THE CONTRASTING PHYSIOLOGICAL MECHANISMS OF TWO DISTINCT MIGRATORY STRATEGIES

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THE CONTRASTING PHYSIOLOGICAL MECHANISMS OF TWO DISTINCT MIGRATORY STRATEGIES

By

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The contrasting physiological mechanisms of two distinct migratory strategies

Chairperson: Dr. Creagh Breuner

Migration allows animals to use resources that vary in time and space. The predictability of this resource variation underlies different migratory strategies. Obligate migrants, with predictable movements, use resources that covary with the seasonal phenology of temperature or rainfall patterns. Facultative migrants, with unpredictable timing and destination of movements, rely on unpredictably variable resources. In this dissertation I explore the physiological changes birds undergo in preparation for obligate vs. facultative migratory flight. Hormones facilitate rapid organismal responses to environmental and internal information, making them an ideal physiological system to understand mechanisms controlling migratory behavior. I focus on corticosterone (CORT), a metabolic hormone that underlies activity patterns and feeding behavior.

Among obligate migrants, I aim to clarify CORT’s role in refueling at and departing from stopover sites. Chapter 1 develops a Stopover-CORT hypothesis, synthesizing current literature on CORT physiology, body condition, and refueling rate during the alternate phases of obligate migration: fuel catabolism (flight) and fuel accumulation (stopover). Chapters 1 and 2 together test this hypothesis in the field. We find that CORT reflects body condition among birds arriving at a spring stopover site and predicts departure among fall migratory birds. We use optimal migration theory to explore observed differences between spring and fall migratory physiology.

Among facultative migrants, I aim to understand how birds respond physiologically and behaviorally to changes in food availability, which is proposed to be the proximate cue initiating departure. In captivity, Chapter 3 tests hypotheses about how body composition, CORT, and activity patterns respond to experimentally manipulated changes in food availability. We find that Pine Siskins, a nomadic, facultative migrant, do not prepare for movement, instead relying on an escape strategy to abandon resource-poor areas. In the field, Chapter 4 tests these same hypotheses and finds that siskins need sufficient—but not large—fuel stores to initiate departure. Body condition and food availability interact to inform siskins’ movement decisions.

Collectively, this dissertation explores the physiology underlying predictable and unpredictable migratory movements. We find that facultative movements are physiologically distinct from obligate movements, though energetic condition can influence both movement types. More broadly, these studies contribute to our understanding of migratory behavior, how it varies with both large-scale resource fluctuations and local food availability, and the role of corticosterone in mediating migratory physiological changes.
Developing a Stopover-CORT hypothesis: Corticosterone predicts body composition and refueling rate in Gray Catbirds during migratory stopover

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**ABSTRACT**

Migratory flight is energetically challenging, requiring alternating phases of fuel catabolism and fuel accumulation, accompanied by dramatic changes in body composition and behavior. Baseline corticosterone (CORT; the primary glucocorticoid in birds) is thought to underlie transitions between fuel catabolism during flight, fuel deposition during stopover, and the initiation of migratory flight. However, studies of CORT on stopover physiology and behavior remain disparate efforts, lacking the cohesion of a general hypothesis. Here we develop a Stopover-CORT hypothesis formalizing the relationships among CORT, body condition, and refueling rate in migratory birds. First, we expect body mass to increase with triglycerides (TRIG) as birds refuel. Second, based on a synthesis of previous literature, we predict a U-shaped CORT curve over the course of stopover, postulating that elevated CORT at arrival is reactive, responding to poor body condition, while CORT elevation before departure is preparative, driving changes in behavior and body condition. We tested these predictions in Gray Catbirds (Dumetella carolinensis) following a trans-Gulf flight during spring migration. We found baseline CORT was negatively correlated with body condition and TRIG, corresponding with our predictions for arriving and refueling—but not departing—birds. It is possible catbirds undergo regional habitat translocations rather than complete the entire stopover phase at our study site. We propose the Stopover-CORT hypothesis as a useful predictive framework for future studies of the mechanistic basis of stopover physiology. By studying the regulation of stopover refueling and departure, we may better understand physiological limitations to overall migration rate and improve assessments of habitat quality for refueling birds.

1. Introduction

Migration is a widespread behavior that has evolved repeatedly and independently in all major branches of the animal kingdom (Dingle and Drake, 2007; Bowlin et al., 2010). This behavior allows animals to take advantage of resources that vary in time and/or space (Alerstam et al., 2003; Dingle and Drake, 2007). For instance, many bird species migrate between wintering and breeding grounds each year, coordinating their movements with seasonal fluctuations in food availability. These migratory journeys are energetically expensive, requiring extensive physiological preparations for flight and recovery after flight at stopover sites along the way.

Both fat and lean mass undergo substantial, cyclical changes during migration: they increase prior to departure (Marsh, 1984; Jenni and Jenni-Eiermann, 1998; Battley and Piersma, 2005; Guglielmo, 2018), are depleted during flight (Moore and Kerlinger, 1987; Piersma and Gill, 1998; Battley et al., 2000; Bauchinger and Biebach, 2001), and are replenished during stopover (Piersma and Jukema, 1990; Carpenter et al., 1993; Gannes, 2002), a cycle that continues until the bird reaches its final destination.

Due to significant in-flight reductions in fat stores and lean mass, stopover refueling is a crucial component of migratory journeys. In fact, a greater proportion of the migration period, and therefore energy, is spent at stopover sites than is spent in flight (Alerstam et al., 2003; Wikelski et al., 2003; Schmaljohann et al., 2012). Upon arrival at stopover sites, birds must replenish and accumulate fat and lean mass to prepare for the next leg of their journey. However, there are often delays in mass gain at stopover (Rappole and Warner, 1976; Karasov and...
Pinshow, 2000), likely imposed by functional losses due to organ cat-
abolism during flight. Therefore, birds must first rebuild lean tissues, especially the digestive tract, before they are able to deposit fat (Carpenter et al., 1993; Karasov and Pinshow, 2000; Gannes, 2002).

Importantly, the rate at which birds are able to recover after a long flight before departing on the next one can determine overall migration rate (Alerstam, 1991; Carpenter et al., 1993; Schmaljohann and Both, 2017).

In order to complete a successful migration, animals must be able to perceive and appropriately respond to environmental and endogenous cues to time their preparations and movements. Corticosterone (CORT), a pleiotropic steroid, is thought to regulate many of these behavioral and physiological responses to both external and internal cues during migration (Cornelius et al., 2013). CORT mediates organizational responses to environmental unpredictability, and supports the increased metabolic demands of life history stage transitions (Landys et al., 2006; Wada, 2008; Cornelius et al., 2013). CORT has been implicated in regulating locomotor activity (Breuner et al., 1998), foraging behavior (Dallman, 1993; Löhmus et al., 2006), and fuel use (Le Ninan et al., 1988; Dallman, 1993), and it has been shown to predict migratory departure from stopover sites (Eikenaar et al., 2017, 2018).

CORT can predict or promote changes in physiology and behavior at two scales: elevations in baseline levels of CORT often induce preparations for predictable life history stage transitions, including fledging or seasonal migration, while much higher stress-induced CORT levels rise in response to unpredictable perturbations, such as a harsh storm (Landys et al., 2006; Wada, 2008; Romero et al., 2009). Because seasonal migration is a predictable life history stage, it is likely not a stressful event: baseline CORT levels can predict and promote stopover physiology, and CORT may only reach stress-induced levels in birds with severely depleted energy stores (Gwinner et al., 1992; Falsone and Jenni-Eiermann, 2009).

This paper has two primary aims. First we synthesize the current state of information on CORT and body composition at stopovers to develop a testable hypothesis about how CORT may reflect or promote changes in body composition in refueling migratory birds at stopover. While previous studies have primarily focused on one phase of the stopover period (i.e. arrival, refueling, or departure from the stopover site), here we provide a framework of hypotheses about the role of CORT over the course of the entire stopover duration. Second, we test this Stopover-CORT hypothesis with a field study in Gray Catbirds (Dumetella carolinensis) captured at a spring stopover site on a barrier island off the Gulf coast of Florida. We propose this hypothesis as a useful predictive framework for future research on stopover physiology that may (1) reconcile previous equivocal studies, (2) uncover the hormonal mechanisms underlying extensive and rapid changes in behavior and physiology, (3) reveal limits on refueling rate (and therefore migration rate), and (4) improve assessments of habitat quality for migrating birds, based on their behavior and body condition at stopover sites. Understanding how CORT reflects physiology and behavior during the arrival, refueling, and departure phases of stopover allows for predictions of birds’ experiences during the previous flight, and its decisions regarding appropriate timing of departure from the stopover site.

1.1. Hypothesis development

Here we review studies examining baseline CORT in migratory birds upon arrival at a stopover site, during refueling, and prior to departure. Within each section we will cover information regarding body condition and CORT physiology. We then use these studies to inform our hypothesis on the role CORT plays in regulating stopover metabolic ecology.

1.1.1. After arrival

1.1.1.1. Body condition. Spring migrants, especially those that cross ecological barriers, often arrive at stopover sites in poor energetic condition, with decreased fat stores and lean mass. Relative to pre-flight individuals, fat stores can be significantly reduced in birds captured after flight (Moore and Kerlinger, 1987; Battley et al., 2000; Bauchinger and Biebach, 2001). For instance, Bar-tailed Godwits (Limosa lapponica) captured at a stopover site had lost an estimated 55% of their body mass during flight (Landys-Cianelli et al., 2002). Great Knots (Calidris tenuirostris) displayed an 85% reduction in fat mass, and about 20–30% reductions in pectoralis muscles, intestines, kidneys, and livers (Battley et al., 2000). However, headwinds or weather events may induce birds with sufficient fat stores to land, such that some arriving birds may not have depleted fuel stores (Moore and Kerlinger, 1987). Additionally, short-hop migrants may not deplete fuel stores and arrive in better condition (Schmaljohann and Eikenaar, 2017).

1.1.1.2. Corticosterone. Baseline CORT has repeatedly been shown to be negatively correlated with measures of body condition across taxa (Schwabl et al., 1991; Kitaysky et al., 1999; Moore et al., 2000; Romero and Wikelski, 2001), and this relationship has emerged in stopover physiology as well. In studies where birds were estimated to be captured within hours of arrival, CORT was negatively associated with body mass and fat stores (Jenni et al., 2000; Schwabl et al., 1991). However, Landys-Cianelli et al. (2002) did not observe a significant relationship between body condition and baseline CORT among Bar-tailed Godwits lured with playback to a false stopover site. Reneerkens et al. (2002) also failed to find a relationship between CORT and body condition in Bar-tailed Godwits that arrived at their breeding grounds but had not yet begun to breed. In this study, however, arrival time is imprecisely measured and includes birds that had been on the breeding grounds for up to 8 days. Importantly, these studies examine variation in baseline levels of CORT. Migratory flight is not stressful in and of itself: the baseline samples of CORT in migrating Garden Warblers (Sylvia borin) and European Robins (Turdus migratorius) only approached stress-induced CORT levels in individuals with severely depleted energy stores (Gwinner et al., 1992; Falsone and Jenni-Eiermann, 2009). Furthermore, captured migrants are able to mount a clear stress response, which is not possible if they are already in a stressed state (Schwabl et al., 1991; Romero et al., 1997; Landys-Cianelli et al., 2002).

1.1.1.3. Predictions for corticosterone and body condition. Given that birds often arrive in poor body condition and begin refueling on arrival, we predict that CORT levels will be elevated reflecting poor energetic condition, and decline as condition improves. In individuals in extremely poor energetic condition, CORT levels will be indicative of a mounted stress response, but in general we expect to observe only variation in baseline levels of CORT.

1.1.2. Refueling

1.1.2.1. Body condition. Birds often spend the stopover phase foraging, rebuilding tissues catabolized during flight, and depositing energy stores for the next leg of their journey. The body mass of Bar-tailed Godwits captured at a spring stopover site increased linearly over the entire stopover period (Piersma and Jukema, 1990). Blackcaps (Sylvia atricapilla) gain mass at stopover, but only after a 3–4 day period of low mass gain (Gannes, 2002). Carpenter et al. (1993) observed a pattern of bi-phasic mass gain characterized by an initial slow phase where lean mass was rebuilt followed by a rapid acceleration of body mass gain, where fat was deposited in refueling Rufous Hummingbirds (Selasphorus rufus). However, Kuenzi et al. (1991) observed mass loss over the course of stopover among several species of migratory passerines, which may be indicative of poor stopover habitat and food availability.

1.1.2.2. Corticosterone. Moderate elevations in CORT may promote foraging behavior (Landys et al., 2006). For example, baseline CORT undergoes daily cyclic rhythms, with peaks occurring before feeding
times in rats and humans, even after the regular feeding schedule is shifted (Dallman et al., 2004). Adrenalectomized rats display reduced daily food intake that can be restored via low doses of CORT (Dallman, 1993). When treated with RU486, which inhibits the low-affinity glucocorticoid receptor, captive Red Knots (Calidris canutus islandica) lowered the rate at which they visited their food dishes (Landys et al., 2004a) and spring migratory White-crowned Sparrows (Zonotrichia leucophrys gambelii) decreased food intake (Landys et al., 2004b). Compared to control birds, CORT-implanted captive White-crowned Sparrows showed prolonged foraging activity after a 3-day fast (Astheimer et al., 1992). The authors suggest CORT may help induce intense foraging behavior after periods of food deprivation, which may be relevant to migratory birds transitioning from extended flight to stopover. Additionally, captive Red-eyed Vireos (Vireo olivaceus) fed CORT-injected mealworms increased the rate at which they visited their food dishes (Löhmu, et al., 2006).

Other experimental manipulations of CORT, however, have not increased foraging behavior. For instance, captive Dark-eyed Juncos (Junco hyemalis) given CORT implants increased fat storage and atrophied flight muscles, but showed no difference in foraging behavior or food consumption (Gray et al., 1990). After experiencing a captive fasting-refeeding simulation of migratory stopover, Northern Wheatears (Oenanthe oenanthe) fed CORT-injected mealworms showed no difference in food intake compared to controls (Eikenaar, 2017).

It is difficult to know whether CORT manipulations appropriately simulate the relevant endogenous hormone levels experienced in the wild. Thus, some studies have taken a more correlorative approach to examining the role of endogenous CORT levels on food intake. Eikenaar et al. (2013) found baseline CORT was negatively associated with fuel deposition rate in Northern Wheatears captured at a migratory stopover site. Similarly, Northern Wheatears experiencing a fasting-refeeding protocol displayed a negative relationship between baseline CORT and food intake while refueling (Eikenaar et al., 2014a, 2014b). Finally, in Red Knots held in captivity throughout the spring migratory period, baseline CORT was another associated with increases in body mass in early spring nor with mass decreases at the end of the migratory phase (Piersma et al., 2000).

In the field, where food intake of individual animals is difficult to record, plasma triglycerides (TRIG) have been shown to accurately represent refueling rate (Guglielmo et al., 2005). As triglycerides are the storage form of lipids, an increase in circulating TRIG predicts body mass gain (Jenni-Eiermann and Jenni, 1994), and TRIG has been observed to increase over the course of stopover (Landys et al., 2005). Liu and Swanson (2014) observed a negative relationship between baseline CORT and TRIG and suggest that high quality habitats with sufficient resources for refueling may lower baseline CORT levels. In a study of partially migratory European Blackbirds (Turdus merula), Fudicar et al. (2013) observed greater fat scores and a trend towards higher baseline CORT levels in migrants compared to sedentary individuals, with no corresponding increase in TRIG.

The studies discussed here relating CORT to refueling behavior appear to have contradictory conclusions, with CORT being either positively or negatively related to foraging rate and behavior. One possible reason for these apparent contradictions is the feedback inherent in these relationships: variation in CORT levels can both cause and respond to foraging behavior. In birds in poor energetic condition, CORT may induce feeding behavior and fat deposition (positive relationship), but as these animals’ body condition improves with increased fuel storage, CORT levels may decline in response (negative relationship).

1.1.2.3. Predictions for cortisol and body mass. The studies discussed here relating CORT and body condition suggest that high quality habitats with sufficient resources for refueling may lower baseline CORT levels. In a study of partially migratory European Blackbirds (Turdus merula), Fudicar et al. (2013) observed greater fat scores and a trend towards higher baseline CORT levels in migrants compared to sedentary individuals, with no corresponding increase in TRIG.

1.1.2.3. Predictions for corticosterone, body condition, and refueling rate. In general, we expect body mass to increase over the course of stopover, with possible delays due to significant in-flight reductions in lean mass. TRIG is expected to increase as birds refuel during stopover, corresponding with increases in body mass. Elevated CORT levels early in the stopover phase may promote refueling, due to both changes in behavior—increasing feeding rate—but also because CORT plays a role in energy mobilization and deposition and during anabolic processes may aid in the deposition of fat. CORT levels may then decline as birds deposit fat and increase body mass and condition.

1.1.3. Before departure

1.1.3.1. Body condition. As discussed above, birds typically increase body mass, fat mass, and lean mass during stopover. Birds are expected to depart their stopover site once they' have acquired sufficient fuel stores for the next leg of their journey (Alerstam, 1991), though departure fuel load also depends on the site-specific fat deposition rate (Landström and Alerstam, 1992). Thus departing birds are often in better body condition than arriving or refueling birds (Biebach et al., 1986; Moore and Kerlinger, 1987; Goymann et al., 2010).

1.1.3.2. Corticosterone. CORT is likely involved in the regulation of migratory departure because glucocorticoids are known to regulate locomotor activity (Breuner et al., 1998; Cash and Holberton, 1999) and elevations in baseline CORT support the increased metabolic demands of life history stage transitions (Landys et al., 2006; Wada, 2008). For instance, nesting baseline CORT levels begin to rise at least a few days before fledging (Heath, 1997; Schwabl, 1999; Sockman and Schwabl, 2001; Quillfeldt et al., 2007; Sprague and Breuner, 2010) and baseline CORT is elevated during periods of increased locomotor activity post-fledging (Belthoff and Dufty, 1998). These elevated levels are hypothesized to facilitate the increased locomotor and foraging activities of a bird outside its nest and/or changes in metabolism prior to fledging (Wada, 2008).

In an analogous fashion, CORT has been shown to increase in birds prior to migratory departure. Baseline CORT is positively associated with migratory restlessness (Löhmus et al., 2003; Landys et al., 2004b), which is nocturnal hopping activity of captive birds, indicative of readiness for flight (Eikenaar et al., 2014b). Landys-Cianelli et al. (2002) observed baseline CORT levels increased with size-corrected body mass among Bar-tailed Godwits, possibly in preparation for migratory flight. Northern Wheatears with higher baseline CORT levels were more likely to depart on a given night, and among departing wheatears, those with higher CORT departed earlier in the night (Eikenaar et al., 2017). Finally, baseline CORT in captive Red Knots was highest when spring body mass reached maximum levels, presumably when they were ready for migratory departure (Piersma et al., 2000).

Increases in baseline CORT levels may “prime” the stress response axis (Sapolsky et al., 2000), such that pre-departure CORT elevations prepare birds for the unpredictable environmental, social, and metabolic challenges of long-distance flights and the breeding grounds (Piersma et al., 2000). Thus elevated CORT prior to predictable life history changes, including the onset of migration, may represent a physiological adjustment to support the increased metabolic demands of flight, and not a reactive response to adverse conditions (McEwen and Wingfield, 2003; Landys et al., 2006; Romero et al., 2009).

1.1.3.3. Predictions for corticosterone and body condition. We thus expect CORT and body condition to be positively associated in birds just prior to departure from a stopover site: these birds have regained body mass catalyzed during flight and deposited energy stores for their next flight, and baseline CORT rises in preparation for migratory departure.

1.2. Hypothesis framework

Based on this review of the literature, we developed a hypothesis regarding the relationships among baseline CORT, body condition, and refueling rate of migratory birds at stopover. This Stopover-CORT hypothesis is depicted by Fig. 1.

We expect birds to improve their body condition and to refuel throughout the duration of stopover, increasing both their TRIG levels
and body condition over time. We expect baseline CORT to be elevated upon arrival—possibly but not necessarily to levels indicating a stress response—and to be negatively associated with body condition, such that birds arriving in poorer condition have higher baseline CORT levels. Additionally, we expect migrants that have completed long flights or crossed ecological barriers to show arrival physiology represented by the far left region of the x-axis in Fig. 1. Short-hop migrants should arrive in better condition (Schmaljohann and Eikenaar, 2017). Thus we expect the arrival physiology of birds arriving in better body condition to be represented by a region of the x-axis further to the right than that of birds arriving in poor condition (Fig. 1).

Elevated CORT may promote refueling in birds shortly after arrival, and baseline CORT is expected to decline as birds continue refueling and improve their energetic condition. Once birds have sufficiently refueled, we expect baseline CORT to increase prior to departure from stopover. This increase may begin a few days before departure, as it does in fledging birds (Sprague and Breuner, 2010), or just within an hour or two of departure (Eikenaar et al., 2020). Importantly, “departure” here means the onset of another migratory flight, not simply departure from the study site, because migrants often use a much broader stopover landscape before resuming migratory flight (Taylor et al., 2011).

For these reasons, we hypothesize inverse relationships between CORT and body condition upon arrival and prior to departure: CORT will be negatively associated with body condition among arriving and refueling birds and positively associated with body condition among birds ready to depart. These expected inverse relationships may be explained by different roles of CORT during different stopover phases. Among arriving birds, CORT levels are likely reactive to body condition: birds with low fuel stores are energetically challenged, which causes CORT elevations; among departing birds, CORT likely serves a preparatory role, inducing migratory behavior.

1.3. Case study

We conducted a case study to test the Stopover-CORT hypothesis by examining the relationships among CORT, body condition, and refueling rate in wild-caught songbirds during spring migratory stopover.

We captured Gray Catbirds (Dumetella carolinensis) on St. George Island, FL, a barrier island in the Gulf of Mexico. Billions of birds pass through the Gulf region each spring on migration (Horton et al., 2019) and St. George Island can provide first landfall for trans-Gulf neotropical migrants (Lester et al., 2016), increasing the likelihood that captured birds are migratory and have just completed a long-distance flight. We analyzed the relationships among baseline CORT, body condition precisely measured using Quantitative Magnetic Resonance (QMR), and TRIG to examine the role of CORT in modulating migratory behavior.

A major inherent difficulty in studies of stopover ecology is producing accurate estimates of stopover duration (i.e. arrival and departure time) for individuals. To account for this issue, some studies have observed arrivals (Jenni et al., 2000), lured birds out of flight with playback or decoys (Landys-Cianelli et al., 2002), or flew trained, captive animals (Haase et al., 1986). Estimations of arrival time can be made based on known site- and species-specific temporal patterns of migratory flight (as in Schwabl et al., 1991; Paxton and Moore, 2017). Additionally, advances in radio telemetry have allowed for accurate recordings of departure time (as in Deppe et al., 2015; Eikenaar et al., 2017). Finally, analysis of stable hydrogen isotopes is a valuable tool to estimate the breeding latitudes and migration destinations of individuals in the absence of bands or transmitters (Hobson and Wassenaar, 1997). The catbirds captured on St. George Island may have completed a trans-gulf flight and used the island as a stopover site before heading to breeding grounds further north. However, St. George Island may not have been a stopover site for catbirds that over-wintered or planned to breed in the area. In this study we use knowledge of typical patterns of migrant body composition as well as feather stable-hydrogen (8D) isotope values to determine which of the Gray Catbirds we captured most likely used St. George Island as a stopover site.

2. Materials and methods

2.1. Study site

St. George Island is a barrier island approximately 8 km from the mainland coast of the Florida panhandle in the Gulf of Mexico (29.6678, −84.8656). This island often provides the first landfall for trans-Gulf neotropical migrants in spring (Lester et al., 2016). Forty-five km long, St. George Island is primarily residential, though the eastern 14 km are protected from development as the Dr. Julian G. Bruce St. George Island State Park. Fragmented lots along 2 km of the bayshore and adjacent to our field site are protected by the Apalachicola National Estuarine Research Reserve (ANERR). Vegetation includes slash pine (Pinus elliottii), live oak (Quercus virginiana), yaupon (Ilex vomitoria), and palmetto (Sabal minor).

2.2. Study species

Gray Catbirds winter throughout Mexico, Panama, and the Caribbean, and occur sparingly in northwest Colombia. Although Gray Catbirds also winter throughout southern Florida, the Central American migrants often use the barrier islands off the Florida coast as their first stopover after crossing the Gulf of Mexico. From there, catbirds continue on to breeding grounds in the southeast, northeast, and central regions of the United States, extending to parts of British Columbia and eastern Washington (Smith et al., 2011).

2.3. Capture and sampling

Gray Catbirds were captured using standard passive mist netting during spring 2017. From 01 April 2017 to 12 May 2017 as many as 16.5 standard size mist nets were opened between 0650–1300 and 1750–2000 daily. Each captured bird received a USGS aluminum leg band following standard banding protocols. We recorded morphometric measurements and body mass for all birds and age was determined according to Pyle (1997) when possible. In addition, each bird was
scanned in a Quantitative Magnetic Resonance (QMR) body composition analyzer, which accurately and non-invasively determines grams of fat and lean mass in a 90–120 s scan (Guglielmo et al., 2005). We collected one tail feather (R3) from each captured bird for stable isotope analysis of deuterium (δD). In all captured catbirds, we collected up to 200 μL of blood via brachial puncture using 27-gauge needles into heparinized capillary tubes. Blood samples taken within 3 min of capture (n = 25) were used to measure baseline CORT (Romero and Reed, 2005). Blood samples were kept on ice in the field and centrifuged later that same day. Plasma was stored in liquid nitrogen for the duration of the field season. Upon return to Massachusetts in May, plasma was stored in a −80 °C freezer until TRIG analysis was completed and remaining plasma was shipped on dry ice to the University of Montana for CORT analysis. All procedures were conducted under the approval of UMass IACUC (#2015-0019) and state and federal permits (USFWS: MB65968B-8; USGS: 23979; Florida: LSSC-16-00033).

2.4. Corticosterone assay

Plasma corticosterone levels were detected using an Enzyme Linked Immunoassay (ELISA) kit (Cat No. 25-0412, Enzo Life Sciences). We conducted parallelism validation assays on a pool of 6 ether-extracted Gray Catbird samples to ensure accurate CORT measurements throughout the range of observed values. Standard dilution of plasma was parallel to the standard curve across dilutions 1:7–1:280. We chose 1:20 and 1:25 to balance plasma volume with placement in the standard curve (data not shown).

CORT was extracted from plasma with a double ether extraction after addition of 2000 cpm of 3H-CORT for recovery; ether was evaporated off in a 50 °C water bath, and sample was reconstituted in 250–500 mL assay buffer for a final dilution of 1:20 or 1:25. Recoveries averaged 78.2 ± 2.0%; samples were corrected to 100% for analysis.

Assays were run as described in Wada et al. (2007). Briefly, 100 μL of extracted, diluted sample was assayed in triplicate if possible, and in duplicate when plasma volume was limiting. A standard curve (20,000 to 15.63 ng/100 μL) was included in triplicate on each of two plates, and an external CORT standard was also run in triplicate on each plate (100 μL/well). Color reaction was read at 405 nm corrected at 595 nm. Within- and inter-plate coefficients of variance were 11.4 and 6.3% respectively. All samples were within the detectability limit for each assay.

2.5. Triglyceride assay

Glycerol and total triglyceride were determined spectrophotometrically using kits and a slightly modified protocol (Sigma Aldrich F6428; Sigma Aldrich T2449) as in Guglielmo et al. (2005). Plasma samples were diluted three-fold with 0.9% NaCl and triglyceride assays were run in duplicate in clear flat-bottom 96-well microplates and read at 540 nm in a microplate spectrophotometer (BioTek Synergy H, VT, USA). Concentrations were determined by comparison to a standard curve as per the kit instructions as in Guglielmo et al. (2005). Within-individual CVs were all < 15%.

2.6. Stable isotopes

Feathers were stored at room temperature until processed. Feathers were washed of surface contaminants including lipids using 3–5 washes of chloroform:methanol 2:1, were allowed to air dry, and were stored in clean envelopes until analysis.

Approximately 0.2 mg of dried feather tissues was sealed in 3x5 mm silver capsules and subjected along with reference materials to benchtop equilibration to local water vapor δD for at least three weeks prior to analysis to account for exchangeable hydrogen (Wassenaar and Hobson, 2005; Bowen et al., 2005). δD values of the non-exchangeable portion of hydrogen were determined by comparative equilibration (Wassenaar and Hobson, 2003) using three internal laboratory keratin reference materials (δD: −55‰, −95‰, −175‰) in which non-exchangeable δD values were measured by comparative equilibration experiments followed by external verification with other laboratories.

δD values were determined using a Thermo-Finnigan high-temperature conversion elemental analyzer (TCEA) coupled to a Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM). Isotopic results are expressed as δ values, δD = 1000{[(Rsample − Rstandard)/Rstandard]}, where Rsample and Rstandard are the 2H/1H of the sample and standard, respectively. The internationally accepted standard for hydrogen is Vienna Standard Mean Ocean Water (V-SMOW) and the units are expressed as parts per thousand, or per mil (%). Precision for δD was determined by analysis of the three exchangeable (keratin) reference materials described above; within-run δD variation (SD) of these reference materials on the mass spectrometer system described above was ≤ 4‰.

2.7. Body composition

We scanned birds in the QMR 2 to 3 times and averaged fat and lean mass across scans. Morphometric data from all 25 birds were used to create a scaled body mass index (as in Peig and Green, 2009). Tarsus was significantly associated with body mass (F1,23 = 5.93, p = 0.023) and lean mass (F1,21 = 4.75, p = 0.04), so tarsus length was used to scale body and lean mass. Fat mass was not significantly associated with any measured morphometrics and thus was not scaled by body size.

2.8. Migratory status

Populations of Gray Catbirds can winter and breed near our field site. To appropriately address our hypothesis, we focused our analyses on birds that most likely used St. George Island as a stopover site en route to breeding grounds further north. We employed two methods to filter our dataset accordingly.

First, we used body composition data to infer birds that were not in post-flight migratory condition, and therefore likely did not complete a trans-Gulf flight. To do so, we plotted the relationship between fat mass (g) and lean mass residuals, calculated from the regression of lean mass against tarsus (Fig. 2). This allowed us to visualize the overall condition of birds upon capture. We identified five individuals with negative lean

Fig. 2. The relationship between fat mass (g) and the residuals of lean mass (g) against tarsus length (n = 21). Five “atypical migrants,” shown in red, are identified with low lean mass residuals and substantial fat mass. These individuals were removed from the dataset. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
mass residuals but > 2.5 g of fat. These individuals are likely not trans-Gulf migrants because of their unusual body condition. Fat is the primary fuel for migratory flight (Jenni and Jenni-Eiermann, 1998), and although supplemental protein catabolism does occur during flight even while fat reserves are high (Gerson and Guglielmo, 2013), substantial protein catabolism is not expected until fat stores are low (Jenni et al., 2000). Furthermore, during stopover, lean mass is typically anabolized before fat deposition can occur (Carpenter et al., 1993; Karasov and Pinshow, 2000; Gannes, 2002). Thus, it is unlikely that long distance migratory birds at stopover would have relatively low lean mass while carrying substantial fat reserves. Therefore, these five individuals may have wintered on the island, close by, or on peninsular southern Florida and are likely not trans-Gulf migrants.

Next, we used stable hydrogen isotopes to remove catbirds that would likely breed near our study site. We collected tail feathers, which were grown on or near the individual’s breeding grounds the previous summer. δD values from these samples estimate the breeding latitude of individuals in the summer prior to being caught, and since the breeding site return rate of catbirds is about 25–30% (Darley et al., 1977; Ryder et al., 2011) birds will likely return to similar latitude. Using the assignR package (Ma and Bowen, 2019), we created a feather δD isoscape by first calibrating feather δD values of birds with known breeding locations to the environmental (precipitation) isoscape using data from Hobson et al. (2012). We then produced posterior probability densities

![Image](image.png)
to estimate the breeding location of each individual. qtlRaster allowed us to visualize the 10% of grid cells with the highest posterior probability for each individual. We eliminated from our dataset samples (n = 5) whose top 10% probability region included our field site, which were the same samples with ΔD values > −45 (Fig. 3.) Wintering ground location cannot be similarly estimated for Gray Catbirds, because the isotopic signatures of southern Florida, the Caribbean, and northern South America are difficult to distinguish isotopically.

By applying these two analytical filters to our dataset, we increased the likelihood that our data reflect birds at a stopover site following a trans-Gulf flight and prior to further travel to the breeding grounds.

2.9. Statistical analysis

Samples were removed from the dataset if there was not corresponding QMR data (n = 2) or ΔD data (n = 2). One bird was removed because its CORT level was >2 standard deviations above the mean. While all other samples were collected before 1300, this sample was collected after 1700. All statistical tests were performed using the mosaic package in RStudio (ver. 1.1.423) and data were visualized using the ggplot2 and gridExtra packages. We used generalized linear models with an alpha level of 0.05 to determine significance. The covariates initially included were Julian date of capture, time of day, age, and days since initial capture; only significant covariates remained in the final model, and these are noted with the results.

3. Results

The following results are from Gray Catbirds that used St. George Island as a stopover site (n = 10).

Eight CORT data points represent the first capture of an individual; two are from individuals captured 21 and 23 days after initial capture. We have repeated CORT measures for one individual, captured on days 0 and 21 of stopover. Of the 3 individuals with recaptures, none gained mass during their time at stopover (Fig. 4). On average, these birds lost 0.30 ± 0.14 g per day.

Baseline CORT reflected differences in body composition. The means, standard errors, and ranges of baseline CORT, plasma TRIG, and body composition are summarized in Table 1. CORT levels declined as scaled body mass increased (p = 0.001; β = −1.31; R² = 0.83; covariate: Julian date of capture; Fig. 5a). There was a non-significant negative association between CORT and fat mass (p = 0.08; β = −1.77; R² = 0.33; covariates: none; Fig. 5b). CORT significantly decreased with increasing scaled lean mass (p = 0.007; β = −1.46; R² = 0.80; covariates: time of day, age; Fig. 5c) and plasma TRIG (p = 0.051; β = −2.91; R² = 0.40; covariates: none; Fig. 5d). These data are also visualized in Fig. S1 in the Supporting Information, which includes the individuals excluded from these analyses based on body composition and ΔD signature.

Table 1

| Summary statistics of CORT, TRIG, and body composition (n = 10). |
|-----------------|------|------|--------|
|                 | Mean | SE   | Range  |
| CORT (ng/ml)    | 8.04 | 1.32 | 2.48–15.10 |
| TRIG (mmol/L)   | 2.23 | 0.29 | 0.79–3.65 |
| Body mass (g)   | 36.45| 0.94 | 31.38–39.81 |
| Fat mass (g)    | 1.67 | 0.43 | 0.43–4.27  |
| Lean mass (g)   | 29.33| 0.57 | 25.89–31.70 |

4. Discussion

Our study aims 1) to formalize the Stopover-CORT hypothesis regarding the relationships among body condition, TRIG, and CORT during migratory stopover and 2) to test this hypothesis in Gray Catbirds during spring migration. We found that baseline CORT predicts body condition. CORT levels decrease as scaled body mass, fat mass, and scaled lean mass increase. This result is consistent with the negative relationship between CORT and body mass seen in Bar-tailed Godwits (Ramenofsky et al., 1995; Landys-Cianelli et al., 2002) and several passerines (Jenni et al., 2000) at stopover. It has been proposed that baseline CORT is elevated during flight due to the metabolic demands of exercise (Ramenofsky et al., 1995; Piersma et al., 2000; Falsone and Jenni-Eiermann, 2009). It is important to note that catalolysis and fuel type are regulated by variations in baseline CORT. Migratory flight is not inherently stressful, and CORT levels may only reach stress-induced levels when the bird is emaciated, with depleted fat stores and flight muscles (Gwinner et al., 1992; Jenni et al., 2006; Falsone and Jenni-Eiermann, 2009).

Contrary to our predictions, we did not observe a U-shaped curve of baseline CORT in relation to body condition. There are three explanations for this observation. First, our modest sample size, limited to morning and early afternoon captures, may make it difficult to observe the expected U-shape, especially if increases in CORT occur only in the hour or two before departure (Eikenaar et al., 2020). Second, Gray Catbirds may not complete their entire stopover period on St. George Island. The birds we captured may only represent the “arrival” and “refueling” phases of stopover, supported by the observed negative relationship between CORT and body condition. In this case, Gray Catbirds may relocate to a different part of the island or to the mainland in search of more suitable habitat to continue refueling before entering the “departure” phase and beginning the next leg of their northward journey. This idea is supported by automated telemetry data from other migratory species at our study site during the same year. The median length of stay on St. George Island of Swainson’s Thrushes (Catharus ustulatus), Northern Waterthrushes (Parkesia noveboracensis) and Yellow-billed Cuckoos (Coccyzus americanus) in 2017 was 14.68 h, while the median stopover duration of these same birds within a stationary telemetry tower array up to 100 km north of the banding site was 107.5 h (M. Gutierrez Ramirez, unpublished data). Taylor et al. (2011) similarly observed such stopover relocations, rather than true departures, across diverse species and taxa during spring and fall migration. Furthermore, St. George Island is impacted by residential development, though the eastern end of the island, about 5 km from our banding site, is protected as the Dr. Julian G. Bruce St. George Island State Park. The mainland, about 8 km away, provides a greater area and diversity of refueling habitat.
Third, we may not have observed the predicted U-shaped curve because Gray Catbirds do not increase baseline CORT prior to departure. We think this is an unlikely possibility. During autumn migration, Holberton et al. (1996) found that catbirds in “migratory condition” (i.e., post-molt and with sufficient fat stores) had higher baseline CORT than those in “pre-migratory condition” (i.e., undergoing molt with little stored fat). Baseline CORT has been found to increase with fat scores or body mass among migratory European Blackbirds (Fudickar et al., 2013), Dark-eyed Juncos (Holberton et al., 2008), Bar-tailed Godwits (Landys-Cianelli et al., 2002), and Northern Wheatears (Eikenaar et al., 2017). In these studies, CORT was proposed to increase prior to departure to help regulate metabolism during flight and/or prepare individuals for unpredictable environmental conditions. It is possible that such an increase in CORT is unnecessary in catbirds completing short-hop flights over stopover areas on their way from St. George Island to their breeding grounds, but we do not know the nature of their flight patterns over the United States (Ryder et al., 2011).

We also observed a nearly significant negative relationship between CORT and refueling rate, as measured by plasma TRIG. The literature provides unclear data on the relationship between CORT and refueling. In this study, we show that CORT tends to be lower in birds with higher TRIG. This result is consistent with our predictions for birds in the earlier stages of stopover: as they refuel and improve their body condition, CORT levels decline.

Surprisingly, we found no mass gain between first and last capture of recaptured catbirds. Other studies have observed birds’ failure to refuel at stopover sites because of physiological limitations due to protein catabolism during flight (Klaassen and Biebach, 1994), territoriality at the stopover site (Rappole and Warner, 1976), or insufficient habitat for refueling (Kuenzi et al., 1991). As noted above, it is possible that St. George Island does not provide sufficient refueling habitat for migratory catbirds. However, the TRIG values of birds in this study are comparable to those of birds at other stopover sites (Guglielmo et al., 2005; Landys et al., 2005; Lyons et al., 2008).

It may be possible to estimate a bird’s stopover phase (e.g., arriving, refueling, or departing) by examining the relationship between CORT and body condition and TRIG, in concordance with our hypothetical framework (Fig. 1). These results can be enhanced and potentially validated in future studies with the use of rapidly advancing tracking technology (McKinnon and Love, 2018). While arrival times are currently best approximated by observing arrivals (Jenni et al., 2000), luring birds out of flight (Landys-Cianelli et al., 2002), or capturing birds in flight (Jenni-Eiermann and Jenni, 1992; Falsone and Jenni-Eiermann, 2009), departure time can be precisely recorded using tracking devices (as in Deppe et al., 2015; Eikenaar et al., 2017). The advancement of efforts on minimization of radio tags will allow greater capacity to investigate stopover duration and habitat use in songbirds, augmenting the data presented here and further developing the Stopover-CORT hypothesis.

Given the difficulty of determining the time since arrival of migratory birds in most systems, and the seemingly contradictory relationships between CORT and body condition that we have reviewed in this manuscript, we believe that our proposed hypothesis provides a framework consistent with the available evidence, which will allow more accurate interpretation and insight into the role of CORT during stopover in migratory birds. Although our case study identified strong evidence in support of the relationship between body condition and CORT during the arrival and refueling period, the short stopovers typical of many songbirds on the barrier island system in the Gulf of Mexico, and the possibility that the island habitat quality is too low, or

Fig. 5. Baseline CORT decreases as (a) scaled body mass, (b) fat mass, (c) scaled lean mass, and (d) plasma TRIG increase (n = 10). The dashed regression line in (b) represents a near significant trend.
competition among species too high, prevented a direct test of the relationship between CORT and departure on a migratory flight. Nonetheless, without regional automated telemetry, departures from the island would be interpreted as migratory departures, whereas in reality these were likely large-scale regional movements in search of better stopover habitat (Mills et al., 2011; Taylor et al., 2011), and this interpretation is supported by our proposed hypothesis.

In conclusion, we present the Stopover-CORT hypothesis as a predictive framework for future studies of stopover physiology and behavior. Examining the relationships between CORT, body condition, and TRIG may indicate which phase of stopover a bird is in. Deviations from the hypothesized pattern, as seen in our Gray Catbird case study, could allude to the stopover movement patterns of migratory birds and to the limitations of poor habitat quality or extensive competition that are worthy of future investigation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2020.104776.

Data availability

Data are available through the Dryad Digital Repository: https://doi.org/10.5061/dryad.41ns1rnbh.

CRedit authorship contribution statement


Declaration of competing interest

The authors have no competing interests.

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References

**Figure S1.** Relationships between baseline CORT and (a) scaled body mass, (b) fat mass, (c) scaled lean mass, and (d) plasma TRIG. Solid circles represent individuals that most likely breed at a latitude north of our field site (based on feather δD values and probability densities); open triangles are birds that may breed near our field site. Red shapes represent those individuals identified as “atypical” migrants by body composition; black shapes represent birds with “typical” migrant body composition. Regression lines are the same as those in Figure 5, fitted to the solid black circles.
Stopover physiology and behavior across multiple spatial scales in two passerines during fall migration

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ABSTRACT

Avian migration involves extensive phenotypic flexibility during alternate phases of flight and stopover. Corticosterone (CORT) is a metabolic hormone that underlies feeding behavior and activity and thus is implicated in regulating refueling at and departure from stopover sites. In this study, we test the predictions of the Stopover-CORT hypothesis by examining the relationships among CORT, body mass, refueling rate (estimated via plasma triglycerides (TRIG)), and activity over the course of fall migratory stopover in two passerine species. We also test whether these physiological parameters predict different spatial scales of movement. During August – September 2019 and 2020, we captured Gray Catbirds (Dumetella carolinensis) and Swainson’s Thrushes (Catharus ustulatus) in Florence, Montana, U.S. We collected physiological data at capture, and continuously monitored activity, departure, and migration speed using automated telemetry Motus stations. Contrary to our predictions, body condition and refueling rate did not predict departure, but CORT levels were higher in Swainson’s Thrushes in the days leading up to departure from the broader stopover landscape. Before departure, Swainson’s Thrush activity levels decreased, and Gray Catbird activity levels increased, with leaner catbirds more active than heavier ones. None of our physiological metrics predicted movement at local or continental scales (i.e. field site activity patterns and migration speed). We discuss these results in the context of optimal migration theory: fall movements may represent an energy-minimization strategy, in contrast to the time-minimization often observed in the spring, explaining the lack of correlation between energetic condition and departure. This study contributes to our understanding of context-dependent variation in migratory physiology.
INTRODUCTION

Seasonal patterns of resources underlie the long-distance movements of billions of birds every year (Lack, 1968; Alerstam et al., 2003; Dingle and Drake, 2007). Due to the relative predictability of these energetically expensive movements, birds can anticipate and therefore prepare for migration. Extensive physiological preparations include fat storage (Guglielmo, 2018) facilitated by hyperphagia (King and Farner, 1965) and increases in lean mass (Marsh, 1984). While fat is the primary fuel for migratory flight (Jenni and Jenni-Eiermann, 1998; Guglielmo, 2018), birds also catabolize lean tissue during flight (Gerson and Guglielmo, 2011), including the digestive tract, kidneys, and flight muscles themselves (Piersma and Gill, 1998; Battley et al., 2000; Bauchinger et al., 2005). Birds spend the majority of their migration period at stopover sites (Hedenström and Alerstam, 1997) that allow birds to refuel between flight bouts or avoid inclement flight conditions (Mehlman et al., 2005; Schmaljohann et al., 2022). Thus birds undergo dramatic changes in physiology and behavior as they alternate between phases of fuel catabolism (flight) and fuel accumulation (stopover).

Migration involves an incredible degree of phenotypic flexibility in response to environmental cues (including photoperiod, local food availability, and weather conditions) and internal information (including the individual’s endogenous time program and body condition), which together inform stopover duration and migratory departure (Jenni and Schaub, 2003). Because migratory birds must fine-tune their behavior and physiology in response to a suite of external and internal cues, researchers have sought to identify the hormonal mechanisms underlying migratory behavior (Cornelius et al., 2013). One hormone in particular, corticosterone (CORT), has long been considered to play an important role in coordinating the migratory phenotype (Ramenofsky et al., 2012; Cornelius et al., 2013). Across taxa and contexts, CORT stimulates increased feeding behavior (Dallman et al., 2004; Landys et al., 2004a; Löhmus et al., 2006) and locomotor activity (Breuner et al., 1998; Cash and Holberton, 1999). Increases in baseline levels of CORT predict life history transitions (Wada, 2008), like those of birth or hatching (Challis, 2000; McLean and Smith, 2001) and fledging and dispersal (Heath, 1997; Belthoff and Dufty, Jr, 1998). Because CORT relates to feeding behavior, activity patterns, and predictable energetic demands, it is a prime candidate in studies of migratory physiology. Indeed, multiple studies have investigated CORT’s influence on stopover refueling (Löhmus et al., 2006; Eikenaar et al., 2013; Liu and Swanson, 2014; DeSimone et al., 2020), migratory
restlessness (Löhmus et al., 2003; Landys et al., 2004b), and migratory departure (Eikenaar et al., 2017, 2020). Studies of migratory endocrinology, however, often examine one phase at a time (i.e. flight, fueling, or departure), and we lack a comprehensive understanding of CORT patterns throughout migration and stopover (DeSimone et al., 2020; Bauer and Watts, 2021).

DeSimone et al. (2020) synthesized our knowledge to date with the Stopover-CORT hypothesis. Overall, the hypothesis predicts a U-shaped curve of CORT over the entire duration of stopover, during which fueling rates and body condition increase. Briefly, CORT is high upon arrival to a stopover site because body condition is typically poor among arriving birds (Moore and Kerlinger, 1987; Piersma and Gill, 1998; Battley et al., 2000) and CORT is elevated in fasted animals and animals in poor energetic condition (Kitaysky et al., 1999; Romero and Wikelski, 2001). As birds refuel, body condition improves, and CORT declines from the high levels present upon arrival. Finally, CORT rises prior to departure, among birds in good energetic condition, facilitating increased activity, energy mobilization, and ultimately migratory flight (Sapolsky et al., 2000; Landys et al., 2006; Eikenaar et al. 2017; Bauer and Watts, 2021).

Though not included in the Stopover-CORT hypothesis, activity patterns are also expected to correlate with body condition and CORT over the course of stopover. Daily activity patterns at stopover often reflect foraging intensity and habitat quality, with birds in poorer condition moving more as they replenish their fuel stores, and birds in better condition moving less to conserve fat stores and reduce predation risk (Yong and Moore, 1993; Cimprich and Moore, 2006; Ktitorov et al., 2010; Matthews and Rodewald, 2010; Cohen et al., 2012). Additionally, daily activity patterns relate to departure timing, with birds often displaying a quiescent period preceding nocturnal departure (Ramenofsky et al., 2008; Schofield et al., 2018a; Morbey et al., 2020). Finally, we expect CORT to relate to daily activity patterns, because in multiple taxa and contexts, CORT predicts locomotor activity (Breuner et al., 1998; Cash and Holberton, 1999; Breuner and Hahn, 2003; Jessop et al., 2018).

The Stopover-CORT hypothesis is useful in broadly considering physiological changes over stopover, and helps reconcile contrasting reports of CORT’s relationship to refueling (Bauer and Watts, 2021) by describing how this relationship is expected to differ depending on whether the bird is newly arrived, refueling, or departing soon. Deviations from the predictions of the Stopover-CORT hypothesis can also be informative. For instance, while many studies show that energetic condition increases over the course of stopover and predicts departure (Moore and
Kerlinger, 1987; Schmaljohann et al., 2013; Cohen et al., 2014; Deppe et al., 2015), other studies have not found a relationship between fuel stores and departure probability (Kuenzi et al., 1991; Salewski and Schaub, 2007; Tsvey et al., 2007). As examples, birds may not gain mass at a stopover site if they landed there due to inclement in-flight weather rather than depleted fuel stores (Moore and Kerlinger, 1987) or if the stopover habitat does not provide adequate fueling opportunities (Kuenzi et al., 1991).

Optimal migration theory can also help explain variation in stopover physiology and deviations from Stopover-CORT predictions (Schmaljohann et al., 2022). Optimal migration theory (Alerstam, 1991; Hedenström and Alerstam, 1997; Alerstam, 2011) predicts that birds either minimize the time spent migrating by adjusting their departure fuel load according to stopover fuel deposition rate, or they minimize the energy costs of migration by departing with a fuel load unrelated to fuel deposition rate (Hedenström and Alerstam, 1997). The selective pressure acting on migration speed in the spring may reward a time-minimization strategy; the higher reproductive success associated with faster migrations and earlier arrivals likely outweigh the energetic costs of time-minimization (McNamara et al., 1998; Schmaljohann et al., 2022). On the other hand, fall migration may tip the balance in favor of a more risk-averse, energy-minimizing strategy (Schmaljohann et al., 2022). Indeed, spring migrations are often faster than fall migrations (Fransson, 1995; La Sorte et al., 2013; McKinnon et al., 2013; Nilsson et al., 2013; Schmaljohann, 2018). Optimal fuel loads at departure are predicted to be higher under a time-minimization strategy compared to an energy-minimization strategy (Hedenström and Alerstam, 1997). The predictions of the Stopover-CORT hypothesis may be most applicable to time-minimized spring migrations, with body mass and fuel deposition rate predicting departure probability, compared to fall movements.

In considering the effect of physiology on migratory movements, it is important to think about different spatial scales of behavior. As previously discussed, body condition, CORT, and refueling rate may all affect daily activity patterns (e.g. movements within a stopover site) and, as the Stopover-CORT hypothesis describes, departure from a stopover site. However, defining the spatial scale of “stopover” has often been limited by field methodology, with stopover duration depending on recapture or resighting of individuals (e.g. Moore et al., 2017) and thus constrained to the study’s field site and limited by recapture/resighting effort. Advances in tracking technology have revealed stopover areas may be much larger than assumed (Taylor et
al., 2011; Smetzer and King, 2018; Gutierrez et al. in review) such that departure from a field site may represent a stopover relocation rather than a true migratory departure. Finally, at an even broader spatial scale, we can examine whether physiological metrics influence migration speed: the time it takes a bird to reach its destination, divided by the total distance traveled. Migration speed has direct fitness consequences because, if it relates to arrival date, it can predict greater reproductive success in the spring (Kokko, 1999; Morbey and Ydenberg, 2001) or high-quality territory acquisition on the non-breeding grounds (Studds and Marra, 2005). If an individual’s ability to rapidly refuel at one stopover site predicts refueling at subsequent stopover sites, physiological metrics at capture could predict overall migration speed, given that flight speeds are less variable (Nilsson et al., 2013).

In addition to examining the relationships among body condition, refueling rate, CORT, and activity during stopover, this study also seeks to identify how physiological metrics may predict varying scales of activity (that is, stopover activity patterns, departure from the local stopover site, departure from the broader stopover landscape, and migration speed). We captured Gray Catbirds (*Dumetella carolinensis*) and Swainson’s Thrushes (*Catharus ustulatus*) during fall migration (August – September 2019-2020) on MPG Ranch in Florence, MT, at the northern end of the Bitterroot Valley. We examined CORT’s relationship with fuelling (plasma triglycerides and body condition) and departure to test the Stopover-CORT hypothesis in the broader context of optimal migration theory. Furthermore, using an array of automated Motus telemetry stations (Taylor et al., 2017), we examined the influence of physiology at the time of capture on four scales of subsequent movement behavior: 1) daily activity patterns at the field site, 2) departure from the local field site (MPG Ranch), 3) departure from the larger stopover landscape (the Bitterroot Valley), and 4) estimated migration speed among individuals detected out-of-state. This study contributes to our understanding of how physiological metrics relate to stopover and migratory behavior during fall migration.

**METHODS**

**Study site**

We conducted fieldwork on MPG Ranch in Florence, MT (46.669, -114.026; Figure 1) from 5 August - 26 September 2019 and 4 August - 25 September 2020. We captured birds using standard mist nets, which we continuously monitored to collect blood samples within 3 min of
the bird hitting the net. In both years, we set up mist nets from morning civil twilight until 1000 or 1100. In 2020, we also attempted evening captures, setting up nets for the last 90 min of daylight about three times a week.

**Blood sampling and morphometrics**

Up to 125 µL of blood was collected from the brachial vein using 26G needles. Blood samples were kept on ice packs in the field, and were centrifuged in the lab within 6 hrs to separate plasma from red blood cells. Plasma was aliquoted: plasma used for CORT analysis was stored at -20°C and plasma for TRIG analysis was stored at -60°C.

We visually scored fat stores on a scale from 0-5 (Moore and Kerlinger, 1987) and pectoralis muscle on a scale from 0-3 (Bairlein, 1995). We measured various morphometrics and weighed each bird to the nearest 0.1 g. We scaled body mass to body size as in Peig and Green (2009). Gray Catbird body mass was scaled with tarsus length ($F_{1,25} = 8.03; p = 0.009$); Swainson’s Thrush body mass was scaled with the length of the bill from the nares to tip ($F_{1,18} = 9.26; p = 0.007$). We calculated the z-score of the scaled mass for each species in order to make easier visual comparisons between species in the same figures.

**Radiotransmitters**

Birds were aged as HY or AHY using plumage characteristics as in Pyle (1997). To increase the probability of survival during migration, we attached transmitters to only AHY birds in 2019, and mostly AHY birds in 2020. 27 (2019: n = 14; 2020: n = 13) Gray Catbirds and 22 (2019: n = 13; 2020: n = 9) Swainson’s Thrushes were fitted with nano-tag transmitters (Lotek NQB2-3-2, <3% body mass) using a standard leg-loop harness (Rappole and Tipton, 1991) and 0.7 mm Stretch Magic® jewelry cord. Tag detections were continuously recorded by an array of automated telemetry Motus stations (Figure 1) on MPG Ranch (2019: n = 6; 2020: n = 5) and throughout the Bitterroot Valley to the south (2019: n = 4; 2020: n = 3). We selected capture sites within eyesight of a Motus station to help ensure tag detections. Daily handheld telemetry monitoring on MPG Ranch confirmed departure day. Two Swainson’s Thrushes dropped their tags near their capture site, and were excluded from movement analyses. Among the remaining individuals, all but one Swainson’s Thrush were detected by at least one Motus station. Detection
data were cleaned and filtered following Crewe et al. (2019): we removed false positive
detections (those with run length < 3 from all sites, and < 5 for “noisy” sites).

*Triglyceride analysis*

Glycerol and total triglyceride concentrations were determined spectrophotometrically
using kits from Sigma Aldrich (Sigma Aldrich F64 28; Sigma Aldrich T2449; Sigma Aldrich
G7793). Samples were thawed on ice, diluted three-fold with 0.9% saline (Sigma Aldrich
S8776), and run in duplicate in clear round-bottomed 96-well microplates (Falcon 353910) with
a glycerol standard curve (2.82 mmol/L to 0.17 mmol/L) and a blank (0.0 mmol/L). 5µL sample
(or standard) and 240µL glycerol reagent were added to each well, incubated for 10min at 37°C
and read at 540nm in a microplate spectrophotometer (ThermoFisher Multiskan Ascent
51118407). Then 60µL triglyceride reagent was added to each well, and the plate was read again
at 540nm after 10min incubation at 37°C. All wells were corrected by blank wells, and
concentrations were determined by comparison to the standard curve. Within-individual CVs
averaged 9.75%.

*Corticosterone analysis*

Plasma corticosterone levels were determined using an Enzyme Linked Immunoassay
(ELISA) kit (Cat No. 25-0412, Enzo Life Sciences). 2,000 cpm 3H-CORT was added to each
sample to estimate recovery, and then CORT was extracted from plasma using a double ether
extraction. Ether was evaporated off in a 50°C water bath. Extracted samples reconstituted in
200-400 µL assay buffer for a final dilution of 1:20 or 1:25. Recoveries averaged 65.2% and
samples were corrected to 100% for analysis.

Assays were run as described in Patterson et al. (2011). 100 µL of extracted, diluted
sample was assayed in triplicate if possible, and in duplicate when plasma volume was limiting.
Each plate included a standard curve (20,000 to 15.63 ng/100µl) and an external CORT standard.
Color reaction was read at 405 nm corrected at 595 nm. Intra- and inter-plate coefficients of
variation averaged 10.6% and 11.5% respectively. All samples were within each assay’s
detectability limit.
**Departure time**

We calculated migratory departure time on two spatial scales: departure from MPG Ranch and departure from the Bitterroot Valley. Departure from MPG was defined as the last time an individual was detected by a Motus station on MPG Ranch (Figure 1), with the exception of one station on the ranch that had a long detection range to the south, and often detected birds simultaneously with the southern, off-ranch stations. Departure from the Bitterroot Valley was defined as the last time an individual was detected by any of our stations. Four birds were detected by handheld telemetry after their last MPG Motus station detection; we adjusted departure time of these individuals to be the last detection via handheld telemetry on MPG Ranch.

**Activity analysis**

We only analyzed the activity patterns of birds during the 2020 season: nano-tagged birds spent most of their time on MPG Ranch in the vicinity of our banding site, and in 2020, we positioned the Motus station adjacent to our banding site in a way that continuously detected these individuals. We quantified the proportion of detections in which an individual was active, as in Schofield et al. (2018a, 2018b). The nano-tags emit a burst with a given signal strength every 25 seconds, and we used changes in signal strength between consecutive bursts to infer activity.

First, we established a baseline of inactivity. We calculated the average change in signal of individuals between 0000 and 0400, when they are presumed to be inactive. Then, we examined changes in signal strength during the day. We only analyzed the signals during bouts of at least 10 min of consecutive detections. We considered any changes in signal strength greater than 2 SD above the baseline, nocturnal mean (1.22 dBM) to represent activity. We calculated the proportion of time spent active as the proportion of changes in signal strength > 1.22 dBM, divided by the total number of detections of each time period. We calculated the daily activity of individuals, as well as investigating different times of day: “morning” (the 3 hrs after sunrise), “midday” (the 3 hrs centered around midday, which was the midpoint between sunrise and sunset), and “evening” (the 3 hrs before sunset).

The magnitude of change in signal strength does not necessarily reflect movement distance. For instance, signal strength may increase because an individual makes a large
movement towards a Motus station, or because it makes a slight movement around vegetation or a geographic barrier that greatly improves signal strength. We therefore can only analyze activity in this categorical way (active or inactive) with no spatial detail about distance traveled.

Migration speed

We estimated the migration speed (distance/time) of birds that were detected by out-of-state Motus stations (n = 15). It is impossible to calculate a precise migration distance for each bird because we know nothing of their route between Motus stations, and only 7 individuals were detected by multiple out-of-state stations. We decided to standardize the migration speed calculation across individuals, regardless of how many Motus stations detected them: we calculated migration time as the difference between the last detection by a Montana Motus station, and the first detection on the last out-of-state Motus station. We coarsely estimated migration distance as the great-circle distance between these two stations using the R package geosphere (Hijmans, 2019). Dividing the estimated migration distance by the migration time produces migration speed. This represents the minimum migration speed, because migratory routes are not straight lines, and the distance these birds actually traveled is greater than what we estimated. See Supplementary Figure 1 for a map of these detections.

Statistical analyses

All analyses were done in RStudio (Version 1.3.1093) and figures were made using ggplot2 (Wickham, 2011). For all analyses, CORT and TRIG were log-transformed to satisfy normality assumptions. We excluded from analysis CORT from blood samples collected > 3min after capture (N = 7; Romero and Reed, 2005), and TRIG samples collected > 10 min after capture (N = 1). We constructed models for each species separately.

To test the relationships among physiological metrics, we constructed GLMs relating logTRIG to logCORT and scaled mass, and logCORT to scaled mass. Initial models included relevant bleed times, capture time of day, Julian day, and year, and only significant covariates were retained in the final model after stepwise removal. We used ANOVAs to test the relationships between fat and muscle scores with each physiological metric (logTRIG, logCORT, or scaled mass).
We investigated whether physiological metrics predicted activity during days 1 – 2 after capture. (We excluded day 0 (the day of capture) from these analyses because the capture event tended to reduce activity that day.) We excluded SWTH from these analyses because the sample size of SWTH for which we had activity data during this time frame was too small for any meaningful interpretation. We constructed GLMs to relate each physiological metric (logCORT, logTRIG, and scaled mass) to the proportion of time spent active for the time bins “morning,” “afternoon,” and “evening.”

Next we related logCORT, logTRIG, and scaled mass to departure at two different spatial scales: departure from MPG Ranch, and departure from the Bitterroot Valley. We constructed GLMs for each species relating each physiological metric to the difference in time between departure and capture. Initial models included the relevant bleed times, capture time of day, Julian day, and year as covariates, and only significant covariates remained in the final model following stepwise removal. In Figure 3, time to departure is displayed on the x-axis, which is inverted because it is intuitive to visualize time progressing from left to right. However, time to departure was a response variable in each of these models.

To test for differences in departure time between species, we first centered the time of day of departure around midnight so that departures in the early morning are considered to occur after departures in the evening. Then we conducted a two-tailed t-test to compare the mean departure times of each species.

Next, we related activity patterns to departure from MPG and the Bitterroot Valley. For each species, we constructed GLMMs (R packages lmerTest (Kuznetsova et al., 2017) and lme4 (Bates et al., 2015), Type III ANOVA with Satterthwaite’s method) to relate the proportion of time spent active to the difference in time between departure and capture, including Bird ID as a random effect. We initially included logCORT, logTRIG, and scaled mass as covariates to test whether these physiological metrics helped explain variation in activity levels, and only retained significant covariates in the final model. Just as in Figure 3, in Figure 4, time to departure is displayed on an inverted x-axis. In each of our models, however, time to departure is a response variable.

Finally, we investigated minimum migration speed. We conducted a two-tailed t-test to compare average migration speed between species. Then we constructed GLMs for each species, relating logCORT, logTRIG, and scaled mass to minimum migration speed.
RESULTS

Body condition

Average CORT, TRIG, and body mass for each species are reported in Table 1. Scaled mass did not predict logCORT of GRCA (\(F_{2,21} = 1.85, \beta = 0.04, p = 0.46\); covariate: CORT bleed time; Figure 2a) or SWTH (\(F_{2,15} = 1.89, \beta = -0.05, p = 0.51\), covariate: capture time of day; Figure 2a). For both species, logTRIG increased with scaled mass (GRCA: \(F_{3,22} = 12.15, \beta = 0.11, p = 0.02\), covariates: Julian Day, trig bleed time; SWTH: \(F_{4,16} = 7.68, \beta = 0.11, p = 0.01\), covariates: Julian Day, capture time of day, year; Figure 2b). logTRIG increased with logCORT in GRCA (\(F_{2,20} = 18.35, \beta = 0.51, p = 0.009\), covariate: trig bleed time; Figure 2c) but not in SWTH (\(F_{3,13} = 5.06, \beta = 0.23, p = 0.19\), covariates: capture time of day and year; Figure 2c).

The results of ANOVAs relating fat and muscle scores to logTRIG, logCORT, and scaled mass are summarized in Table 2. Fat scores predicted TRIG of both species (Table 2).

Physiology and activity

The following results are only for GRCA due to small sample sizes of SWTH activity patterns. We found no significant relationships between the physiological metrics (CORT, TRIG, and scaled mass) and activity on days 1-2 after capture during the morning (\(F < 0.62, p > 0.44\)), afternoon (\(F < 0.57, p > 0.46\)), or evening (\(F < 1.35, p > 0.27\)).

Physiology and local departure

No physiological metrics predicted MPG departure in either species. MPG departure was not predicted by logCORT (GRCA: \(F_{2,21} = 13.88, p = 0.08, \beta = -171.874\), covariate: Julian Day; SWTH: \(F_{1,13} = 0.77, p = 0.40, \beta = -113.90\)), logTRIG (GRCA: \(F_{2,23} = 12.75, p = 0.52, \beta = -50.36\), covariate: Julian Day; SWTH: \(F_{1,16} = 0.80, p = 0.39, \beta = -125.2\)), or scaled mass (GRCA: \(F_{2,24} = 11.62, \beta = -24.3, p = 0.28\), covariate: Julian Day; SWTH: \(F_{1,17} = 0.49, \beta = 22.8, p = 0.49\)).

Physiology and landscape departure

LogCORT predicted Bitterroot departure in SWTH (\(F_{3,12} = 5.00, \beta = -282.6, p = 0.01\), covariate: cort bleed time, year; Figure 3a) but not GRCA (\(F_{2,21} = 8.89, \beta = -136.01, p = 0.19\), covariate: Julian Day; Figure 3a). LogTRIG did not predict Bitterroot departure in either species (GRCA: \(F_{2,23} = 12.93, \beta = -84.2, p = 0.27\), covariate: Julian Day; SWTH: \(F_{1,17} = 3.10, \beta = -228.0\), covariate: Julian Day; Figure 3b).
Scaled mass tended to increase with approaching Bitterroot departure in GRCA (\(F_{2,24} = 12.88, \beta = -40.8, p = 0.06\), covariate: Julian Day; Figure 3c) but did not predict Bitterroot departure in SWTH (\(F_{1,18} = 2.67, \beta = 51.73, p = 0.12\); Figure 3c).

**Departure time**

There was no significant species difference in the time of day individuals were last detected by one of our Motus stations (\(t_{32.5} = -1.23, p = 0.23\), Supplementary Figure 2). GRCA departure time averaged 22:21; SWTH departure time averaged 23:36.

**Activity and departure**

GRCA daily activity increased as both MPG and Bitterroot departure approached (\(MPG: F_{1,72.67} = 3.99, \beta = -0.01, p = 0.0496\), covariate: scaled mass; \(Bitterroot: F_{1,75.25} = 4.04, \beta = -0.01, p = 0.048\), covariate: scaled mass; Figure 4a). Scaled mass helped explain variation in daily activity in these models: individuals with lower mass were more active than heavier individuals (Figure 4a). When looking at each time bin independently, we see that this increase in daily activity is driven by afternoon activity patterns. Afternoon activity increased as MPG departure approached (\(F_{1,47.81} = 5.13, \beta = -0.01, p = 0.03\); Figure 4b), but not as Bitterroot departure approached (\(F_{1,46.26} = 2.43, \beta = -0.01, p = 0.13\)). Changes in GRCA morning and evening activity did not predict MPG or Bitterroot departure (\(F < 2.83, p > 0.09\)).

SWTH daily activity did not change in the days leading up to MPG departure (\(F_{1,5.8} = 1.23, \beta = 0.004, p = 0.31\), no covariates), but tended to decrease as Bitterroot departure approached (\(F_{1,47} = 3.29, \beta = 0.08, p = 0.076\); Figure 4c). This overall pattern is driven by evening activity patterns, which decreased as both MPG departure (\(F_{1,21} = 6.07, \beta = 0.01, p = 0.02\)) and Bitterroot departure approached (\(F_{1,21} = 8.93, \beta = 0.02, p = 0.007\); Figure 4d). Morning and afternoon activity did not change in the days leading up to MPG or Bitterroot departure (\(F < 2.30, p > 0.13\)).

**Physiology and migration speed**

Estimated minimum migration speed was significantly greater in SWTH than GRCA (\(t_{7,13} = -2.42, p = 0.046\); GRCA mean: 88.38 km/day; SWTH mean: 157.97 km/day; 95% CI for
difference: [-137.43, -1.74]; Supplementary Figure 3). No physiological metric predicted migration speed for either species (F < 3.80, p > 0.10).

DISCUSSION

This study examines the relationships among corticosterone, body mass, and triglycerides to test hypotheses about how CORT underlies stopover refueling and migratory departure. We also investigate how these physiological metrics relate to multiple scales of movement behavior: daily activity, local departure, landscape departure, and migration speed. Understanding the mechanisms underlying movement ecology can clarify the external and internal factors shaping migratory behavior and the degree of its flexibility.

We expected our physiological metrics (body mass, TRIG, and CORT) to be positively correlated with each other in the days leading up to departure. We found that TRIG and mass were positively correlated in both species, with TRIG predicting fat score in both GRCA and SWTH. This result is not surprising as TRIG is known to predict mass gain in multiple species (Jenni-Eiermann and Jenni, 1994; Guglielmo et al., 2002, 2005). We also found that CORT and TRIG were positively correlated, but only in GRCA. The literature does not report consistent relationships between CORT and fuel stores or food intake (reviewed in Bauer and Watts, 2021), possibly because different relationships are expected for different phases of the entire stopover period (DeSimone et al., 2020). Here, these results support the idea that CORT facilitates feeding behavior and fat storage in GRCA (Landys et al., 2004a, 2006; Löhmus et al., 2006). Contrary to our predictions and the pattern commonly observed in the literature (Schwabl et al., 1991; Kitaysky et al., 1999; Moore et al., 2000; Romero and Wikelski, 2001), CORT was not related to body mass in either species.

Next, we examined whether physiological metrics predicted movement and activity at multiple spatial scales. At the finest scale, we expected birds with greater body mass and TRIG levels would be less active (Yong and Moore, 1993; Cimprich and Moore, 2006; Ktitorov et al., 2010; Matthews and Rodewald, 2010; Cohen et al., 2012), and CORT would positively correlate with activity (Breuner et al., 1998; Cash and Holberton, 1999; Breuner and Hahn, 2003). However, none of these physiological metrics related to daily activity patterns in the days following capture. Then, we expected birds in better energetic condition would be closer to departure, and that CORT would be elevated in these birds. However, none of our physiological
metrics predicted departure from the local field site (MPG Ranch). In terms of landscape departure from the Bitterroot Valley, CORT was higher in SWTH closer to departure, and GRCA with greater body mass tended to depart from the Bitterroot Valley sooner after capture. Surprisingly, TRIG did not correlate with departure from MPG Ranch or the Bitterroot Valley. There are three main implications of these results. First, we emphasize the differences between stopover on MPG Ranch and the Bitterroot Valley. Adopting a broader view of stopover land use may be critical to studies trying to relate physiology to movement. Our study corroborates others that have found that departure from the immediate field site does not necessarily indicate initiation of migratory flight, but could simply be a stopover relocation (Taylor et al., 2011; Smetzer and King, 2018; Gutierrez et al. in review), obscuring the predicted relationships between physiology and migratory departure. Second, we did not observe a relationship between body condition and departure. If the lack of correlation between departure and body condition were because the habitat was too poor for preparatory fueling, we would expect birds to depart quickly in search of better habitat (Gwinner et al., 1985; Alerstam and Lindström, 1990; Schaub et al., 2008), but our focal birds stayed on MPG Ranch for a relatively long time (up to 27 days after capture). The lack of relationship between body condition and departure supports the idea that fall migratory flights are more likely to be guided by an energy-minimizing strategy than a time-minimization one (Schmaljohann et al., 2022). Fall migratory GRCA and SWTH in Montana, flying over the continental United States without ecological barriers, may not deposit large fuel stores. Heavier birds face more energetically costly flights (Alerstam and Lindström, 1990) and increased predation risk (Witter and Cutthill, 1993). Thus these birds may instead undergo short-hop flights without excessive fuel storage to minimize flight costs and predation risk. Finally, the fact that CORT predicts SWTH departure despite no relationship between CORT and body condition is interesting. This result implies that CORT can still underlie migratory departure even when uncoupled from energetic condition and appetite-regulating processes that have previously been proposed to influence departure timing (Holberton and Dufly, Jr, 2005; Cornelius et al., 2013; Goymann et al., 2017).

While we found that SWTH migrate almost twice as fast as GRCA, we found no relationship between stopover physiology and migration speed. Because most of the migration time period is spent at stopover sites (Hedenström and Alerstam, 1997), it is possible that an individual’s refueling ability at one site predicts its stopover duration at subsequent sites, and
ultimately migration speed. That was not the case here, which may not be especially surprising, as not all stopover habitats are the same, such that refueling rate at one site does not predict refueling rate at another. Furthermore, we have rather small sample sizes in these analyses, and technological limitations of the Motus network preclude more precise estimates of migration speed. Migration speed is more accurately assessed with archival or satellite tags (McKinnon et al., 2013) or with a more extensive network of Motus stations (as in Duijns et al., 2019).

We found species-specific changes in activity leading up to departure. GRCA increased afternoon activity as departure approached, and lighter individuals were more active on average than heavier ones. Perhaps the increase in afternoon activity indicates increased foraging intensity in preparation for departure. Body condition has previously been shown to influence activity patterns at stopover, with leaner birds more active and foraging at greater intensities than fatter birds (Matthews and Rodewald, 2010; Cohen et al., 2012), at least in habitat where there is sufficient food available for profitable foraging (Graber and Graber, 1983; Schofield et al., 2018b). Birds with more stored fuel may be less active in order to conserve fat stores and reduce predation risk (Cimprich and Moore, 2006). On the other hand, SWTH decreased evening activity before departure. Perhaps a reduction in activity is an energy-conservation strategy, a result of having found optimal habitat after an exploratory period (Cohen et al., 2012), or indicative of a quiescent period before migratory restlessness (Schofield et al., 2018a). We don’t have enough information to conjecture why there are species differences in the relationship between activity and departure. We can, however, rule out an effect of differences in departure time, as there was no difference in departure time between species.

Overall, we find that CORT does not relate to body condition in either species, but does predict SWTH migratory departure. We observed species-specific changes in activity patterns leading up to departure from the Bitterroot Valley that, in GRCA, were partly explained by differences in body mass. Of the various spatial scales we examined, physiological metrics most often predicted landscape-level migratory departure. We thus encourage scientists to consider broader stopover landscapes in their study designs and analyses. Additionally, we argue that the mostly non-significant relationships between body condition and departure are informative in the context of optimal migration theory: fall migrations may be less governed by refueling rate and body condition than other supplementary information, such as predation risk, social information, and weather conditions, as these birds seek to minimize the energy costs of movement.
ACKNOWLEDGMENTS

Thanks to Sunny Domschot for assistance in the field. Alex Gerson provided helpful advice on the triglyceride assay protocol. Thanks to the Motus Wildlife Tracking collaborative network, enabling long-distance re-detections of birds tagged in this study. Funding for this project was provided by MPG Ranch and the University of Montana Bird Ecology Lab, and JGD was supported by an NSF GRFP and an award from the Drollinger-Dial Foundation.

REFERENCES


Figure 1. These maps show A) all active western Montana Motus stations at the time of this study and B) an inset of Motus stations on MPG Ranch. Yellow: stations that detected signals from “MPG Ranch”; Red: stations that detected signals from the “Bitterroot Valley”; Blue triangles: capture locations of birds in this study.
Table 1. Summary statistics (mean ± SE) of corticosterone, triglycerides, and body mass of Gray Catbirds (GRCA) and Swainson’s Thrushes (SWTH).

<table>
<thead>
<tr>
<th></th>
<th>CORT (ng/mL)</th>
<th>TRIG (mmol/L)</th>
<th>Scaled Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRCA</td>
<td>3.87 ± 0.41</td>
<td>1.82 ± 0.24</td>
<td>39.76 ± 0.58</td>
</tr>
<tr>
<td>SWTH</td>
<td>5.76 ± 0.95</td>
<td>2.30 ± 0.30</td>
<td>32.27 ± 0.83</td>
</tr>
</tbody>
</table>
Figure 2. The relationships among CORT, TRIG, and scaled mass for Gray Catbirds (GRCA) and Swainson’s Thrushes (SWTH). Scaled mass predicted logTRIG for both species; logCORT predicted logTRIG in GRCA only.
Table 2. Summary of ANOVAs relating visual fat and muscle scores to TRIG, CORT, and scaled mass of each species. Statistically significant p-values shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>GRCA</th>
<th>SWTH</th>
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<tbody>
<tr>
<td></td>
<td>F-statistic</td>
<td>p-value</td>
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<tr>
<td>logTRIG ~ Fat</td>
<td>$F_{(1,24)}$ = 20.33</td>
<td><strong>0.0001</strong></td>
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<td>$F_{(1,22)}$ = 0.07</td>
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<tr>
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<td>$F_{(1,25)}$ = 0.69</td>
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<td>$F_{(1,24)}$ = 0.97</td>
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<td>scaled mass ~ Muscle</td>
<td>$F_{(1,25)}$ = 0.72</td>
<td>0.40</td>
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Figure 3. Relationships between Bitterroot departure and (A) logCORT, (B) logTRIG, and (C) scaled mass of Gray Catbirds (GRCA) and Swainson’s Thrushes (SWTH). The x-axis is inverted, so that time progresses from left to right. CORT increased in the days leading up to Bitterroot departure in SWTH. TRIG did not predict departure in either species. Scaled mass of GRCA tended to increase as Bitterroot departure approached.
**Figure 4.** Model main effects displaying activity patterns of Gray Catbirds (A and B) and Swainson’s Thrushes (C and D) in the days leading up to departure. The x-axis is inverted so that time progresses from left to right. (A) Gray Catbird daily activity increases in the days leading up to Bitterroot departure, and individuals with lower scaled body masses were more active than heavier birds. (B) Gray Catbird afternoon activity increased in the days leading up to MPG departure. (C) Swainson’s Thrushes had a non-significant negative relationship between daily activity and Bitterroot departure. (D) In the evening, Swainson’s Thrush activity significantly decreased as Bitterroot departure approached.
Supplementary Figure 1. Map of detections used to estimate minimum migration speed (n = 15) between Motus stations (yellow points). Each line represents an individual Gray Catbird (GRCA; purple) or Swainson’s Thrush (SWTH; orange); the lines are slightly jittered to more clearly display individual, overlapping tracks.
Supplementary Figure 2. Histogram of the time of the final Motus detection in Montana of each species: Gray Catbirds (GRCA) and Swainson’s Thrushes (SWTH). There was no significant difference in departure time between species.
**Supplementary Figure 3.** Minimum migration speed (km/day) was significantly greater in Swainson’s Thrushes (SWTH) than Gray Catbirds (GRCA).
RESEARCH ARTICLE

Physiology and behavior under food limitation support an escape, not preparative, response in the nomadic pine siskin (Spinus pinus)
Joely G. DeSimone*, Bret W. Tobalske and Creagh W. Breuner

ABSTRACT
Migration allows animals to use resources that are variable in time and/or space, with different migratory strategies depending on the predictability of resource variation. When food varies seasonally, obligate migrants anticipate and prepare for migration. In contrast, facultative migrants, whose movements are unpredictable in timing and destination, may prepare for either migration or escape when resources are depleted. We propose and test two alternative hypotheses regarding the behavioral and physiological responses of facultative migrants to declining food availability. (1) The prepare hypothesis predicts that facultative migrants prepare for departure by increasing fuel stores in response to declining food availability, and elevations of baseline corticosterone (CORT) facilitate increased activity. (2) The escape hypothesis predicts that facultative migrants do not prepare for departure, body condition declines as food availability declines, and stress-induced levels of CORT induce escape behavior when both energetic condition and food resources are low. We conducted a 16-day experiment, measuring body composition (using quantitative magnetic resonance), activity (using force perches) and baseline CORT in pine siskins (Spinus pinus) given ad libitum food or a slow decline, fast decline or randomly changing amount of food. Our results support the escape hypothesis: body condition declined as food declined, decreases in body and fat mass were associated with increases in baseline CORT, and activity increased only when food availability was low. This work suggests that facultative migration in autumn allows birds to escape low-resource areas and that the underlying physiological mechanisms differ from those driving both seasonal, obligate migrations and spring nomadic movements.

KEY WORDS: Body composition, Corticosterone, Irruption, Locomotor activity, Migration, Pine siskin

INTRODUCTION
Migration enables animals to use resources that are heterogeneous in time and/or space (Lack, 1968; Alerstam et al., 2003; Dingle and Drake, 2007; Sonneville et al., 2019). Different migratory strategies largely depend on whether resource variation is predictable or unpredictable (Mueller and Fagan, 2008; Newton, 2012). For instance, the seasonal migrations of birds are often synchronized with the seasonal phenology of temperature or rainfall patterns that correlate with food availability (Alerstam and Enckell, 1979; Newton and Dale, 1996a,b), and which can be predicted by photoperiod and endogenous rhythms (Gwinner, 1996; Dingle and Drake, 2007; Moller et al., 2008; MacPherson et al., 2018). These movements – called obligate migration – are highly predictable in terms of timing, distance and destination within and among individuals and years (Newton, 2012).

When migratory departure is predictable, animals can anticipate and therefore prepare for the energetic challenges of a long-distance journey, depositing fuel stores while local food resources are still present. Before the onset of obligate migrations, birds eat more (King and Farner, 1965), increase fat and muscle mass (King and Farner, 1965; Marsh, 1984), and exhibit nocturnal restlessness in captivity, which is hopping or wing-whirring behavior indicative of readiness for flight (Agatsuma and Ramenofsky, 2006; Eikenaar et al., 2014). Changes in photoperiod, which serve as a reliable indicator of seasonal changes in food availability, proximately cue these migratory preparations (Jenni and Schaub, 2003; Cornelius et al., 2013), though the timing of departure is often refined by supplementary and synchronizing cues (Jacobs and Wingfield, 2000; Jenni and Schaub, 2003; Cornelius et al., 2013).

In contrast, when resources vary unpredictably in space and/or time, the animals that rely on these resources are often facultative migrants, with characteristically unpredictable timing and destination of movements (Newton, 2012). Facultative migrations include nomadic and fugitive migrations: nomadic migrations are associated with potentially abundant but ephemeral resources, while fugitive movements are characterized by an escape response to a labile perturbation (Watts et al., 2018). Facultative migrants include nomadic rapplers that prey on rodents (Lack, 1954; Galushin, 1974), desert birds that track unpredictable rainfall events and subsequent patches of food (Ward, 1971; Davies, 1984; Wiens, 1991), and birds that feed on conifer seeds (Newton, 2006). Conifers produce cone crops sporadically in time and space, though often synchronously across broad geographic ranges (Koenig and Knops, 1998). Birds that rely on conifer seeds are often nomadic, irruptive migrants and may travel hundreds of kilometers in search of an abundant food source (Koenig and Knops, 1998; Newton, 2006).

Although the physiology of obligate migration has been studied for decades, the physiology of facultative migrations remains more obscure. Low food availability, exacerbated by competition, is thought to be the proximate cue initiating many facultative migrations (Lack, 1954; Bock and Lepthien, 1976), because in these species, photoperiod is not always an informative indicator of spatial and temporal patterns of food availability. Observational studies reveal that years with poor seed crops correspond with irruptions of boreal seed-eating birds (Bock and Lepthien, 1976; Koenig and Knops, 2001). Interestingly, many nomadic migrants are sensitive to photoperiod, and they accumulate fat and become more active in response to spring (but not autumn) photoperiod (Pohl and West, 1976; Cornelius and Hahn, 2012; Robart et al., 2018). However, low food availability may still be necessary to
Captive red crossbills (*Loxia curvirostra*) and pine siskins (*Spinus pinus*) experiencing food restriction increase activity in winter and spring but not autumn (Cornelius et al., 2010; Robart et al., 2019).

Like obligate migration, facultative migrations likely involve the integration of both external cues (e.g. food availability, environmental conditions, social information) and internal cues (e.g. energetic stores) to time migratory behaviors. Thus, in developing hypotheses about the regulation of facultative migratory behavior and physiology, we include the endocrine system, because hormones coordinate rapid, whole-organism responses to both external and internal challenges (Martin et al., 2011). Corticosterone (CORT), the main avian glucocorticoid, is a prime candidate because it can mediate locomotor behavior at two distinct levels that can be differentiated experimentally.

First, elevated levels within the baseline range of CORT support the increased energetic demands of predictable challenges and life history stage transitions (Wada, 2008), such as hatching and parturition (Challis, 2000; McLean and Smith, 2001), fledging and dispersal (Heath, 1997; Bellthoff and Dufly, 1998; Sprague and Breuner, 2010), and, importantly, obligate migratory departure (Lõhmus et al., 2003; Eikenaar et al., 2017, 2020). In obligate migrants, CORT stimulates fat deposition and mobilizes protein for fuel (Gray et al., 1990). Baseline CORT increases with body mass (Piersma et al., 2000) and migratory restlessness (Lõhmus et al., 2003), and predicts departure from a stopover site (Eikenaar et al., 2017). In summary, elevations in baseline CORT in obligate migrants with sufficient fuel stores can promote migratory departure.

Second, CORT levels can significantly increase in response to unpredictable perturbations, such as harsh storms (Wingfield et al., 1983, 1998; Landys et al., 2006). These stress-related levels initiate an emergency life history stage, whereby the animal diverts energy away from immediately unnecessary processes, such as reproduction, and toward short-term survival (Wingfield et al., 1998). In these situations, CORT promotes escape behavior and fugitive migration in response to a stressor (Breuner et al., 1998; Breuner and Hahn, 2003).

Thus, CORT can mediate increases in activity either in anticipation of predictable life history stage transitions or in reaction to unpredictable situations. We propose two competing hypotheses to explain the regulation of facultative migration by examining the relationships among CORT, body condition and locomotor activity in response to declining food availability: the prepare hypothesis and the escape hypothesis.

Under the prepare hypothesis, facultative migrants anticipate and prepare for departure just as obligate migrants do. Individuals could prepare if they are sensitive to changes in food availability such that they deposit fuel stores before local food availability is depleted. In this case, (1) we expect body mass to increase as food availability declines, possibly mediated by an increase in food intake and/or a reduction in energetic expenditure and activity. (2) We expect baseline CORT to be elevated in anticipation of departure, playing a similar role as in obligate migrants ready for flight or nestlings ready to fledge. Therefore, (3) we predict body condition and CORT to be positively correlated as food availability decreases and migratory activity increases. Under this scenario, facultative migration is similar to obligate migration in terms of physiological preparation, but the birds respond to a different proximate cue: rather than preparing in response to changing photoperiod like an obligate migrant, facultative migrants initiate migratory preparations in response to changing food availability.

Alternatively, under the escape hypothesis, facultative migrants may express a stress response and escape behavior in low-resource areas. In this case, we would not expect the birds to perceive and respond to changes in food availability, but rather to the absence of food when resources are very low. Here, (1) we predict that body condition will decline as food availability does. A bird in poor energetic condition in an area with low food availability will initiate an emergency life history stage, so that (2) stress-elevated CORT levels (i.e. circulating levels that approximate those achieved in response to a standardized stressor) promote increased locomotor activity indicative of escape behavior. Therefore, (3) we predict body condition and CORT to be inversely related as food declines and activity increases.

We tested the physiological and behavioral responses to declining food availability in a facultative migrant, the pine siskin. To distinguish the hypotheses, we experimentally manipulated food availability for captive pine siskins and measured hopping activity, body composition and CORT responses. Two groups received food that declined at two different steady rates over the course of the 16-day experiment, allowing us to detect any rate dependence to the birds’ responses. An additional random group received an unpredictable amount of food each day so that we could differentiate the effects of declining versus fluctuating food availability. A control group received *ad libitum* food throughout the experiment.

Captive red crossbills and pine siskins exposed to sudden reductions in food quantity or quality have shown decreases in body condition, increases in CORT and, at certain times of year, increases in activity (Cornelius et al., 2010; Robart et al., 2019). Here, we slowly reduced food availability over 16 days to more explicitly test the sensitivity of birds’ responses to changes – rather than simply reductions – in food availability. Furthermore, the continuous activity data we collected allow for a more comprehensive and nuanced analysis of the effect of changing food availability on behavior. Finally, the novel addition of a random group in this study enables us to (1) differentiate responses to predictable versus unpredictable changes in food availability and (2) assess whether physiological responses to food restriction are sensitive to changes in food availability over multiple days, or just the food received on a given day.

**MATERIALS AND METHODS**

**Bird capture and housing**

Twenty-eight pine siskins (*Spinus pinus*) were captured using mist nets and playback in Missoula, MT, USA, between 5 and 19 October 2018 and banded with unique color band combinations for identification in captivity. They were collected under permits from US Fish and Wildlife Service (permit 23228) and Montana Fish, Wildlife, and Parks (permit 2018-089-W). Birds were housed at the Field Research Station at Fort Missoula, with two individuals per cage, separated by an opaque plexiglass cage divider, allowing for auditory, but not visual, contact between pairs. Cage space per bird was 30.5×25.5×33 cm. Cages were placed inside sound-attenuating chambers (MED-OF-022, Med Associates Inc., Fairfax, VT, USA) so that a quiet researcher could be in the room without disturbing the birds, though birds could still hear each other’s calls.

Birds were held under an approximately 10 h:14 h light:dark photoperiod, similar to that of 1 November 2018 in Missoula, MT, with no dim light overnight. Ambient temperature in the housing rooms was ~22°C. Birds were provided with *ad libitum* water and RoudyBush Small Bird Daily Maintenance Diet (RoudyBush, Woodland, CA, USA) until the beginning of the food manipulation, as described below. Birds were given at least 2 weeks to acclimate to captivity and 1 week to acclimate to the isolation chambers before the start of the experiment. All housing
and experimental protocols were approved by the University of Montana Institutional Animal Care and Use Committee (Protocol 010-18CBOBE-032018).

**Food manipulation**
After acclimation to captivity, we measured the food intake of each bird for 3 days before the start of the experiment; we weighed the amount of food provided to each bird at lights on (∼300% of their daily intake), and subtracted the amount of food remaining in their food dish or on the floor of their cage the next morning. The average of these 3 days of *ad libitum* feeding represents each bird’s daily average food intake (3.85±0.04 g; mean±s.e.m.).

We randomly assigned birds to one of four treatment groups (Fig. 1): control, slow decline, fast decline and random. Control birds received 300% of their daily average food intake; slow decline birds received 2% less food each day (98% of their daily average food intake on day 0–68% on day 15); fast decline birds received 4% less each day (96–36%); and random birds received a random amount of food between 72 and 135% each day. On experimental day 15, all birds received *ad libitum* food when returned to their cages after blood sampling and body composition analyses.

On day 3 among the slow decline group, the amount of food provided was mistakenly calculated as 92% of one pre-experimental day’s food intake, rather than 92% of the average daily pre-experimental food intake, such that the group’s average provided food on day 3 was 89.9%, but ranged from 76.1 to 100.4%.

Because finches are known to communicate information about food quantity to each other (Cornelius et al., 2010, 2018), birds in different food treatments were housed in different rooms. Owing to space limitations, the experiment was conducted in two parts: control and slow decline birds 1–20 November 2018; fast decline and random birds 29 November–18 December 2018. Photoperiod remained the same throughout the study. Four first-round control birds were used as two fast decline and two random individuals in the second round of the experiment.

**Physiological measurements**
Five days before and every 4 days after the start of the food manipulation, we collected three physiological measurements. Starting approximately 1.5 h after lights on, we took baseline blood samples from each individual for hormone analyses. Blood samples were collected by puncturing the brachial vein with a 26.5 gauge needle within 3 min of opening the door of the sound-attenuation chambers. Up to three-quarters of a heparinized capillary tube (about 55 µl) was collected each time to limit blood loss over the course of the experiment. Additional bleeding was staunched with cotton, or when necessary, cotton and styptic powder. Next, we weighed each bird to the nearest 0.01 g and visually scored fat stores on a scale of 0 to 5 (Moore and Kerlinger, 1987) and pectoralis muscle size on a scale of 0 to 3 (Bairlein, 1995). Finally, we scanned them in a quantitative magnetic resonance (QMR) machine (EchoMRI, Houston, TX, USA). The QMR reports the grams of fat mass, lean mass and body water of an individual after a rapid (∼90 s), non-invasive scan (Guglielmo et al., 2011). Physiological data collection was completed within 4 h of lights on.

**Activity monitoring**
We used custom-made force perches instrumented with 120 Ω strain gauges (EA-06-125BT-120, Micrometrics-Measurements, Raleigh, NC, USA) arranged in a full-bridge configuration to measure any vertical force made upon the perch (Tobalske et al., 2004). Thus hops onto and off of the perch were recorded as peaks in the data, and two distinct baselines represented either a bird sitting still on the perch or an empty perch. The perches consisted of a 36 cm wood dowel, 1 cm in diameter, with two brass strips 2.5 cm long, 6 mm wide and 0.08 mm thick (5024682, K&S 0.032 in wide, 0.0032 in thick, Vishay 2100 signal conditioner and 2120B amplifier). Analog output from the amplifier was sampled at 100 Hz using Axoscope (v8.1) and an Axon Instruments Digidata 1322A analog/digital converter, and stored for analysis on a computer. Resonant frequency of the unloaded perches was ~100 Hz, significantly higher than the time intervals of interest for hops. Thirty minutes of pre-experimental visual observation confirmed that almost all of the hops birds made were either onto or off of the perch; thus the perch data reflect total hopping activity.

**Activity analysis**
Perch output was analyzed in RStudio (Version 1.2.1335) by calculating the derivative squared of the reported voltages with respect to time, such that the steep increases/decreases in voltage of hops on/off the perch were represented by highly positive squared derivatives. We then calculated the number of local maxima above a threshold value of 20 (determined by visualization of a subset of the data), with the limitation that local maxima must be more than 0.25 s apart to avoid counting a single hop as multiple ones.

Daytime data were divided into blocks of time representing each hour after lights came on. Nighttime data blocks represent each hour after lights went off. Because data recorded while a researcher was in the bird room were removed, each hour chunk does not always represent 60 min of continuous data collection. Thus we converted the number of hops per time block to the number of hops per 10 min for each block. Activity on day 15 (the last day of the study) was removed from analyses because all birds received *ad libitum* food after physiological data were collected that morning. Changes in
activity (Δactivity) represent the hops per 10 min on a given experimental day at a given hour after lights on minus the average hops per 10 min at that same time over the course of four pre-experimental days.

We plotted average Δactivity by hours after lights on for the control, slow decline and fast decline treatment groups, with a different line per experimental day. The random group was excluded from this analysis because their activity patterns depended on food availability on a given day, and examining changes in activity over time does not make sense for this group. We fit non-linear sine curves (nls in R) to these data for each individual bird (i.e. a curve for every experimental day, for every individual), assuming that the entire 10 h period represented one cycle (i.e. 2π radians). We extracted from these curves the amplitude (which represents the height of the curve) and phase (which represents the midpoint between the trough and peak of the cycle). We averaged the amplitude and phase for each experimental day by treatment. We fitted a linear regression to assess the relationship between the amplitude and phase of these sine curves over the course of the experiment for each treatment.

In post hoc analysis, we divided the day into ‘morning’ and ‘afternoon’ to separately investigate activity patterns during these times. ‘Morning’ represents the first 5 h after lights on, and ‘afternoon’ is the latter 5 h.

CORT analysis

Plasma CORT levels were detected using an Enzyme Linked Immunoassay (ELISA) kit (cat. no. 25-0412, Enzo Life Sciences). First, CORT was extracted from plasma using a double ether extraction after addition of 2000 cpm of 3H-CORT to estimate recovery. Ether was evaporated off in a 50°C water bath. Sample was reconstituted in 135–425 µl assay buffer for a final dilution of 1:20 or 1:25. Recoveries averaged 75.8±0.01%; samples were reconstituted in 135 µl of extracted, diluted sample was assayed in triplicate if corrected to 100% for analysis.

Intra- and inter-plate coefficients of variation (CVs) were 10.2 and 20.5%, respectively. Average assay detectability was 0.514 ng ml⁻¹; samples below the assay detectability limit (CD) were assigned the value on the pre-experimental days, so no activity value was possible.

Assays were run as described in Patterson et al. (2011). In brief, 100 µl of extracted, diluted sample was assayed in triplicate if possible, and in duplicate or singly when plasma volume was limiting. A standard curve (20,000 to 15.63 pg ml⁻¹) was included in triplicate (100 µl per well) on each plate. An external CORT standard was also run in triplicate on each plate to assess inter-plate variation. Color reaction was read at 405 nm corrected at 595 nm. Intra- and inter-plate coefficients of variation (CVs) were 10.2 and 20.5%, respectively. Average assay detectability was 0.514 ng ml⁻¹; samples below the assay’s detectability limit (n=6) were assigned the mean detection limit of their respective assay.

Statistical analysis

We calculated changes in various physiological metrics (i.e. ΔCORT, Δbody mass, Δfat mass and Δlean mass) as the value on a given experimental day subtracted from the value on the pre-experimental day −5. We used GLMMs (R package lmerTest in conjunction with lme4, Type III ANOVA with Satterthwaite’s method) to analyse: the relationship between visual muscle score and experimental day; the relationship between Δlean mass and experimental day; the relationships between ΔCORT and Δbody mass, Δfat mass, and Δlean mass; and the change in morning and afternoon activity in relation to percentage food reduction. In these models, bird ID was included as a random effect unless otherwise noted. Overall models included treatment as an interaction term when significant, but when calculating treatment-specific P- and β-values, treatment groups were analyzed separately. In assessing changes in lean mass on day 15, 95% confidence intervals of the average Δlean mass that do not include 0 are considered to represent significant (P<0.05) changes in mass. When we compared the slopes of the linear relationships between percentage food reduction and changes in morning and afternoon activity across treatment groups, non-overlapping 95% confidence intervals for the slope parameters were considered to be significantly different. Figures were created using the ggplot2 and cowplot packages.

Sample sizes

Eight birds were assigned to each treatment group. One random bird was removed from the experiment owing to a pre-existing illness. Birds in very poor body condition (i.e. depleted fat stores, concave pectoralis muscles, and body mass ~75% of their initial mass) were removed from the experiment: three fast decline birds were removed on experimental day 12 and two on day 13.

The refrigerator where assay reagents were stored malfunctioned during the time that the second assay plate was run, and the samples from this plate had higher CVs and had markedly higher CORT levels than the other five plates. These samples included control birds on experimental days 3, 7 and 11 (n=16), and Slow Decline birds on day 7 and 11 (n=14). We removed these samples from analyses involving CORT or ΔCORT values.

One bird from the fast decline group was removed from analyses involving Δactivity because its perch was broken and repaired between the pre-experimental and experimental days, and the Δactivity was an outlier likely because of a change in perch sensitivity. One bird from the control group was not included in activity analyses because its perch was non-functional during the pre-experimental days, so no Δactivity value was possible.

RESULTS

Body composition and food availability

Treatment groups differed in their change in body and fat mass over the course of the experiment. As food availability declined, birds in food reduction treatments lost body mass and fat mass (Fig. 2A,B, Table 1); fast decline birds lost body and fat mass at a faster rate (Table 1). Control and random birds showed no change in body

![Image](https://example.com/image.jpg)

**Fig. 2. Changes in body composition compared with pre-experimental values for each group throughout the experiment.** Slow decline (n=8) and fast decline (n=8) birds lost body mass (A) and fat mass (B), and had decreased muscle scores (D) as food availability declined. Among control (n=8) and random birds (n=7), there was a significant increase in lean mass (C) over time. Means and 95% CI are shown for each group. See Table 1 for statistical analyses of these data.
mass over time (Fig. 2A, Table 1). Control birds showed a slight, significant increase in fat mass and random birds showed a decrease in fat mass over time (Fig. 2B, Table 1). Absolute values of body, fat and lean mass for each treatment group over the course of the experiment are shown in Fig. S1B–D.

Visual scores of pectoralis muscle size declined for fast decline and slow decline birds, but not control or random birds as the experiment progressed (Fig. 2D, Table 1). In spite of this, all treatments showed a significant, slight increase in average lean mass – as measured by the QMR – when comparing day 15 with the pre-experimental day –5 (day 15 mean ±Δlean mass [95% CI]; control: 0.39 g [0.16, 0.61]; slow decline: 0.25 g [0.02, 0.49]; fast decline: 0.20 g [0.09, 0.31]; random: 0.39 g [0.45, 0.73]; Fig. 2C). Over the course of the experiment, there was an overall significant increase in whole body lean mass, driven by the control and random groups (overall: $F_{1,88.62}$=6.97, $\beta$=0.01 [0.00, 0.02], $P$=0.01; Fig. 2C; see Table 1 for statistics per treatment; interaction term not included in model).

### Table 1. Statistics for Δbody mass, Δfat mass, Δlean mass and Δmuscle score over the course of the experiment (days 3–15) for each treatment group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment group</th>
<th>$F$</th>
<th>$\beta$ [95% CI]</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔBody mass (g)</td>
<td>Control</td>
<td>$F_{1,23}$=5.4</td>
<td>0.01 [−0.03, 0.05]</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Slow</td>
<td>$F_{1,23}$=5.5</td>
<td>−0.12 [−0.14, −0.09]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Fast</td>
<td>$F_{1,18.34}$=2.9</td>
<td>−0.19 [−0.22, −0.15]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Random</td>
<td>$F_{1,20}$=5.7</td>
<td>−0.02 [−0.04, 0.00]</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Treatment×day</td>
<td>$F_{3,84.58}$=3.3</td>
<td>−0.02 [0.01, 0.04]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔFat mass (g)</td>
<td>Control</td>
<td>$F_{1,23}$=7.6</td>
<td>0.02 [0.01, 0.04]</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Slow</td>
<td>$F_{1,23}$=7.6</td>
<td>−0.11 [−0.13, −0.08]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Fast</td>
<td>$F_{1,18.2}$=19.0</td>
<td>−0.18 [−0.20, −0.15]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Random</td>
<td>$F_{1,20}$=15.9</td>
<td>−0.03 [−0.05, −0.02]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΔLean mass (g)</td>
<td>Control</td>
<td>$F_{1,23}$=4.5</td>
<td>0.02 [0.00, 0.03]</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Slow</td>
<td>$F_{1,23}$=1.8</td>
<td>0.01 [−0.01, 0.03]</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Fast</td>
<td>$F_{1,25}$=0.3</td>
<td>0.01 [−0.04, 0.02]</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Random</td>
<td>$F_{1,20}$=1.3</td>
<td>0.02 [0.01, 0.04]</td>
<td>0.002</td>
</tr>
<tr>
<td>ΔMuscle score</td>
<td>Control</td>
<td>$F_{1,23}$=1.2</td>
<td>0.03 [0.00, 0.06]</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Slow</td>
<td>$F_{1,23}$=3.3</td>
<td>−0.09 [−0.13, −0.06]</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>Fast</td>
<td>$F_{1,18.7}$=18.1</td>
<td>−0.11 [−0.16, −0.06]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Random</td>
<td>$F_{1,20}$=0.4</td>
<td>−0.011 [−0.04, 0.02]</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Treatment×day</td>
<td>$F_{3,84.73}$=1.3</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

$F$- and $P$-values are shown for the treatment×day interaction term in the overall model. See Fig. 2 for visualization of these data.

### Activity over time

Changes in daytime activity over the course of the experiment differed among treatment groups (Fig. 4; day×treatment: $F_{3,380.05}$=8.00, $P$<0.0001). The slow decline and fast decline groups slightly, though significantly, decreased morning activity as the experiment progressed (slow: $F_{1,97}$=24.37, $\beta$=−5.85 [−8.18, −3.51], $P$<0.0001; fast: $F_{1,103}$=4.37, $\beta$=−1.97 [−3.83, −0.11], $P$=0.04). There was no relationship between change in morning activity and experimental day in the random ($F_{1,97}$=0.0, $\beta$=0.00 [−1.15, 1.14], $P$=0.99) or control groups ($F_{1,83}$=3.3, $\beta$=−1.36 [−2.86, 0.14], $P$=0.08).

Changes in afternoon activity also differed among treatment groups (Fig. 4; day×treatment: $F_{3,375.61}$=23.96, $P$<0.0001). Slow decline and fast decline groups significantly increased afternoon activity as the experiment progressed (slow: $F_{1,97}$=23.46, $\beta$=7.38 [4.38, 10.38], $P$<0.0001; fast: $F_{1,103}$=89.24, $\beta$=17.34 [13.71, 20.94], $P$<0.0001; random: $F_{1,97}$=3.55, $\beta$=2.73 [−0.12, 5.59], $P$=0.06; control: $F_{1,83}$=0.07, $\beta$=−0.31 [−2.58, 1.95], $P$=0.79). The mean
amplitude of the sine curves fitted to the change in activity significantly increased over the experimental days in the fast decline and slow decline groups, and at a faster rate within the fast decline group (slow: $\beta=9.56 \ [7.98, 11.14], P<0.0001$; fast: $\beta=13.88 \ [9.06, 18.69], P<0.0001$; control: $\beta=1.74 \ [-1.52, 5.00], P=0.27$). The mean phase of the sine curves increased (shifted earlier in the day) over time in the fast and slow decline groups, and at a faster rate within the fast decline group (slow: $\beta=0.05 \ [0.03, 0.08], P=0.001$; fast: $\beta=-0.07 \ [0.06, 0.08], P=0.0001$; control: $\beta=0.05 \ [-0.61, 0.72], P=0.86$), such that the phase occurred 1 h earlier between days 9 and 10 in the fast decline group and by day 12 in the slow decline group. Almost no nocturnal activity was observed in any treatment group, and with no change over time. The birds were not provided with low levels of light overnight, which enhances nocturnal activity (Ramenofsky et al., 2008). We therefore cannot test for an effect of experimental treatment on nocturnal behavior, and do not present these data here.

**Activity and food availability**

Among experimental days involving food reductions in the slow decline, fast decline and random groups, reductions in morning activity as percentage food reduction increased differed among treatment groups (Fig. 5A; percentage food reduction*treatment: $F_{2,240.39}=10.43, P<0.0001$). There was no difference in slope between the random and fast decline groups (mean $\beta$ [95% CI]; fast: $\beta=-0.49 \ [-0.95, -0.02]$, random: $\beta=0.28 \ [-0.74, 1.30]$), but the slow decline group had a significantly steeper negative slope ($\beta=-2.93 \ [-4.12, -1.74]$). In the afternoon, activity increased with food reduction across all three treatment groups (Fig. 5B; $F_{1,246.7}=139.14, P<0.0001$; % food reduction*treatment interaction term not included) and to a similar extent across groups (fast: $\beta=4.33 \ [3.43, 5.24]$; slow: $\beta=3.61 \ [2.07, 5.15]$; random: $\beta=4.81 \ [2.62, 6.99]$).

**Activity and CORT**

We found no significant relationship between change in CORT and the change in morning activity (overall: $F_{1,43}=2.53, \beta=4.92 \ [-1.48, 10.86], P=0.12$; ΔCORT×treatment interaction not included) or change in afternoon activity (overall: $F_{1,62}=0.49, \beta=-4.87 \ [-18.14, 8.39], P=0.48$; ΔCORT×treatment interaction not included).

**Random group food intake**

On the four experimental days when random birds were provided >100% of their pre-experimental daily average food intake immediately following days of food restriction (<100%), random birds did not increase food intake above their pre-experimental level. The average difference in food consumed on these post-food restriction days and their pre-experimental average food intake was $-0.044\pm0.08$ g (mean±s.e.m.).

**DISCUSSION**

Our results support the escape hypothesis, suggesting that irruptive migratory physiology is distinct from obligate migratory physiology: whereas obligate migrants undergo extensive preparations for flight, pine siskins express escape behavior to flee areas with insufficient resources, even when provided with ‘advance notice’ of deteriorating conditions through a gradual decline in food availability. Consistent with our predictions, we found that body mass and fat mass declined with food availability (Fig. 2A,B) and, in birds experiencing prolonged food reductions, declines in body and fat mass predicted increases in CORT (Fig. 3B,C). Afternoon activity increased with food reduction, and to the same extent across the slow decline, fast decline and random groups (Fig. 5B), indicating that changes in activity were not sensitive to changes in food availability over multiple days, but rather to the amount of food received on a given
day. Pine siskins were also sensitive to the time of day when they ran out of food. As the experiment progressed and they received less food, birds in the fast decline and slow decline groups increased activity earlier in the day (Fig. 4B,C). Finally, birds in the random group did not compensate for days of food restriction by increasing food intake when provided with abundant food the following day. Other studies show that birds often increase food intake, mass or fat stores when experiencing unpredictable food regimes (Witter et al., 1995; Cuthill et al., 2000; Reneerkens et al., 2002; Bauer et al., 2011; Cornelius et al., 2017), further suggesting that siskins respond to insufficient food availability by moving rather than fattening. One caveat is that these captive birds carried greater fat stores than they did in the wild, possibly limiting the effect of intermittent reductions in provided food. In summary, pine siskins exposed to experimental declines in food supply in autumn do not show preparative fueling, they are not sensitive to declines in food availability but rather its absence, and low food availability reduces body condition, increases CORT levels and initiates increased activity.

Under a different food manipulation protocol, Robart et al. (2019) also concluded that siskins do not prepare for autumn movements. In their study, captive siskins in the spring and autumn experienced a reduction in food quality and then a 25% reduction in food quantity. Food-restricted birds showed increased baseline CORT and decreased body condition, and in the spring (but not the autumn), exhibited increases in activity. Robart et al. (2019) suggested that siskins are less behaviorally sensitive to changes in food availability in the autumn, or that their lower body condition in the autumn limits any increases in energy expenditure and activity. Our results are not consistent with these possibilities. We observed marked increases in activity in the autumn, and increases in activity became even more pronounced as food availability declined and body condition worsened. In Robart et al.’s (2019) study, changes in activity may have occurred outside the time it was recorded, or the exposure of food-restricted birds to the vocalizations of control birds may have attenuated their behavioral response to food reductions (Cornelius et al., 2010).

In our study, although the visual muscle scores of slow and fast decline birds decreased over time, there was no change in total lean mass as measured by the QMR. It is possible that the pectoralis muscles appeared smaller over time owing to a depletion of intramuscular fat rather than a loss of lean tissue. Redfern et al. (2004) found that fat and muscle scores were correlated among sedge warblers (Acrocephalus schoenobaenus) captured at a banding station, but fat and pectoralis muscle mass were unrelated among dissected individuals. This discrepancy suggests that fat stores may affect visual muscle scores. Another possibility is that there was an increase in some other component of lean mass, such that the QMR reported no overall change in lean mass despite a decrease in pectoralis mass.
CORT underlies increases in activity in two ways, with elevations of baseline CORT promoting increased activity associated with predictable life history stage transitions (as in the prepare hypothesis), and stress-related elevations promoting escape behavior in response to unpredictable stressors (as in the escape hypothesis) (Landys et al., 2006). CORT binds with different affinity to two tissue-level receptors, which allows for these distinct organismal responses to different concentrations of CORT (Reul and de Kloet, 1985; Sapolsky et al., 2000). While our other results support the escape hypothesis, CORT levels did not reach the stress-related elevations expected under this hypothesis and previously measured in pine siskins (mean±s.e.m. 12.20±1.86 ng ml⁻¹; J. DeSimone unpublished data; Asheimer et al., 1992; Knutie and Pereyra, 2012). This result is consistent with that of Robart et al. (2019), with food-restricted siskins exhibiting only modest elevations in CORT. One possibility is that CORT does not play a mechanistic role in siskin movements in response to food reductions, or that downstream processes, such as receptor levels, are responsible for their migratory behaviors (but see Watts et al., 2019). However, across the avian literature, CORT is correlated with activity during the processes of fledging (Sprague and Breuner, 2010), dispersal (Belthoff and Dufty, 1998), obligate migration (Lõhmus et al., 2003; Eikenar et al., 2020) and escape behavior (Breuner et al., 1998; Breuner and Hahn, 2003). We therefore think it more likely that we did not observe a relationship between CORT and activity as a function of experimental design rather than biology. We collected blood samples approximately 2 h after lights on, when birds always had food remaining in their dishes, even among fast decline birds on day 15. Sampling birds while they had food would have allowed us to detect changes in physiology in response to birds’ perception of and sensitivity to changes in food availability over time, rather than their response to the absence of food. Instead, changes in activity were not apparent until later in the day, when birds had little or no food remaining. CORT physiology during these times could have been very different from what we measured.

Pine siskins gained significant body mass while acclimating to captivity. The average mass of these siskins when captured in the wild was 12.65±0.11 g (mean±s.e.m.), while their average pre-experimental mass (5 days before the start of the food manipulation) was 14.56±0.22 g. Had birds started the experiment in their original body condition, we may have observed even stronger responses to food declines, stronger relationships between changes in CORT and body composition, and declines in lean mass once fat stores were depleted.

The apparent lack of preparation for migration in pine siskins has several possible, non-mutually exclusive implications, all related to the idea that they may not need to make long-distance flights before finding the next available food source. First, siskins may be sufficiently generalist such that, even if their current food source is depleted, they are likely to find patches of suitable alternative food sources on their way to their next destination, negating the need to deposit fuel before departure. Second, areas of low food availability may not be as synchronous as suggested by Koenig and Knops (1998), so that there are patches of food across the landscape and siskins never have to travel far before encountering a food source. Third, siskins could further reduce the need for additional fuel stores if they know where they are going, so they reach their next destination efficiently. This option could be possible if siskins can remember the locations of food patches encountered in the past, or remember areas with past environmental conditions that may favor present food availability. They could also make exploratory excursions from their current area, while local food remains and refueling is possible, to gather information about broader-scale food availability before departing (Bennetts and Kitchens, 2000). Decisions about flight direction or destination could be enhanced by social information if flocks are able to come to a consensus based on their collective information (Seeley and Buhman, 1999; Cornelius et al., 2010). Finally, search efficiency of scarce, patchy food sources can be improved with social behavior, as information about food availability can be transferred among neighboring conspecifics, and because foraging conspecifics are likely easier to detect while in flight than consier seeders (Egert-Berg et al., 2018).

Facultative migratory finches are often social, and public information shared among individuals can alter the behavior and physiology of group members (Smith et al., 1999; Cornelius et al., 2010, 2018). Thus, group behavior in the wild could alter the behavioral and physiological relationships we observed in captive individuals. However, group membership of pine siskin flocks is highly dynamic in the wild (J. DeSimone, personal observation). It is more likely that departure decisions are made individually based on public information, rather than groups arriving at a democratic consensus.

In conclusion, our experimental test supports the escape hypothesis and excludes the prepare hypothesis. Our study characterizes autumn facultative movements as distinct from obligate migrations, and even from spring nomadic movements, and more representative of escape behavior in response to low food availability. A clearer understanding of facultative migratory physiology can open the door for future integrative hypotheses and studies of the broad spectrum of migratory behaviors, how they relate to one another, and their ecological and evolutionary implications.

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Competing interests
The authors declare no competing or financial interests.

Author contributions

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Data availability
Raw data are available in the Dryad digital repository (DeSimone et al., 2021): q2f12jmmd.

Supplementary information
Supplementary information available online at https://jeb.biologists.org/lookup/doi/10.1242/jeb.238774.supplemental

References


Fig. S1. Absolute values (mean ± 95% CI) of CORT, body mass, fat mass, and lean mass for each treatment group over the course of the experiment.
Fig. S2. Linear regressions for the relationships between $\Delta$ CORT and (a) $\Delta$ body mass, (b) $\Delta$ fat mass, and (c) $\Delta$ lean mass for each individual across the four treatment groups.
Body mass and triglycerides predict departure of free-living nomadic pine siskins

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ABSTRACT

1. Facultative migrations are observed across vertebrate taxa, include irruptive and nomadic movements, and occur in response to ephemeral and unpredictably variable resources. While the physiology underlying seasonal, obligate migrations is thoroughly studied, much less is known about the physiological mechanisms of facultative movements. We test two hypotheses in a free-living, nomadic bird, the pine siskin (Spinus pinus).

2. The Prepare Hypothesis predicts that, like obligate migrants, siskins increase fuel stores to prepare for migratory movements and elevations of baseline corticosterone (CORT) support departure. The Escape Hypothesis predicts that siskins do not prepare for departure, body condition declines as food availability declines, and stress-related levels of CORT induce escape from resource-poor areas.

3. Under the controlled lab conditions of previous studies, food restriction induces declines in body condition and increases in CORT and locomotor activity, supporting the Escape Hypothesis. This study evaluates the ecological relevance of these captive findings by testing the Prepare and Escape Hypotheses in the field for the first time.

4. During two fall field seasons, we radio-tagged siskins and tracked their local movements using handheld and automated telemetry. We assess how body condition and CORT relate to feeding behavior (estimated via plasma triglycerides (TRIG)), space use, and departure.

5. We do not find support for either the Prepare or Escape Hypothesis, but rather observe an intermediate pattern. Birds with higher TRIG, and therefore greater food intake, are more
likely to depart. Birds in poor condition stay longer near the field site; however, above a threshold body mass, body condition does not predict departure.

6. These findings suggest moderate energy stores are necessary for departure but movement decisions depend on other factors among birds with sufficient fuel. Siskin movements are physiologically distinct from both obligate and fugitive movements, and we discuss how food availability and body condition interact to drive different types of movement.

INTRODUCTION

Facultative migrations often occur in response to resource availability that varies unpredictably in space and time. Thus these movements, observed across taxa and around the globe, are characterized by unpredictable timing and destination (Watts et al., 2018; Teitelbaum and Mueller, 2019). Facultative movements occur in species ranging from snail kites (Rostrhaus sociabilis; Bennetts and Kitchens, 2000) to sperm whales (Physeter macrocephalus; Mizroch and Rice, 2013), and are commonly observed among birds in arid environments (Davies, 1984; Ward, 1971) and ungulates in drylands (Mueller et al., 2011; Ito et al., 2013). Nomadic and irruptive movements, both types of facultative migration, are also common among birds that feed on conifer seeds (e.g. red crossbills (Loxia curvirostra), pine siskins (Spinus pinus), and common redpolls (Acanthis flammea); (Newton, 2006a)). Conifer trees produce seed crops in boom-bust cycles, and the fall and winter southward movements of boreal seed-eating birds occur during years of low seed availability (Bock and Lepthien, 1976; Koenig and Knops, 2001). Because the masting cycles of cone production are often synchronous across large areas (Koenig and Knops, 1998), birds dependent on these seeds may need to travel thousands of kilometers in search of an abundant source of food (Koenig and Knops, 1998; Newton, 2006b), raising questions about how they accomplish long-distance flights from areas with presumably poor feeding conditions and through potentially unfamiliar landscapes.

Captive studies have investigated facultative migratory physiology by manipulating food availability (i.e. the presumptive proximate migratory cue) and recording the effects on body condition, activity, and corticosterone (CORT) levels (Cornelius et al., 2010; DeSimone et al., 2021; Robart et al., 2019). These studies focus on CORT, the primary glucocorticoid in birds, because it underlies increases in activity and coordinates animals’ responses to energetic demands, making it a likely candidate hormone underlying facultative migrations. Baseline
CORT levels rise in anticipation of the energetic demands of predictable life history transitions, including fledging and dispersal (Belthoff and Dufty, Jr, 1998; Heath, 1997; Sprague and Breuner, 2010) and seasonal, obligate migrations (Eikenaar et al., 2017, 2020; Landys et al., 2006; Piersma et al., 2000). In response to unpredictable stressors, stress-induced levels of CORT initiate an emergency response (Wingfield et al., 1998). For instance, food-restricted white-crowned sparrows (Zonotrichia leucophrys gamelii) have higher levels of CORT and are more active than fed controls (Lynn et al., 2003). Furthermore, exogenous CORT increases locomotor activity in captivity (Breuner et al., 1998; Cash and Holberton, 1999) and activity ranges in the field (Breuner and Hahn, 2003). Thus birds exhibit two types of movement with distinct physiological mechanisms: predictable movements that can be anticipated and prepared for, and escape movements made in direct response to a stressor.

These two patterns of CORT action are incorporated into the Prepare and Escape Hypotheses, which make explicit predictions about the relationships among body condition, CORT, and food availability prior to departure in facultative migrants (DeSimone et al., 2021). The Prepare Hypothesis predicts that individuals are sensitive to changes in food availability such that they can anticipate departure and prepare by depositing fuel stores before local food availability is depleted. We would expect body mass to increase as food availability declines, and baseline levels of CORT to rise in anticipation of departure, as in obligate migrants. The Escape Hypothesis predicts that birds respond directly to insufficient food, rather than changes in food over time. Thus, body condition would decline as food becomes scarce, and stress-related CORT levels would initiate an escape response (i.e. departure).

The patterns predicted by the Escape Hypothesis represent a physiological stress response observed across vertebrates (Sapolsky et al., 2000; Wingfield et al., 1998). However, based upon the fuel storage patterns observed among obligate migrants, the Prepare Hypothesis is likely only applicable to flying and swimming—rather than walking—facultative migrants. Obligate migratory birds demonstrate the capacity for extensive fuel storage (Guglielmo, 2018) that is not observed to a similar extent in terrestrial migratory mammals. Foraging on-the-go is likely more feasible for terrestrial migrants than birds that, with the exception of aerial insectivores, must choose between flight and fueling. For instance, migrating mule deer (Odocoileus hemionus) spend 95% of their migrations at stopover sites, with a stopover for about every 5 km travelled (Sawyer and Kauffman, 2011). Marine animals can fuel their long-distance migrations with fat
accumulated before departure, without foraging along the way (Braithwaite et al., 2015; van Ginneken and van den Thillart, 2000), because the energetic cost of swimming is significantly lower than that of flying and walking (Schmidt-Nielsen; Hedenstrom 2003b). In sum, while the patterns predicted by the Escape Hypothesis are a possibility across taxa, the anticipatory fueling predicted by the Prepare Hypothesis is not expected for terrestrial mammalian facultative migrants.

Studies of captive pine siskins and red crossbills in the fall and winter found overall support for the Escape Hypothesis, with lower body condition, greater hopping activity, and higher CORT levels among birds experiencing marked decreases in food availability (Cornelius et al., 2010; DeSimone et al., 2021; Robart et al., 2019). Furthermore, siskins’ increases in activity were not sensitive to changes in food availability over multiple days, but rather to the amount of food received on a given day, and the time of day when they ran out of food (DeSimone et al. 2021). However, it is important to note that pine siskin and red crossbill migratory behavior differs significantly between spring and fall. Both species exhibit migratory preparations—increased fat stores and activity levels—in response to spring photoperiod, but not fall (Cornelius and Hahn, 2012; Robart et al., 2018). Spring movements are more predictable, involve migratory preparations, and likely function to return an individual to its breeding range after fall and winter movements (Robart et al., 2018; Watts et al., 2017).

The unpredictable nature of facultative movements makes them logistically challenging to study in the field. Previous studies have related the nomadic and irruptive movements of birds and ungulates to resource availability and climatic conditions (Bock and Lepthi, 1976; Koenig and Knops, 2001; Mueller et al., 2011; Olson et al., 2010; Pedler et al., 2014; Strong et al., 2015), or have examined the physiology of avian nomads in captivity (Cornelius et al., 2010; DeSimone et al., 2021; Robart et al., 2019). But studies relating physiology to the movement patterns of free-living nomadic species are generally lacking (Watts et al., 2018). This study aims to test the Prepare and Escape Hypotheses in free-living nomadic birds. During August – September of 2019 and 2020, we tracked the local movements of pine siskins in relation to their body condition, feeding behavior, and CORT levels. Because it is difficult to manipulate and measure food availability at a large scale, we instead measured triglyceride (TRIG) levels of each individual. Plasma triglycerides reflect fat deposition, and can be used to assess habitat quality and foraging intensity (Guglielmo et al., 2005). We aim to determine whether pine
siskins prepare for movements (i.e. increase body mass, TRIG, and baseline levels of CORT prior to departure), or rather escape (i.e. low body mass and TRIG, with stress-induced CORT levels, prior to departure). In contrast to captive research, studying free-living siskins ensures that both food availability and movement behaviors vary in biologically relevant ways. This is the first study that we are aware of that integrates physiological and movement data to better understand nomadic movements in the wild.

MATERIALS AND METHODS

Capture and sampling

We conducted fieldwork on MPG Ranch in Florence, MT (46.669, -114.026) during August – September of 2019 and 2020. We captured birds between 0700 and 1100 using standard mist nets and pine siskin playback recordings. We continuously monitored the nets to note exact capture time and extract birds immediately.

Up to 125 µL of blood were collected from the brachial vein using 30G needles. Blood samples were kept on ice in the field and were centrifuged in the lab within 6 hrs to separate plasma from red blood cells. Plasma was aliquoted: plasma used for CORT analysis was stored at -20 C and plasma for TRIG analysis was stored at -60 C. Only blood samples collected < 3 min from capture were included in analyses involving CORT (Romero and Reed, 2005); all blood samples were included in TRIG analyses.

Birds were aged as HY or AHY using plumage characteristics as in Pyle (1997). We visually scored fat stores on a scale from 0-5 (Moore and Kerlinger, 1987) and pectoralis muscle on a scale from 0-3 (Bairlein, 1995). We measured various morphometrics and weighed each bird to the nearest 0.1 g on a digital scale. We scaled body mass with the length of the exposed culmen (Peig and Green, 2009) ($F_{1,37} = 5.92; p = 0.02$).

All capture and handling methods were approved by the University of Montana Institutional Animal Care and Use Committee, and conducted under the appropriate state and federal permits.

Radiotelemetry

40 pine siskins (2019: n = 21; 2020: n = 19) were fitted with nano-tags (Lotek NTQB2-2) using standard leg-loop harnesses (Rappole and Tipton, 1991) and elastic thread (Gütermann
elastic thread #5019). Tags weighed < 3% body mass of each individual. An array of automated telemetry Motus stations (www.motus.org) on MPG Ranch (2019: n = 6; 2020: n = 5) and throughout the Bitterroot Valley to the south (2019: n = 4; 2020: n = 3) continuously monitored the presence/absence of tagged individuals. 38 birds were detected by at least one Motus station.

Birds were localized using handheld telemetry receivers (Lotek SRX-800). We localized each bird at least 2 hrs since its previous localization to 1) minimize disturbance to the bird and 2) ensure greater independence among points. When possible, we recorded the exact location of the individual using GPS units (189 of 426 localizations; Garmin GPSMAP 64st). When the bird was inaccessible or on private property, we triangulated its position using the coordinates and azimuth of 3 or more detections. We used Locate III software (Nams, 2005; Version 3.34) to calculate the triangulated locations; points with large MLE estimates (> 0.05 km²) were excluded.

We analyzed telemetry data in five ways to assess movement patterns and space use. 1) We estimated departure time as either the time of the last handheld telemetry detection of the individual, or the last time it was detected by a Motus station in Montana, whichever came later. We calculated “Days to Departure” as the time difference between capture and departure. 2) We used ArcMAP (version 10.6.1) to calculate the minimum convex polygon area for all of the localizations of each bird (“total polygon”) and 3) for only the localizations 0-2 days after capture (“3-day polygon”). 4) We used the distHaversine function in R package geosphere (Hijmans, 2019) to calculate the distance between consecutive localizations for each bird. 5) We estimated a total distance traveled per unit time, by summing the distances among consecutive points for each bird, and dividing by the amount of time (hrs) between the first and last localizations.

Corticosterone assay

Plasma CORT levels were measured using an Enzyme Linked Immunoassay (ELISA) kit (cat. No. 25-0412, Enzo Life Sciences). 2000 cpm of 3H-CORT was added to samples to estimate recovery. CORT was extracted from plasma using a double ether extraction. Ether was evaporated off in a 50°C water bath. Sample was reconstituted in 260 – 400 µL assay buffer for a final dilution of 1:20 – 1:25. Recoveries averaged 64.1 ± 0.05% (mean ± SD); samples were corrected to 100% for analysis.
Assays were run as described in Patterson et al. (2011). Briefly, 100 µL of extracted, diluted sample were assayed in triplicate if possible, and in duplicate or singly when plasma volume was limiting. Each plate included a standard curve (20,000 to 15.63 pg/mL). An external CORT standard was run in triplicate on each plate to quantify inter-plate variation. Plates were read at 405 nm corrected at 595 nm. Intra- and inter-plate coefficients of variation (CVs) were 11.25 and 12.33%, respectively. All samples were within the detectability limit for each assay.

Triglyceride assay

We quantified glycerol and total triglyceride using kits and a slightly modified protocol, as in Guglielmo et al. (2002). We thawed plasma samples on ice and diluted them three-fold with 0.9% saline (Sigma Aldrich S8776). Triglyceride assays were run in duplicate in 96-well microplates (Falcon 353910). A glycerol standard curve (Sigma Aldrich G7793) was included on each plate, with values ranging from 2.82 mmol/L to 0.17 mmol/L and a blank (0.0 mmol/L). 5 µL sample (or standard) and 240 µL glycerol reagent (Sigma Aldrich F6428) were added to each well, and read at 540 nm in a microplate spectrophotometer (ThermoFisher Multiskan Ascent 51118407) after incubating for 10 min at 37°C. 60 µL of triglyceride reagent (Sigma Aldrich T2449) were added to each well, and the plate was read again at 540 nm after another 10 min incubation at 37°C. All wells were corrected by blank wells, and concentrations were determined by comparison to the standard curve. Within individual CVs averaged 8.08%.

Statistical analysis

All statistical analyses were run in RStudio (version 1.3.1093), models were assessed using the performance package (Lüdecke et al., 2021), and figures were produced using ggplot2 (Wickham, 2016).

We log-transformed CORT, TRIG, Days to Departure, and Distance Traveled Per Time in all analyses to satisfy normality assumptions. To test the relationship between CORT and energetic state, we built GLMs examining the effect of logTRIG on logCORT, and the effect of scaled body mass on logCORT. We also constructed a GLM to test whether logTRIG predicts scaled body mass. Initial models included capture Julian day, capture time, and year as covariates, but only significant covariates remained in the final model, as noted in the Results section.
We used GLMs to test the effect of physiology on departure timing. Models tested whether logCORT at capture predicted logDays to Departure, and whether logTRIG at capture predicted logDays to Departure. We initially included capture Julian day, capture time, and year as covariates, but only retained significant covariates, which are noted in the Results section. A GLM relating scaled body mass to logDays to Departure had heteroskedastic and non-normally distributed residuals, leading us to try a breakpoint analysis. Using the chngpt package (Fong et al., 2017), we identified a breakpoint in the relationship between logDays to Departure and scaled body mass. We used GLMs to test the relationship between logDays to Departure and scaled body mass for points above and below this breakpoint. We used an F-Test to compare the variances of points to the left and right of the breakpoint.

Next we used GLMs to assess the effect of physiological metrics on various movement metrics. Each model tested the effect of one physiological metric (logCORT, logTRIG, or scaled body mass) on one movement metric (Total Polygon Area, 3-day Polygon Area, or Distance Traveled Per Time). We initially included capture Julian day and year as covariates, but only retained significant variants in the final model, as noted in the Results. We also initially included the total number of localizations of an individual as a covariate in the models with Distance Traveled Per Time, but removed it because it was non-significant and did not contribute to the model fit. We included a quadratic term in the model relating scaled body mass to Distance Traveled Per Time because this term had a p = 0.050 and greatly improved the model’s R².

Finally, to test the relationship between logDistance Traveled Per Time and logDays to Departure, we built a GLMM with Bird ID as a random effect and Year as a covariate.

RESULTS

Physiological Metrics

The range, mean, and standard error of body mass, triglycerides, and CORT are summarized in Table 1.

CORT levels were not predicted by TRIG (F_{1,27} = 0.33; β = -0.08; p = 0.57; Figure 1a) or by scaled body mass (F_{1,28} = 0.37; β = 0.01; p = 0.55; Figure 1b). TRIG levels did not predict scaled body mass (F_{1,35} = 0.37; β = 0.69; p = 0.55; Figure 1c).

Physiology and Departure Timing
CORT levels did not predict days to departure ($F_{2,27} = 2.20; \beta = -0.08; p = 0.91$; covariate: Year; Figure 2a). Birds with higher TRIG levels departed sooner after capture ($F_{2,34} = 6.36; \beta = -0.81; p = 0.04$; covariate: Year; Figure 2b).

We identified a breakpoint in the relationship between scaled body mass and days to departure (Figure 2c). Higher scaled body mass predicted fewer days to departure among birds with scaled body masses below 13.32 g ($F_{1,22} = 5.03; \beta = -0.17; p = 0.04$), while days to departure did not vary with scaled body mass among heavier birds ($F_{1,13} = 0.58; \beta = -0.22; p = 0.46$). The variance in days to departure was also significantly smaller among birds lighter than 13.32 g compared to birds with scaled body masses higher than the breakpoint ($F_{14,23} = 6.01; p = 0.0002$).

**Physiology and Movement Patterns**

Scaled body mass predicted the distance birds traveled over time ($F_{2,29} = 2.19; p = 0.048$; covariate: quadratic term; Fig. 3), with birds of intermediate mass traveling further than both lighter and heavier individuals. Scaled body mass did not relate to our other spatial metrics: total polygon area ($F_{2,25} = 3.99; \beta = 1.17; p = 0.13$; covariate: Julian day of capture) or 3-day polygon area ($F_{1,19} = 0.10; \beta = 0.05; p = 0.75$). We found no relationships between CORT and the total polygon area ($F_{2,19} = 4.43; \beta = 7.25; p = 0.35$; covariate: Julian day of capture), 3-day polygon area ($F_{1,18} = 0.37; \beta = 0.67; p = 0.55$), or the distance traveled per time ($F_{1,22} = 0.87; \beta = 0.436; p = 0.36$). TRIG did not relate to these spatial metrics either: total polygon ($F_{2,23} = 2.86; \beta = -2.48; p = 0.60$; covariate: Julian day of capture); 3-day polygon ($F_{1,17} = 1.38; \beta = -1.19; p = 0.26$); distance traveled per time ($F_{1,28} = 3.39; \beta = 0.52; p = 0.08$).

**Movement and Departure**

On average, for each bird, the distance between consecutive localizations increased as departure time approached ($F_{1,325.8} = 9.53; \beta = -0.18; p = 0.002$; covariate: year; random effect: Bird ID; Figure 4).

**DISCUSSION**

Facultative and nomadic movements are poorly understood, and clarifying the environmental and physiological cues underlying these movements is critical to understanding
the evolution and diversity of migratory strategies. This study is the first to investigate the physiology of nomadic migrants in relation to their movement in the wild. We tested whether birds fuel up before flight (Prepare Hypothesis) or rather leave an area when food resources and internal body stores are depleted (Escape Hypothesis). We found that individuals had higher TRIG levels as they neared departure (Fig. 2b), indicating that foraging success or energetic condition relate to departure decisions. However, we found that scaled body mass only predicts departure among birds below a breakpoint mass (Fig. 2c). Birds with lower body mass stayed around the field site longer, but at masses above the breakpoint, mass did not predict departure. Together these results suggest that moderate energy stores are necessary for departure, but movement decisions depend on other factors among birds with sufficient fuel. Our results do not fully support either the Prepare or Escape Hypotheses. Instead, we observed an intermediate pattern: siskins need adequate—but not large—fuel stores to initiate departure.

In contrast to this field study, captive studies demonstrate support for the Escape Hypothesis and show clearer relationships among body condition, food availability, and activity (DeSimone et al., 2021; Robart et al., 2019). In these captive studies, siskins experiencing low food availability in the fall lose body mass and show increases in CORT and activity levels. Bennetts and Kitchens’s (2000) framework relating behavior to food availability (Fig. 5) helps explain the discrepancy between captive studies and our field results. Bennetts and Kitchens hypothesize that the probability of movement or departure is expected to vary in relation to food availability. When food availability is critically low, birds must leave their current area or die. At moderately low food availability, birds are less likely to depart because they do not have sufficient fuel stored for flight and can still subsist on local food resources. As food availability increases, the risk of movement decreases and thus the probability of departure increases. At high levels of food availability, the probability of departure decreases again because birds benefit from maintaining high-quality territories.

We believe this framework helps reconcile captive and field results and can help predict facultative departure probability. Captive studies have tested siskins’ responses to low food availability and found support for the Escape Hypothesis. Birds in these studies were likely in the “escape or starve” region (Fig. 5). Captive siskins increase activity when they have little or no food remaining each day, and CORT levels only increase among birds experiencing sustained food reductions (DeSimone et al. 2021). Our field results fit within the central region of Figure 5.
Birds in poor energetic condition stayed in the area for up to 27 days (“high-risk for marginal benefits”). Birds with heavier body masses had the fuel necessary to depart if they chose to, but body mass did not relate to departure among these birds (“low-risk for marginal benefits”). In our study, birds with higher TRIG levels were closer to departure time, corresponding with the transition from high-risk to lower-risk movements.

Furthermore, the combined results of this study and those of DeSimone et al. (2021) suggest that, as departure probability increases, variation of departing individuals’ body condition increase. For instance, the average mass of pine siskins at the start of the DeSimone et al. (2021) captive study was 14.56 g, much heavier than the wild-caught birds of this study, and siskins experiencing a variety of food restriction regimes increased activity during times of day when food availability was critically low or absent. In the present field study, departure was limited to those individuals with sufficient stored fuel. In other words, when food availability is critically low, all individuals depart, regardless of their energetic condition, while only those in good condition depart areas with moderate food availability.

We found no relationships between CORT levels and various metrics of movement behavior. This was unexpected, as previous studies have linked CORT with locomotor activity in captivity (Breuner et al., 1998; Cash and Holberton, 1999), activity ranges in the field (Breuner and Hahn, 2003; Jessop et al., 2018), and predictable transitions to life stages associated with increases in activity, such as fledging (Heath, 1997; Sprague and Breuner, 2010), dispersal (Belthoff and Dufty, Jr, 1998), and obligate migration (Eikenaar et al., 2017, 2020; Löhmus et al., 2003; Piersma et al., 2000). In this study, we do not know CORT levels at the moment of departure, and physiology at capture may be too far removed temporally from physiological drivers of departure. However, CORT did not relate to area use in the few days immediately following capture, either. Future experimental work with exogenous CORT could explicitly test its effect on nomadic and irruptive movements.

On average, siskins increased the distance travelled between consecutive localizations as they neared departure time (Fig. 4). This result suggests that siskins may explore a broader area when deciding when to depart and where to go next. We also found that birds of intermediate body masses traveled the furthest distances around our field site (Fig. 3). One explanation is that light and heavy birds may restrict their movement to conserve energy or remain in an area with abundant resources, respectively. Finer-scale temporal and spatial data could reveal more
information about what kind of environmental or social information siskins may gather during these pre-departure movements. Furthermore, because body condition did not predict departure among birds with sufficient fuel, future studies could investigate other climatic, environmental, or social factors that may inform siskins’ departure decisions. Nomadic finches are often social, form flocks, and likely share information with each other about food availability or to coordinate group movements. Captive pine siskins and red crossbills vocalize more frequently when food-restricted (Robart et al., 2019; Smith et al., 1999), and wild pine siskins increase their call rate before departing from a foraging area (S. Sriraman, unpublished data).

By integrating physiological and movement data, we conclude that siskins’ local movements are clearly distinct from both obligate migrations, which involve extensive physiological preparations, and fugitive migrations, which are initiated in response to a large and sudden disturbance. Our study also illustrates the importance of testing captive findings in the wild, where both food availability and movement behavior vary in ecologically relevant ways. Finally, we demonstrate the use of Bennetts and Kitchens’ (2000) framework in relating physiology and food availability to different movement probabilities.

ACKNOWLEDGMENTS
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REFERENCES


Table 1. Range, mean, and standard error of the body mass, triglyceride, and corticosterone levels of all pine siskins included in analyses.

<table>
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<th>Range</th>
<th>Mean</th>
<th>SE</th>
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<tr>
<td>Body mass (g)</td>
<td>11.43 – 14.60</td>
<td>12.78</td>
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<tr>
<td>TRIG (mmol/L)</td>
<td>0.55 – 5.29</td>
<td>1.51</td>
<td>0.14</td>
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<tr>
<td>CORT (ng/mL)</td>
<td>1.15 – 3.61</td>
<td>1.99</td>
<td>0.13</td>
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Figure 1. Corticosterone was not predicted by (A) plasma triglycerides or (B) scaled body mass. Plasma triglycerides did not predict scaled body mass (C).
Figure 2. Physiology and departure timing. (A) Corticosterone did not predict departure timing. (B) Birds with higher levels of triglycerides departed sooner after capture. (C) Scaled body mass negatively correlated with days to departure among birds below the breakpoint at 13.32 g; departure did not relate to scaled body mass among heavier birds.
Figure 3. An individual’s scaled body mass predicted the estimated total distance traveled during their time spent at the field site. On average, birds of intermediate body mass traveled further distances than both lighter and heavier individuals.
Figure 4. The distance (m) between consecutive localizations of an individual increased as departure time neared. Note the inverted x-axis, with departure time approaching from left to right.
**Figure 5.** Hypothesized departure probability along a gradient of food availability. The light shaded area indicates the region previously tested and observed by captive studies. The dark shaded area indicates the region likely observed in this field study. See main text for further explanation. Adapted with permission from Bennetts and Kitchens (2000), © OIKOS 2000.