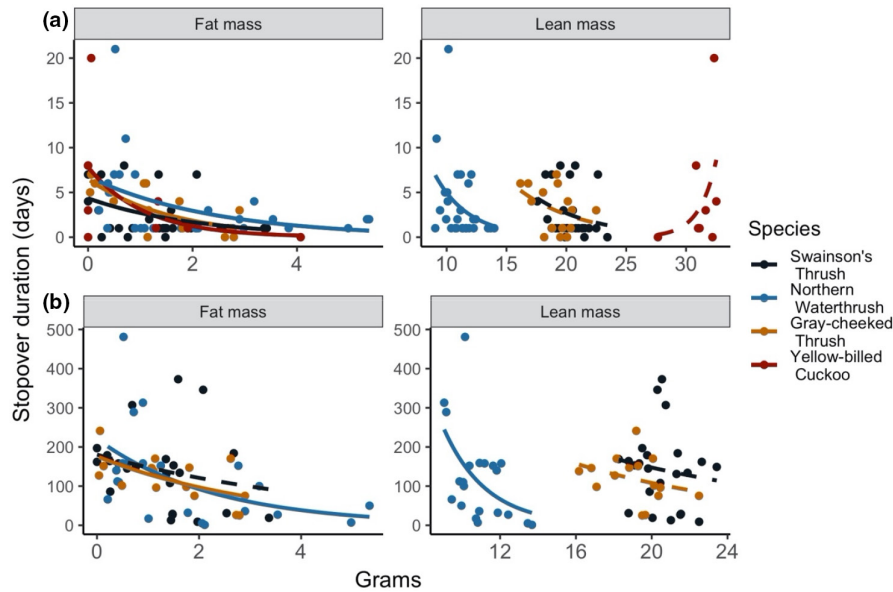


FIGURE 3 Relationship between body composition at first capture (fat mass and lean mass) and stopover duration on (a) St. George Island and (b) northern Florida for Swainson's Thrush (black), Northern Waterthrush (blue), Gray-cheeked Thrush (orange), and Yellow-billed Cuckoo (red). We were unable to estimate regional stopover duration for Yellow-billed Cuckoo (SI Methods). Fat mass (g) and lean mass (g) measured via quantitative magnetic resonance and stopover duration determined via automated radio telemetry. Lean mass was corrected for body size separately for each species (SI Methods). Solid lines represent statistically significant relationship and dashed lines represent non-significant trends. Model means and relationships were estimated from generalized linear models fitted with a Poisson distribution.

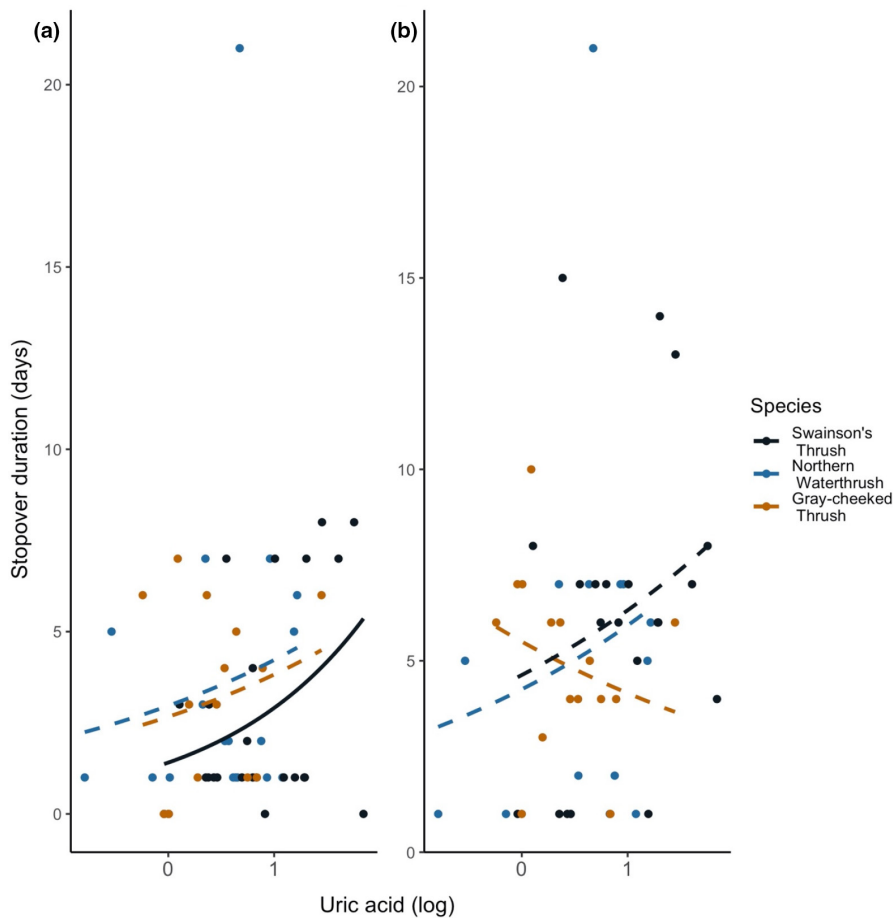


FIGURE 4 Relationship between (log) plasma uric acid concentration and stopover duration on (a) St. George Island and (b) northern Florida for Swainson's Thrush (black), Northern Waterthrush (blue), and Gray-cheeked Thrush (orange). We did not examine uric acid concentration in Yellow-billed Cuckoo. Solid lines represent statistically significant relationships and dashed lines represent non-significant trends. Model relationships were estimated from generalized linear models fitted with a Poisson distribution.

Waterthrush. We found no relationship between plasma uric acid and regional stopover duration for any species (SWTH: $F_{1,19} = 1.85$, $p = 0.06$; NOWA: $F_{1,12} = 1.5$, $p = 0.13$; GCTH: $F_{1,13} = -1.0$, $p = 0.3$). Limited sample size precluded analysis of lean mass breakdown for Yellow-billed Cuckoo.

3.4 | Influence of refuelling rate on stopover duration

Island stopover duration increased by 200% for each unit increase of residual triglyceride in Northern Waterthrush ($F_{1,24} = 3.39$, $p < 0.001$). There was no effect of residual triglycerides on island stopover duration for the other species (Figure 5; SWTH: $F_{1,25} = 0.5$, $p = 0.6$; GCTH: $F_{1,13} = -0.8$, $p = 0.4$; YBCU: $F_{1,6} = -0.46$, $p = 0.6$). Regional stopover duration increased by 48% for Swainson's Thrush for each unit increase of residual triglyceride ($F_{1,22} = 4.7$, $p < 0.001$).

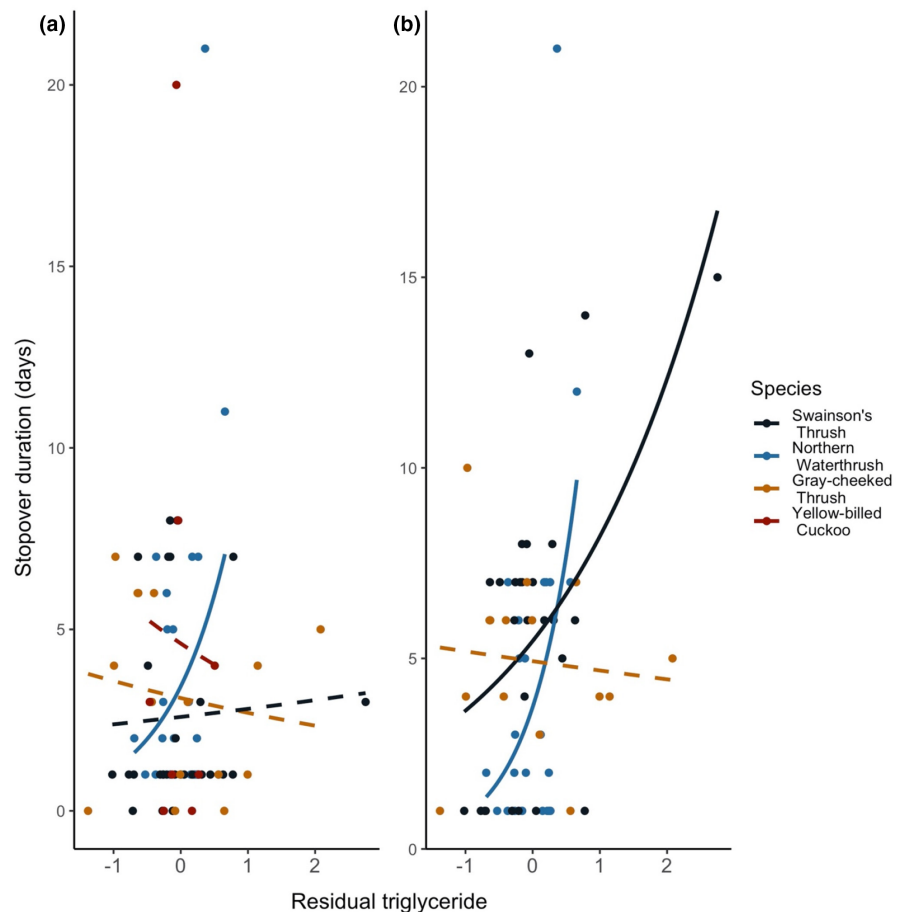
4 | DISCUSSION

Using an extensive automated telemetry array, QMR body composition analysis, and plasma metabolite profiling, we link individual measures of physiological condition to stopover behaviour in free-living migrant birds after a non-stop long-distance migratory flight.

We found species-specific relationships between multiple components of precisely measured body condition on first capture and subsequent stopover duration, refuelling rate, and stopover relocation. While lean mass was important for one species, the fat mass was negatively associated with stopover duration across all species. Fat is the primary fuel of migration and is the main determinant of stopover duration independent of weather conditions (Goymann et al., 2010), and our results confirm its importance to stopover duration and the continuation of migration. Although universally influential, fat mass alone did not explain stopover behaviour.

We found strong evidence that lower lean mass leads to longer stopover duration in Northern Waterthrush, presumably to rebuild muscle or organ masses, as has been shown in other species (Biebach, 1998; Carpenter et al., 1993; Karasov & Pinshow, 1998). Average lean mass of Northern Waterthrush on first capture was nearly 3 g (~20%) lower than previously estimated (Cano et al., 2020; Rogers & Odum, 1966), and the large range in lean mass (9.05 g–13.98 g) may indicate that Northern Waterthrush that arrive at our site have varying winter origins. Though wintering populations are most common throughout the Greater Antilles, they also winter from Mexico through Colombia and Venezuela (Whitaker & Eaton, 2020). Northern Waterthrush can acquire sufficient fuel loads to complete a non-stop flight from northern Colombia to the Gulf of Mexico (Cano et al., 2020), so it is plausible that some of the very depleted birds we captured

FIGURE 5 Relationship between residual triglycerides and stopover duration on (a) St. George Island and (b) northern Florida for Swainson's Thrush (black), Northern Waterthrush (blue), Gray-cheeked Thrush (orange), and Yellow-billed Cuckoo (red). We were unable to estimate regional stopover duration for Yellow-billed Cuckoo (SI Methods). Solid lines represent statistically significant relationship and dashed lines represent non-significant trends. Model relationships were estimated from generalized linear models fitted with a Poisson distribution, using the residuals of triglycerides ($\log_{10} + 1$) and fat mass as the predictor variable in the models to account for the effect of fat mass (SI Methods).



departed directly from South America. The quality of overwintering and/or staging habitat influences the body condition and fuel load of birds prior to departing across an ecological barrier (Gómez et al., 2017) and has repercussions for subsequent phases of the annual cycle (González-Prieto & Hobson, 2013; Smith et al., 2010). So, we cannot reject that very depleted individuals had relatively short flights but originated from poorer quality habitat. It is also plausible that individuals with higher lean mass had been at the site for a few days when we first captured them and had a chance to recover some of their lean mass. The rate of fat and lean mass breakdown during migration is also dependent on environmental conditions (Gerson & Guglielmo, 2011; Groom et al., 2019) or relative exercise intensity (McWilliams et al., 2004). Exploring the relationship between arrival body composition and quality conditions of departure sites is an opportunity and need for future research.

Lean mass influenced stopover duration for Northern Waterthrush, as expected, but this relationship was not apparent in Swainson's Thrush, Gray-cheeked Thrush, or Yellow-billed Cuckoo, even though these species also arrived with depleted lean body mass. In fact, these species were captured with very low fat/lean ratios (6.25%, 7.5% and 3.4%, respectively), well under the point where birds experience reduced flight performance (Schwilch et al., 2002). A possible explanation for this differential response to lean mass could be related to body size, as Northern Waterthrush is the smallest species examined in this study and has less chance for drastic reductions in lean mass. The lack of relationship between lean mass and stopover duration in Swainson's Thrush, Gray-cheeked Thrush, or Yellow-billed Cuckoo may point to a time-minimizing migration strategy, where individuals may depart in sub-optimal body condition. Northern Waterthrush may instead adapt an energy-minimizing strategy, suggested by the report that this species defends territories during stopover (Rappole & Warner, 1976).

Yellow-billed Cuckoo captured on St. George Island during stopover may have just completed a non-stop transoceanic flight, departing from northern South America, across the Caribbean Sea and arriving in the northern Gulf of Mexico coast (Cano et al., 2020; Clipp et al., 2021; Gómez et al., 2017). Remarkably, we document that the average lean mass of Yellow-billed Cuckoo on arrival on St. George Island was 13–16 g lower than previously estimated for post-flight individuals (Cano et al., 2020). Many Yellow-billed Cuckoo acquire sufficient fuel loads for non-stop flights from northern Colombia to the northern Gulf of Mexico (Cano et al., 2020), and most individuals likely overwintered in South America (Hughes, 2020; McNeil et al., 2015; Sechrist et al., 2012). Gray-cheeked Thrush acquire sufficient fuel load to complete a long-distance flight from northern South America to the northern Gulf of Mexico (Bayly et al., 2013; Gómez et al., 2017), and it is estimated that they reach the US Midwest without much time to stop to refuel (Gómez et al., 2017). Though we recorded a large range of stopover duration for Gray-cheeked Thrush (1–10 days), the median total stopover was 5 days after crossing an ecological

barrier. Feather deuterium confirmed the individuals we studied are long-distance migrants with breeding ranges in the boreal forests. With a minimum ~1500 km yet to travel, boreal breeders may choose to depart in sub-optimal body condition to minimize the time required to arrive at their destination. Given that the Gulf of Mexico is the only ecological barrier for these species, departing our site in Florida with sub-optimal body condition may carry minimal risk, and may represent a transition to single night (6–12 h) flights which are much less demanding than their prior trans-oceanic flights from South America. The migratory routes and wintering sites of the birds we tracked were unknown; clearly, migratory connectivity is a key missing element for understanding the mechanisms of lean mass dynamics during stopover. We call for further inquiry into performance of overlapping subpopulations of migrants during spring stopover.

Northern Waterthrush are territorial during stopover, defending areas for up to 7 days (Rappole & Warner, 1976), but will relocate if a site is inadequate and will actively move in response to changing resources during the non-breeding season (Smith et al., 2010). During stopover, Northern Waterthrush primarily used the barrier island and resumed migration from there, passing over the inland habitat, suggesting they met the energetic needs of preparing for a migratory flight there. This differed from the *Catharus* thrushes, where ~50% used an island-mainland strategy. On the mainland, birds likely concentrated along the Apalachicola River bottomlands as the surrounding matrix is predominately open pine savanna, and migrants prefer taller, more structurally diverse vegetation types (Petit, 2000). It is possible that some birds left the island for the mainland, but went undetected by our receivers, either by tag failure or using habitat outside our detection range. Total stopover duration was significantly longer for birds that used an island-mainland strategy. On average, Swainson's Thrush that used an island-mainland strategy stayed an additional 121 h (5 days) relative to an island-only strategy. This extra stopover time may result in improved body condition, allowing birds to make up for 'lost' time by minimizing time at subsequent stopovers. Whether these strategies lead to variation in body condition upon departure is an important question in determining the contribution of stopover habitat to subsequent migratory performance.

Swainson's Thrush with higher levels of uric acid concentration had longer stopover duration, as expected, indicating that individuals that burned more lean mass in flight stayed significantly longer. However, plasma uric acid is not necessarily only from endogenous sources and could indicate consumption of a high-protein diet (Guglielmo et al., 2017) if an individual has been on stopover (and not just arrived). Since we found no effect of residual triglycerides on stopover duration for Swainson's Thrush, we suspect that this may be indicative that uric acid levels were not caused by consuming a high-protein diet. Unfortunately, we did not measure beta-hydroxybutyrate, an indicator of fasting, which would have helped to confirm this. Protein diet manipulation experiments in free-living birds at stopover would aid in improving

our understanding of avian migration physiology and behaviour, particularly the role of high-protein diets and lean mass on stopover duration. Uric acid is an antioxidant that may help birds deal with increased free radicals and avoid oxidative stress caused by high intensity aerobic exercise during long-distance migration (Tsahar et al., 2006). Since we do not see a significant relationship between lean mass at capture and stopover duration for Swainson's Thrush, perhaps the key determinant here is not total grams of lean mass, but rather the rate of lean mass loss or total grams lost from the onset of flight.

We expected that birds with higher refuelling rates would have shorter stopover duration. Contrary to this, we found Northern Waterthrush with higher residual triglyceride levels had longer stopover durations and found no indication that stopover duration was affected by circulating triglyceride concentration for Swainson's Thrush, Yellow-billed Cuckoo, and Gray-cheeked Thrush. The lack of a relationship between stopover duration and residual triglycerides could indicate that these birds are in the first phase of a biphasic mass gain pattern, which has been shown during refuelling in the Gulf coast (Carpenter et al., 1993). Biphasic mass gain occurs when individuals initially gain mass without measurable increases in fat stores, as lean tissues catabolized in flight are rebuilt. During the initial phase, birds experience a physiological digestive limitation (Bauchinger & Biebach, 1998; Gannes, 2002). The positive relationship between stopover duration and residual triglycerides in Northern Waterthrush possibly means they are finding enough resources to remain and refuel, prolonging stopover to 'take advantage' of these resources, and further confirming suspicion that some individuals may have been on site for a few days prior to capture.

We recognize that attributing total stopover duration to measurements collected from birds on arrival is complicated by the changeable nature of these measurements. Undoubtedly, the fat mass, lean mass, and metabolite levels of individuals change the longer they stopover. Since we did not recapture and remeasure these individuals outside of the island, we assume that body condition on first capture reflects body condition further into the stopover or of when they decide to continue migration. We also cannot rule out completely that some individuals may not have been sampled immediately right after arrival and may have been already on stopover. However, the emaciated state of most of the individuals captured makes this unlikely.

We found multiple lines of support for our hypothesis that lean mass breakdown during flight increases stopover duration. Using measures of lean mass, we found a significant negative relationship between stopover duration and lean mass in Northern Waterthrush, but not in the other three study species. Swainson's Thrush with higher levels of protein catabolism, measured using uric acid, had longer stopover durations, and although not statistically significant, the direction of the relationship was similar for Northern Waterthrush and Gray-cheeked Thrush. Our observations of the effect of body composition on stopover duration of birds after crossing an ecological barrier represent a major advance

in understanding the unique pressures affecting migratory populations in the Nearctic-Neotropical system, particularly those that likely take a trans-Caribbean route in spring. When analysing all migratory birds together, we fail to realize species-specific differences and the strategies to cope with the physiological pressures of migration. Studies like ours may inform research focused on understanding the impacts of shifting phenology due to climate change on the physiology and migration of long-distance migratory birds. Our finding of species-specific impacts of lean mass on stopover duration has implications on the study of the evolution of migratory strategies; physiological studies of the unique metabolism of migratory birds should continue to explore differences among species that may be shaped by these differing ecological and evolutionary pressures. Despite arriving with very low lean mass, stopover duration did not increase in the ultra-long-distance transoceanic migrants, Yellow-billed Cuckoo and Gray-cheeked Thrush. Rapid recovery of lean mass may be an adaptation in species that use a transoceanic strategy when departing the wintering range. Our findings also highlight the value of staging and stopover habitats that birds rely on for fuel deposition both before and after crossing ecological barriers, especially considering evidence that birds are burning their lean body mass to physiological limits beyond what was previously estimated possible. The loss of stopover habitats in the northern Gulf of Mexico could affect landing opportunities (Lester et al., 2016), with potentially fatal results if birds attempted to make long over-water flights with insufficient energy reserves.

AUTHOR CONTRIBUTIONS

Mariammar Gutierrez Ramirez and Alexander R. Gerson designed the research and developed the hypotheses. Mariammar Gutierrez Ramirez performed the research and collected the data with assistance from Michael S. Griego, Cory R. Elowe and Joely G. DeSimone. Mariammar Gutierrez Ramirez curated the data, performed the laboratory analyses, performed the formal analyses and wrote the manuscript with input from Alexander R. Gerson, Michael S. Griego, Cory R. Elowe and Joely G. DeSimone. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank our numerous volunteers for assistance in the field and the many undergraduates who aided this project in the laboratory. We thank our federal, state, and private partners around the Apalachicola Bay for permissions to install and operate telemetry equipment. We owe many thanks to the Apalachicola National Estuarine Research Reserve and to Lauren Levi and Kenny Piotrowski for their hospitality and support, for generously providing use of their property for mist netting, and for galvanizing support from neighbouring properties to expand our trapping and tracking efforts, including the Edmiston Family, Ashmore Family, Joanne Lewis, Rolande Cummins, Sandy England, Bob and Marny Ramont, and John and Linda Pettit. We thank Pam Loring, Justin Boyles, Liam McGuire, and Stu Mackenzie and

Motus WTS. Patricia Brennan, David King, and Emily Cohen provided insightful comments on this manuscript and in the development of the project. This work was supported by funds provided to ARG by the University of Massachusetts Amherst (UMass) and National Science Foundation (NSF; IOS-1656726) and to MGR by the American Ornithologists' Union, Wilson Ornithological Society, and UMass (Graduate School Dissertation Fieldwork Grant, Graduate School Dissertation Research Grant, OEB Student Research Grant). MGR was supported by the Northeast Alliance for Graduate Education and the Professoriate (NEAGEP), Joseph L. Boscov Fellowship, UMass Biology Distinguished Graduate Student Summer Research Fellowship, Ford Foundation Dissertation Fellowship, and Spaulding-Smith Dissertation Fellowship. MSG was supported by NEAGEP and an NSF GRFP Award (2017210719). We thank T. Piersma and an anonymous reviewer for helpful comments that improved the manuscript. Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Bird banding, sample collection, and radio-tagging were authorized through US Bird Banding Laboratory (permit 23979) and the Florida Fish and Wildlife Conservation Commission (LSSC-16-00033). University of Massachusetts Amherst IACUC (2015-0019) approved of all methods conducted in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.6wwpzgn2m> (Gutierrez Ramirez, Griego, et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Gutierrez Ramirez, M., Griego, M. S., DeSimone, J. G., Elowe, C. R., & Gerson, A. R. (2022). Depleted lean body mass after crossing an ecological barrier differentially affects stopover duration and refuelling rate among species of long-distance migratory birds. *Functional Ecology*, 36, 2995–3006. <https://doi.org/10.1111/1365-2435.14203>