

Automated Serial Sampling in  
Hummingbirds Obviates Laborious Ecological Studies of  
Behaviourally Induced Rises in Departure Stores

by

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A thesis submitted in conformity with the requirements  
for the degree of Master of Science  
Graduate Department of Ecology and Evolutionary Biology  
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## Abstract

I examined the feasibility of automating the collection of free-ranging hummingbird mass data facilitated by low-cost, low-power radio frequency identification (RFID) technology. Wild hummingbirds were captured, subcutaneously implanted with passive integrated transponder (PIT) tags, and released. Detection of tagged hummingbirds perched on balances at feeder stations outfitted with RFID readers initiated the recording of masses at regular intervals, continuing for as long as the birds remained. Mass data showed consistent trends at multiple temporal scales: the individual feeder visit, single days, and whole seasons. Using these data, I modelled changes in foraging behaviour during the morning, mid-day and evening, and examined rates of overnight mass change to determine how wild hummingbirds achieve premigratory fattening. Premigratory hummingbirds increased the duration of visits and thus total time spent at the feeders during the mid-day only and exhibited greater use of overnight torpor, as evidenced by a reduced rate of overnight mass loss.

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## Chapter 1

### General Introduction

Seasonal migration is an important life history event marked by changes in both behaviour and physiology. Migration presents a considerable selection pressure due to the large energetic demands and the many challenges an individual faces during a migratory journey, including ecological barriers, competition for food at stopover sites, predation, and unfavourable weather (Moore et al., 2003). Moreover, improper timing of a migratory journey can lead to a cascade of effects with severe consequences, where individuals arriving too early or too late at a destination could encounter adverse weather and suboptimal or insufficient food resources (Coppack and Both, 2002) leading to a reduction in fitness or possibly death.

The challenges faced by migrants are often energetically demanding; common across many migratory species is the amassing of energy stores prior to departure. For many organisms, migration is a time during which foraging activities are suspended to allow for continuous movement. Migrants require energy reserves above that necessary for the journey to the next stopover site such that an energetic buffer exists in the event that variable conditions en route result in an energetic shortfall (Moore et al., 2003). Moreover, insufficient energy stores could prolong migration, resulting in protracted exposure to migratory costs and risks, and contributing towards delayed arrival at the destination whereby time necessary for recovery and maintenance or breeding is potentially abbreviated (Alerstam, 2003). Amassing adequate energy stores prior to and during migration is therefore paramount to survival and can provide an added benefit; energy reserves in excess of that required for migration can be redirected towards other activities upon arrival at the destination (Moore et al., 2003), such as establishing and defending territories and reproduction. However, mass gain and the storage of fat have associated costs, such as increases in energetic expenditure related to locomotion, and decreases locomotor performance (e.g. acceleration and agility) that can increase predation risk (Alerstam et al., 2003). Migratory individuals must therefore balance the costs and benefits of fattening in order to ensure successful migration.

Regulating and balancing energy intake and expenditure is vital for all forms of life. Energetic demands can vary seasonally with changing environmental conditions, and for life history events

such as reproduction, migration, and hibernation. In the study of energetics, hummingbirds are a uniquely important and fascinating study system where their highly energetic existence due to small size, large thermoregulatory costs, and their use of hovering flight is largely fueled by ephemeral food sources. For migratory species such as the ruby-throated hummingbird (*Archilochus colubris*), seasonal migration is an additional energetic challenge. As the storage of energy prior to departure is often necessary to fuel migration, premigratory energy needs increase substantially in individual hummingbirds and necessitates changes in energy intake and expenditure.

Ecological studies examining physiological and behavioural aspects of energy acquisition in the field have traditionally been time- and labour-intensive. Logistical hurdles related to the identification of unique individuals, and the capture and recapture of individuals for data collection can be onerous. Moreover, stress induced by repeated capture and handling can disrupt normal behaviour and physiology, thereby biasing data.

In this work, I present a method that permits the automated serial collection of relevant physiological and behavioural data on uniquely identified individuals while minimizing the need for repeated captures, and greatly reducing time- and labour-investments. Using this method, I was able to gain insights into foraging strategies necessary for the adequate fueling of migration in one of the most metabolically active vertebrates, the ruby-throated hummingbird.

# Chapter 2

## Automated Tracking of Wild Hummingbird Mass and Energetics Over Multiple Time Scales Using Radio Frequency Identification (RFID) Technology

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### 1 Introduction

The collection of physiological data from wild animals often requires the repeated capture of individuals, which is typically opportunistic and limited by a low probability. Handling can be a physiological stressor to individuals (e.g. Cabanac and Aizawa, 2000; Remage-Healey and Romero, 2001) and can cause persistent changes in behaviour and energy balance, such as learned aversion to traps (Zarnoch and Burkhart, 1980), delayed egg-laying (Buttler et al., 2011), impaired territorial defense (Carpenter et al., 1983), or sustained mass gain (Macloed and Gosler, 2006). In a study of wild animals, such changes in behaviour and physiology can bias data. It is therefore necessary to develop alternative methods to collect relevant data from wild animals while minimizing the need for repeated captures.

Physiological studies of free-living animals have been constrained by several logistical limitations. In many cases, recapture rates have been frustratingly low, such as in hummingbirds with recapture rates ranging from 17 to 32% (Brewer et al., 2011; Calder III et al., 1983; Hilton Jr. and Miller, 2003; Powers and Nagy, 1988; Powers and Conley, 1994). Moreover, the efficiency of data collection can be limited by the recapture of individuals within and between seasons. Furthermore, the efficacies of certain techniques (e.g. the use of doubly-labeled water) are temporally constrained, requiring recaptures to occur within brief temporal windows (e.g. Powers and Nagy, 1988; Powers and Conley, 1994; Weathers et al., 1996). Even approaches that do not rely on the recapture of individuals have drawbacks. Unique identifiers that are remotely detectable by observers can wear or fall off as birds molt, thus individuals are not easily tracked across seasons. Moreover, the tracking of marked individuals in the field can be both time- and labour-intensive (e.g. Carpenter et al., 1991).

More recently, it has become possible to overcome these logistical hurdles and gather both physiological and behavioural data on free-living birds using battery powered transmitters and geolocators, some of which can monitor variables such as heart rate and temperature (Kettlewell et al., 1997), track home range movement (Dunn and Gipson, 1977), and migration (Stutchbury et al., 2009). However, such approaches have been limited to use with larger avian species, as the Bird Banding Laboratory (BBL) of the United States Geological Survey only permits the attachment of devices that are 3% or less of a bird's body weight. While dataloggers and transmitters can allow for the collection of spatial, temporal, physiological and behavioural information of individuals (Cooke et al., 2004), these devices rely on an internal battery, and therefore have a limited lifespan.

Unlike other technologies, passive integrated transponder (PIT) tags as small as 5mm in length show promise as unique identifiers in animals as small as hummingbirds (Brewer et al., 2011). Because PIT tags do not rely on an internal battery, they can potentially provide data collection over the lifetime of a tagged individual (Brewer et al., 2011). To date, RFID technology has been used in many long-term ornithological studies that examine presence-absence, movement, mating, nesting, reproduction, provisioning, foraging, and homing behaviours of birds (reviewed by Bonter and Bridge, 2011).

Serial collection of physiological data facilitated by the use of PIT tags has previously been successful in larger avian species, allowing for the collection of visitation and body weight records daily (Boisvert and Sherry, 2000; Macleod, 2006). However, the feasibility of this approach has not yet been explored for the smallest avian species. My objective was to determine whether RFID technology could be successfully harnessed to permit the serial collection of hummingbird mass records in the field. The tracking of mass over short time periods in hummingbirds can be a reliable indicator of energetic status (Beuchat et al., 1979; Gass et al., 1999). Hummingbirds exist at the extreme end of the energetic spectrum, where a high mass-specific metabolic rate (Suarez, 1992), small size, and highly energetic hovering lifestyle mean changes in energy balance quickly manifest as changes in mass.

I tested the efficacy of the smallest commercially available PIT tag (7mm) compatible with common readers used in biological research and examined if it could be used to automate the serial monitoring of hummingbird mass. With the hope of reducing overall equipment costs, I

tested the use of a low-cost, low-power RFID reader, as the financial burden of commercial RFID technology can be prohibitive (Bonter and Bridge, 2011). I predicted that data collected using this approach would have high temporal resolution permitting observations of hummingbird mass change at time scales of seconds to entire seasons. Specifically, this method would permit consistent observations of a) hummingbird mass gain over the course of individual feeding events, b) changes in hummingbird mass over the course of a day from sunrise to sunset (Beuchat et al., 1979; Calder et al., 1990), and c) seasonal changes in hummingbird mass such as exponential mass gain prior to migration (Carpenter et al., 1993). The identification of patterns of mass change similar to those reported in existing literature (e.g. Beuchat et al., 1979; Calder et al., 1990; Carpenter et al., 1993) would allow us to conclude that mass variation is reliably measured in the field using this approach, thereby revealing physiologically and ecologically relevant phenomena.

## 2 Methods

### 2.1 Study Site

The study area, in King City, Ontario, was approximately 8 hectares of irregularly shaped land, and centrally located on the Koffler Scientific Reserve (KSR) at Joker's Hill ( $44^{\circ} 1' 47''$  N,  $79^{\circ} 32' 2''$  W). The reserve was surrounded by mixed forests and comprised of open meadows and two ponds.

### 2.2 Automated Stations

Six free-standing stations were constructed on the reserve. In addition to the RFID antenna and reader (see below), each station consisted of a Perky-Pet® commercial hummingbird feeder (Model #: 220, Perky-Pet, Lititz, Pennsylvania, USA) with five of six “flowers” sealed so that only one was available to the bird and a Denver MAXX digital scale (model no: MXX-212, Sartorius, Bohemia, New York, USA) onto which a perch was mounted for mass measurements (figure 1). Tag detection and mass measurements were recorded to a notebook PC via direct serial communication. All electronic components were housed in weatherproof boxes.

Of the six stations constructed on the reserve, one employed a commercially-available Biomark Reader (FS2001F-ISO, Biomark Inc., Boise, Idaho, USA) and racquet antenna to detect PIT tags (hereafter referred to as ‘station 1’). The remaining five stations were constructed using a low-cost, low-power RFID reader. This involved a custom-made circuit board designed by Dr. Eli Bridge (Bridge and Bonter, 2011) with programmable poll, pause and data logging times. Upon receipt of tag information from the reader, a custom designed MATLAB script (Mathworks Inc., Natick, Massachusetts, USA) queried the digital balance seven consecutive times at 0.5 second intervals. Unique tags were recorded, when in range, as often as every five seconds. This allowed hummingbird mass to be recorded nearly continuously as long as individuals remained within the read range of the antenna (Biomark: ~9cm; low-power RFID: ~1cm). In the absence of a bird, mass readings were automatically obtained every 10 minutes in a pattern identical to that above. This mass record (referred to as the ‘baseline’) permitted corrections for balance drift and triggered automated balance taring when the reported “empty” mass was more than  $\pm 0.3$  g. A

12V deep-cycle battery attached via a trickle-charger to AC power was used to power the RFID board, antenna, and balance at each station. Notebook computers were attached to AC power. While a total of six stations were constructed in 2012, a fatal error on one of the electronic balances reduced the number of functional stations to five in 2013.

Except for station 1, I manufactured a low-cost, low-power antenna for use with each low-power reader. The antennas were constructed using 28-gauge copper magnetic wire wound to create a coil of approximately 3.2cm in diameter, and with an inductance of 1.17mH corresponding to the frequency of the PIT tags. Each antenna was secured in four spots with small strips of duct tape and coated with silicon glue and clear lacquer. Antennas were then attached to the artificial flower of a Perky-Pet® feeder perpendicular to the ground.

## 2.3 Tagging in the Field

Capture and use of ruby-throated hummingbirds were approved under the auspices of the University of Toronto Local Animal Care Committee (protocol numbers: 20008398, 20008930, 20009510, 20010080) and the Canadian Wildlife Service in Ontario (permit number: 10813).

Hummingbirds were captured, banded and tagged between June and August in 2011 and between mid-May and mid-September in 2012 and 2013 for 1 to 2 days every week. Trapping occurred between sunrise and 1000h and was suspended during periods of moderate to intense rainfall. During trapping, RFID readers were removed from the stations and replaced with wire-mesh trap-door cages and Perky-Pet® artificial feeders supplied with ~25% (w/v) sucrose solution. Hummingbirds were captured at six stations on the reserve. Captured birds were extracted from the cage, placed in a mesh bag and transported to the KSR lab for processing. The age (juvenile: < 3 months old; adult: ≥ 1 year) and sex of the bird was first determined followed by collection of morphological data and tarsal banding according to standard BBL practices.

I chose to employ the smallest commercially available PIT tag, the 7mm UNO PICO ID ISO transponder (~0.026 g). Tag implantation was performed in the field using a method similar to that reported by Brewer et al., (2011). With an assistant gently restraining the bird, I proceeded to implant the transponder. A cotton swab was used to disinfect and anesthetize the skin on the

dorsal surface of the bird between the scapulae with betadine and 1% Lidocaine solution, respectively. Using a pair of forceps to lift the skin, the PIT tag was implanted subcutaneously with a syringe at a shallow angle. The injection site was then sealed using 3M™ Vetbond™ Tissue Adhesive to promote tag retention. The bird was then allowed to feed freely from a feeder while being held. To obtain the unique alphanumeric code of the PIT tag, the bird was passed through an antenna before being released at the station where it was originally captured. Implantation was typically accomplished in approximately 5 minutes and total bird handling time was approximately 15 minutes.

## 2.4 Data and Statistical Analyses

I identified individual visits to each feeder station by unique hummingbirds as all sequential readings occurring within 10 seconds of each other. As the response of the electronic balance is not instantaneous, and because hummingbirds could, in the case of station 1, be detected by the RFID reader while not on the perch, it was necessary to filter mass readings associated with each visit. Erroneous or artifact mass readings were filtered by regressing mass versus time within each visit. Readings with Cook's distance values greater than  $4/n$ , where  $n$  is the number of points used, or where the calculated rate of change in mass between sequential readings was  $> 0.5 \text{ g/sec}$ , were excluded. Mass values were corrected for balance drift. Corrected mass values that were outside the biologically realistic range (i.e. less than 1.5 grams or greater than 10 grams) were also excluded. The biologically realistic range of male and female ruby-throated hummingbirds was determined by examining recorded masses of ruby-throated hummingbirds at the time of banding by a fellow hummingbird bander for over 300 males and over 300 females between 2003 and 2012. The threshold values were set at 117% above the maximum recorded mass value and 37% below the minimum recorded mass value such that mass values outside this range are highly unlikely.

I analyzed a subset of visits greater than 30 seconds in length to obtain a net rate of mass change over one visitation, recognizing that this integrated both mass gain via nectar intake and mass loss due to urination. Least-squares linear regressions were developed for each visitation to determine average rate of integrated mass gain.

Daily mass variation of hummingbirds was assessed by selecting individuals on days for which there were more than 50 mass records. I then created two-hour time bins between 0500h and 2200h and selected individuals on days for which I had four or more mass records in at least seven time bins. Masses and times were averaged for each two-hour time bin. A linear least-squares and parabolic model was fitted to these variables for each individual on each day. From this I obtained adjusted  $R^2$  ( $R^2_{adj}$ ) values and assessed the relative quality of the two models using the Akaike information criterion (AIC) scores.

Pre-migratory mass gain in hummingbirds was examined beginning 14 days prior to the presumed date of migration, identified as the date of last recorded mass. Median mass measurements for each visit obtained between 1900h and 2000h were averaged and modeled exponentially as a function of the number of days before departure using a nonlinear mixed effects model with individual included as a random factor. These same variables were modeled using a least-squares linear mixed effects model. The relative quality of the two models was determined using AIC scores. Percentage mass gain and rate of mass gain for each individual was calculated using the difference in mass between day 0 and day 4, where mass gain appeared to be linear.

All statistical analyses were conducted using R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Linear and nonlinear mixed-effects models were developed using the R packages, lme4 version 1.0-5 (Bates et al., 2013) and nlme version 3.1-113 (Pinheiro et al., 2013), respectively. Data are presented as mean  $\pm$  SEM, except where noted.

## 3 Results

### 3.1 Tagging in the Field

A total of 118 ruby-throated hummingbirds were tagged at KSR – 12 of which were tagged during a pilot study in 2011, 50 were from 2012, and 56 were from 2013. Of the 118 birds, 79 were female and 39 were male. By the end of 2013, ~70% of all tagged hummingbirds (82 individuals) were redetected at the stations at least once after initial capture. Annual hummingbird redetections since 2011 have been summarized in table 1. The return rate in 2012 of individuals originally banded during the pilot study in 2011 was 33% (table 1), while the return rate in 2013 of individuals originally banded in 2012 was 24% (table 1). Overall, 26% of individuals were redetected in years subsequent to the initial year of tagging. Over 27,100 separate visits were recorded from tagged hummingbirds at five active feeder stations between May and September of 2013. Ten hummingbirds were identified as regular visitors, each exceeding 600 recorded visitations at the stations. The most frequent visitor was an adult female with nearly 8000 recorded visitations.

In 2012, three females returned from 2011, but only one fed at the stations regularly throughout the summer – of the remaining two females, one was redetected 20 May to 22 May 2012, and the other was redetected on 25 May and 27 May 2012.

### 3.2 Patterns of Mass Change Through Time

Mass readings consistently exhibited a positive linear relationship with time over the duration of a visitation (figure 2). I calculated that hummingbirds gained mass at a median and average rate of  $7.0 \pm 0.3$  mg/s (6383 observations, 40 hummingbirds, min = 4 mg/s and max = 12 mg/s).

Mass records of 7 hummingbirds for 99 total days (min = 1 day/individual, max = 53 days/individual) were selected, based on the criteria outlined above, to determine the pattern of mass variation in relation to time of day. Parabolic models fit patterns of mass change throughout the day better than linear models for 42 of 99 days, as determined using AIC scores, where mass variation presented as U-shaped curves over time (figure 3 and figure 4a;  $0.12 < R^2_{adj} < 0.92$ ).

Inversely parabolic models performed better than linear models in 12 additional analyses (figure 4b;  $0.06 < R^2_{adj} < 0.87$ ). For 30 of 99 days, linear models best represented the variation of mass in relation to time (figure 4c;  $0.05 < R^2_{adj} < 0.70$ ). The remaining 15 days analyzed produced negative  $R^2_{adj}$  values for both linear and parabolic models (figure 4d).

For 3 individuals for which large numbers of mass records were available between late August and early September, I observed consistent, predictable patterns of exponential mass gain prior to migration (figure 5; table 2). Average percentage mass gain beginning 4 days prior to migration was calculated to be  $23.56 \pm 2.57\%$ , at an average rate of  $0.23 \pm 0.02$  g/day.

## 4 Discussion

In the field study, the use of PIT tags and RFID technology allowed the redetection of 70% of tagged individuals, which is much greater than the recapture rates reported previously. A smaller field study by Brewer et al. (2011) using a similar method of PIT tagging in ruby-throated hummingbirds reported redetections after initial capture to be 55.5%, while redetections of hummingbirds in years subsequent to the year of initial tagging were reported to be 26%. These values are comparable to those found in the study. Reasons for failing to redetect individuals after initial tagging may include tag loss, the movement of individuals away from the study location, individuals becoming averse to feeder stations as a result of capture or handling, and death of the individual. Hummingbirds tagged early and late in the season may have been migrating through the area and thus may not have remained at the study site after release. This could explain the redetection of two females in late May 2012 for only two to three days. A 70% redetection rate and the return of individuals in subsequent breeding seasons are evidence for the lack of long-term negative effects and the successful retention of subcutaneously implanted PIT tags.

Analyses of the data produced results consistent with what has been found in other physiological studies of hummingbirds that employ highly labour intensive methods of data collection. Hummingbirds have been found to exhibit feeding restraint during the day to maximize aerial performance and only gained mass at dusk (Calder et al., 1990). For the majority of days examined, patterns of mass versus time of day exhibited either a linear or a U-shaped relationship. U-shaped relationships best described data on 42 of 99 days and suggest that this approach generally revealed patterns of mass variation throughout a day that are consistent with laboratory (Beuchat et al., 1979) and smaller field studies (Calder et al., 1990). Where mass data were best described by a linear increase over time, data from 7 of 30 days pertained to an adult female between 23 August 2013 and 5 September 2013. Because these dates are near the presumed date of migration, I hypothesize that this individual may have switched from prioritizing flight maneuverability to mass gain prior to migration. While other patterns of daily mass change were observed, there were no immediately obvious temporal or environmental variables coinciding with the observed variation. With continued data collection, future studies can better analyze this variation. In addition to demonstrating patterns of daily mass change, I was able to identify a pattern of exponential mass gain across multiple days prior to

hummingbird migration. Fall migration of hummingbirds typically begins as early as mid-August for males and September for females in Ontario, Canada (Sandilands, 2010). Of the ten hummingbirds frequenting the stations, three provided continuous records of mass between late August and early September. The data show that body mass of these three ruby-throated hummingbirds increased exponentially several days prior to migration and is similar to that found by Carpenter et al. (1993) in rufous hummingbirds. Premigratory mass gain is further examined in the next chapter. For all other tagged hummingbirds, the records show that the date of last recorded mass was prior to August 26<sup>th</sup> in 2013. Pre-migratory mass gain was not observed in these individuals suggesting that individuals may move away from the breeding site prior to migration. Such movement has been previously reported by Saunders (1936) who observed the arrival of males after mid-July in New York coinciding with a change in food availability and is suggestive of a switch in breeding habitats.

The automated system employed in this study eliminates the need for repeated captures, thereby minimizing the disturbance this experiment has on study subjects. In addition to collecting usable physiological data, this technology permits the monitoring of certain life history events such as migratory arrival and departure while requiring minimal labour input (three hours of personnel time per week for the maintenance of six stations). Moreover, the lower power consumption of the low-cost RFID reader at 5 volts compared to the Biomark FS2001F-ISO unit at 12 volts provides an additional advantage by permitting greater longevity if the unit draws from a battery supply. Commercial RFID readers intended for use in animal studies offer many advantages to the low-power reader used in five of the stations (e.g. superior read range, greater portability or durability). Our intention is not to imply that low-cost readers can replace the need for such specialized equipment in most or all applications. However, this study does demonstrate that lower-cost, low-power readers can be successfully used to monitor hummingbird visits and, when coupled with a balance, mass of individuals. Equipment cost per station has been summarized in table 3. By substituting the commercial Biomark Reader (Model#: FS2001F-ISO) with a custom RFID circuit board and low-power antenna, I have reduced the equipment cost of a station, which includes a precision electronic balance, from \$3680 to \$791 CAD. A cost savings of 79% makes this miniaturized design a cost-effective technology for the physiological and ecological study of wild hummingbirds. Ongoing work aims to replace the cumbersome notebook computer used in the setup with smaller, less expensive, and lower power computing

circuitry. In doing so, one can further reduce reader station cost and rely exclusively on 12V battery (coupled with solar) power.

While this design employs an antenna with a much smaller read range than that of the Biomark antenna (~1cm and ~9cm perpendicular to the plane of the antenna, respectively), the miniaturization of the antenna does not compromise its ability to detect PIT tags that are subcutaneously implanted into hummingbirds. Particularly for small birds, strategic placement of the antenna in relation to food resources can ensure the successful detection of subcutaneous PIT tags. In some instances, such as when multiple birds are competing to feed simultaneously at one feeder, a smaller reading range is desirable for identification accuracy. Though tag migration has been a reported disadvantage of subcutaneous implantation (Bonter and Bridge, 2011), in small birds any migration is unlikely to position the tag out of an antenna's detection range. Given the large number of hummingbird redetections within a season, and the redetection of hummingbirds in subsequent breeding seasons, antenna read range and tag migration are not issues of concern.

Implementation of a low-cost, low-power RFID reader in concert with an electronic balance in the field has successfully permitted the automated, serial collection of hummingbird physiological data. The return of tagged hummingbirds in subsequent years and the large quantity of data collected lends support to my hypothesis that ruby-throated hummingbirds are not adversely affected by tagging. Moreover, tagged hummingbirds consistently incorporate nectar from the artificial feeding stations into their diet thereby permitting consistent data collection. As predicted, the regular, repeated recording of mass generated high-resolution data that revealed trends in mass change at time scales of seconds, hours, and days in the wild, demonstrating that this approach can be used to track individual and population-level energetics in even the smallest of avian species.

Given the success of this approach, I contend that the automated and repeated collection of additional types of data from hummingbirds and other small birds is feasible. For example, it may be possible to automate the collection of feather samples for genetic analyses, collect exhaled breath for respirometry (Bartholomew and Lighton, 1986), and obtain feather, tissue, or breath samples for isotopic analyses.

## Chapter 3

### Premigratory Energy Needs Increase Substantially: *Archilochus colubris* Use the Mid-Day to Engorge on Nectar

## 1 Introduction

Migration is an important life history event for many species in which behavioural and physiological changes are necessary to facilitate relatively large-scale movement. Individuals migrating over long distances typically risk mortality and incur significant time and energetic costs (Alerstam et al., 2003). While for most organisms, seasonal migration offers advantages to fitness; these advantages can only be realized upon the successful completion of migration.

For many migrating individuals, mass gain is a prerequisite change that includes the hypertrophy of locomotor structures, such as flight muscles for avian species (e.g. Dietz et al., 1999; Marsh, 1984), as well as the deposition of fat, which is used as a primary fuel source for flight (Lindstrom, 1991). Migration involves sustained movement over extended periods of time, where the act of moving precludes foraging. Migrating individuals also encounter inhospitable environments, unpredictable resource availability, and extreme climatic conditions that limit opportunities to consume food or water. Moreover, some migratory journeys include periods during which there are no foraging opportunities, such as when volant animals traverse large bodies of water or deserts. Amassing adequate energy reserves prior to the initiation of migration is therefore paramount to individual survival.

Premigratory fattening is made possible through seasonal changes in behaviour and physiology that alter energy intake and expenditure. A shift in foraging behaviour that allows for greater food intake is often observed in fattening individuals (Scott et al., 1994; Bairlein, 2002). This behavioural shift can also be accompanied by physiological changes that facilitate the storage of energy as fat, such as increased efficiencies in energy and nutrient absorption (Scott et al., 1994). In addition to increased energy intake and assimilation, energy balance can be altered through reductions in energy expenditure. Use of nocturnal hypothermia and torpor permits energy conservation (Hiebert, 1990), where metabolic rate and body temperature can be reduced to a

fraction of normothermic values (Geiser, 2004; Bartholomew et al., 1957; Carpenter and Hixon, 1988; Carpenter et al., 1993; Butler and Woakes, 2001)

Rates of premigratory mass gain are highly variable; whereas for many avian species, mass gain occurs over the course of several weeks (Bairlein, 2002), hummingbirds achieve the majority of premigratory mass gain within approximately 4 days leading up to migration (Carpenter et al., 1993; Hou et al., 2015). Given their high mass-specific metabolic rate (Suarez, 1992), high thermoregulatory costs due to small size, and the use of energetically expensive hovering flight, such rapid mass gain illustrates the exquisite control hummingbirds maintain over the balance of energy intake and expenditure.

Hummingbirds provide a unique study system where changes in energy balance manifest as changes in mass over short time frames (Beuchat et al., 1979; Gass et al., 1999). The pattern of daily mass change for hummingbirds has been assessed both in the laboratory (Beuchat et al., 1979) and in the field (Calder et al., 1990) during non-migratory periods and characterized as having a U-shaped pattern where mass is high in the early morning as a result of compensatory feeding after overnight fasting, maintained at a reduced level during the day, and increases in the evening prior to overnight fasting. It was hypothesized that this pattern of mass change observed in males during the breeding period indicated the prioritization of flight agility for territory defense and courtship displays over energy acquisition during the day (Calder et al., 1990). However, as the breeding season wanes, hummingbirds are faced with a trade-off between investing in immediate versus future fitness gains, where mass gain necessary for successful migration and survival into the next breeding season is achieved at the expense of current aerial performance. Given the fine control hummingbirds exert over daily mass change, the initiation of premigratory fattening indicates a shift in priorities and the daily balance of energy intake and expenditure.

Currently, there is a dearth of knowledge as it relates to how hummingbirds alter foraging behaviours during different periods of the day in order to facilitate premigratory fattening. Using artificial feeding stations outfitted with radio-frequency identification (RFID) technology coupled with an electronic balance, I aimed to elucidate how rapid premigratory fattening can be achieved in the wild. I hypothesized that premigratory hummingbirds would forgo feeding restraint during the day in order to achieve sufficient amounts of stored energy for migration, and

predicted that premigratory hummingbirds would therefore exhibit positive linear mass change over the course of a day. To accomplish this, hummingbirds would increase energy intake throughout the morning and mid-day by a) feeding from the feeders more frequently and b) increasing the proportion of available time spent feeding from the feeders. I did not expect premigratory hummingbirds to exhibit increased energy consumption during the evenings compared to non-premigratory hummingbirds, as hummingbirds regularly engage in hyperphagia in preparation for overnight fasting. Additionally, I hypothesized that premigratory hummingbirds would increase the use of torpor during the night to conserve energy (Carpenter and Hixon, 1988), and predicted that premigratory hummingbirds would therefore exhibit lower rates of overnight mass loss compared to non-premigratory hummingbirds. To evaluate these hypotheses, I examined foraging behaviour and mass change of ruby-throated hummingbirds in a semi-natural study while taking into account the effects of confounding biotic and abiotic environmental factors, including age, sex, intraspecific competition, temperature, and precipitation.

## 2 Methods

### 2.1 Data Collection

Hummingbird trapping and data collection occurred between mid-May and mid-September in 2013 and 2014 as described in the previous chapter. Additionally, raw mass data were filtered and culled as previously described. I established sex specific ranges of biologically realistic mass to cull erroneous mass readings; mass values falling outside the range of minimum recorded mass minus 2 standard deviations, and maximum recorded mass plus 2 standard deviations ( $\text{min}_{\delta} = 2.4 - 0.6\text{g}$ ,  $\text{max}_{\delta} = 4.6 + 0.6\text{g}$ ,  $n_{\delta} = 338$ ;  $\text{min}_{\varphi} = 2.7 - 0.6\text{g}$ ,  $\text{max}_{\varphi} = 4.6 + 0.6\text{g}$ ,  $n_{\varphi} = 409$ ) were excluded from the dataset.

Temperature and precipitation data were collected using a HC-S3 Temperature and Relative Humidity Probe (Campbell Scientific, Edmonton, AB, Canada), and a Tipping Bucket Rain Gauge (model no. 52202-10-L, R. M. Young Company, Traverse City, MI, USA) at an on-site weather station every 15 minutes. During periods of local weather station malfunction, I used data collected by the nearest station located at Buttonville Airport (Toronto Buttonville Municipal Airport, ON, Canada).

In late-July and early-August 2014, data collection was interrupted by highly disruptive nectar thievery by rogue raccoons. Our stations were subsequently redesigned as sturdier wood enclosures with wire caging to exclude these nectar-robbing fiends. The new station design did not appear to impede hummingbird visits, and data collection continued as previously described.

### 2.2 Premigratory Status of Hummingbirds

For this analysis, I selected a subset of hummingbirds whose last recorded visit was on or after August 1<sup>st</sup> of the respective year, as autumnal migration for this species in Ontario, Canada begins in mid-August (Sandilands, 2010). The date of migration was presumed to be the date of last recorded visit and designated as ‘day 0’. Following the finding in Hou et al. (2015) in which linear mass gain was observed across the 4 days leading up to the day of migration, I created a subset of records from 0 to 4 days prior to migration. A least-squares linear regression of average

daily mass as a function of the number of days before migration was built for each individual with 8 or more mass records per day in at least 4 of 5 days. A total of 61 individuals were assessed from across two years (2013: 35 hummingbirds; 2014: 26 hummingbirds); individuals with a positive slope were designated as exhibiting premigratory mass gain in the 4 days leading up to migration.

In all subsequent analyses, the premigratory status of individual hummingbirds on a given day was designated as ‘premigratory’ if the individual exhibited significant mass gain as determined above, and the day in question fell within the 4 days leading up to migration for that individual. The premigratory status of hummingbirds was otherwise designated as ‘non-premigratory’.

## 2.3 Model Selection Protocol

I assessed differences in presence/absence, foraging frequency, proportion of available time and total time spent feeding, and rates of overnight mass loss by premigratory and non-premigratory hummingbirds using mixed effects models. Unless otherwise stated, I began all model selection by creating a full model with all available explanatory variables and interactions that were deemed biologically relevant. This included age, sex, premigratory status, average temperature, total precipitation, and number of unique IDs detected as a proxy for the amount of competition experienced at the feeders. I also included interaction terms for premigratory status and average daily temperature, premigratory status and total daily precipitation, as well as premigratory status and sex. Because all premigratory hummingbirds were adults, the interaction term between premigratory status and age was omitted from the models.

The best random effects structure for the model was determined using corrected Akaike information criterion (AICc), where the model with the lowest AICc indicated the best-fit model (Burnham et al., 2011). I subsequently assessed the multicollinearity of the fixed effects using variance inflation factors (VIF), where fixed effects with VIF values greater than 3 (Zuur et al., 2009) were sequentially excluded from the model beginning with the highest value interaction term. I then performed backwards selection on the fixed effects to determine the best-fitting model based on AICc. Where the exclusion of a fixed effect did not increase the AICc of the model by more than 2 points, the model with the higher AICc value but with fewer fixed effects

was deemed the best-fitting model. Through 10,000 bootstrap replicates where resampling was stratified for premigratory status, I obtained the 95% confidence intervals (CI) for all regression coefficients using the bias-corrected and accelerated (BCa) method. Where possible, I include R<sup>2</sup> values for models (Nakagawa and Schielzeth, 2013). All analyses were performed on data collected on or after June 1<sup>st</sup> of 2013 and 2014 in order to exclude patterns that may reflect post-migratory recovery in May.

All analyses were performed using R ver. 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Linear mixed-effects models and generalized mixed-effects models with a binomial distribution were built using the R package lme4 ver. 1.1-7 (Bates et al., 2014). Generalized linear mixed-effects models with a truncated negative binomial distribution were built using R package glmmADMB ver. 0.8.1 (Skaug et al., 2015). Variance inflation factors were assessed using the vif.mer function (Frank, 2011). Marginal and conditional R<sup>2</sup> values were calculated using the method described by Nakagawa and Schielzeth (2013). Bootstrapping was performed using the R package boot ver. 1.3-16 (Canty and Ripley, 2015).

## 2.4 Patterns of Daily Mass Change

For this analysis, I selected individuals on days with 2 or more mass records in 7 or more two-hour time bins spanning from 0500h to 2300h to ensure that mass records spanned the entirety of a day. For each individual on each day, I built a parabolic regression and a least-squares linear regression of mass values versus time. To determine which shape (linear or parabolic) best fit the data, I assessed the quality of fit of the linear model compared to the parabolic model using AICc and Akaike weights.

## 2.5 Foraging Strategy

Hummingbird visits recorded before 1100h, between 1100h and 1500h, and after 1500h were classified as morning, mid-day and evening records, respectively. For each individual hummingbird, on each day, during each time period, I tallied frequency of visits, amount of time

spent at the feeders, and calculated proportion of available time spent at the feeders beginning with the date of first recorded visit and ending with the date of last recorded visit. Frequency, proportion of time and total time were identified as ‘0’ for time periods during which an individual was not detected. Periods of station malfunction during which all stations failed to detect a hummingbird ID for 4 or more consecutive hours (excluding overnight hours), were omitted from the dataset.

Mean temperature for each morning was calculated as the average temperature beginning 1 hour before sunrise, rounded to the nearest 15-minute interval, and ending at 1100h. Mean temperature for mid-day was calculated as average temperature between 1100h and 1500h, while mean temperature for the evening was calculated as the average temperature between 1500h and 1 hour after sunset, rounded to the nearest 15-minute interval. Temperature values were centered for each analysis. Total precipitation and number of unique IDs detected for each time period were similarly calculated based on the time divisions described above.

To assess the foraging strategy of hummingbirds, I examined the frequency of visits and total time spent at the feeders. Because the data were found to be zero-inflated, I built zero-altered models in which zero and non-zero data were first assessed in a binomial model as the presence/absence of hummingbirds at the feeders, followed by a separate analysis of the non-zero data.

### 2.5.1 Presence/Absence

I coded non-zero values of frequency and total time ‘1’ for present, while zero values represented absences. Due to the way in which ‘premigratory status’ was defined, where a hummingbird was required to have 8 or more mass records per day in at least 4 of 5 days leading up to the day of migration, incidences of absent premigratory hummingbirds were rare. This resulted in complete separation of premigratory status during certain time periods. Presence/absence data for mornings, mid-day, and evenings were pooled together and assessed using a single model, rather than as 3 separate models.

I built a binomial generalized linear mixed effects model using the aforementioned fixed effects in addition to time period, and determined the best random effects structure from various random slopes (number of unique IDs, centered average temperature, total precipitation, time period),

while hummingbird ID nested within year served as a random intercept. To determine 95% confidence intervals, I used the percentile method as the BCa method produced unstable confidence intervals in this analysis.

### 2.5.2 Frequency of Visits

I built generalized linear mixed effects models to assess the frequency of visits by hummingbirds for the morning, mid-day, and evening time periods. Data for 2013 and 2014 were analyzed separately and collectively. Only positive count values were used for these analyses.

Models were built using a truncated, negative binomial distribution to account for overdispersion of the data. For all models the best random slope was determined from among 3 options: centered average temperature, total precipitation, and number of unique IDs. For models in which I analyzed data from 2013 and 2014 collectively, hummingbird ID nested within year was used as a random intercept, whereas for the separate analyses of 2013 and 2014 data, only hummingbird ID was used as the random intercept. Due to limitations on available computing power, the number of bootstrap replicates used to determine the 95% CIs was reduced to 1000. Where the BCa method produced unstable CIs, I report CIs obtained using the percentile method instead.

### 2.5.3 Proportion of Available Time and Total Time Spent at Feeding Stations

Linear mixed effects models were built to assess the proportion of available time spent at the feeders for the morning, mid-day, and evening time periods. As above, data for 2013 and 2014 were assessed separately and collectively, and only positive values were used. Proportion of time was log transformed as the data exhibited a right skew. The best random effects structure for each model was determined from among the same options listed for the frequency of visits analyses.

As the window of time representing the morning and evening periods changed with sunrise and sunset, I assessed total time spent at the feeders to determine if changes in the proportion of time spent at the feeders were due to changes in total time spent at the feeders or due to reduced daylight hours during premigratory days relative to non-premigratory days. Total time spent at the feeders was assessed using the same procedure.

## 2.6 Rate of Overnight Mass Change

I created a dataset of hummingbirds for which the last recorded mass on a given day was matched with the first recorded mass of the subsequent day. To ensure I was obtaining the first and last mass records of the day, I only used records occurring up to 30 minutes after sunrise, or 30 minutes before sunset. Rate of overnight mass change was calculated as the difference between last mass of the day and first mass of the subsequent day divided by the difference in time between first and last mass. Positive rates of mass change were excluded from the analysis, representing either measurement error or records that were not true first or last masses.

Average overnight temperature and total overnight precipitation were calculated beginning 30 minutes before sunset, and ending 30 minutes after sunrise, for which both were rounded to the nearest 15-minute interval. Average overnight temperature and hummingbird last mass were centered.

A linear mixed effects model was constructed with overnight mass change as a function of sex, age, total overnight precipitation, centered average overnight temperature, centered hummingbird last mass, premigratory status, with interaction terms between premigratory status and centered average overnight temperature, premigratory status and total overnight precipitation, and premigratory status and centered hummingbird last mass. Hummingbird ID nested within year was used as a random intercept, while a random slope was selected from among centered last mass, overnight average temperature, and total overnight precipitation.

## 3 Results

### 3.1 Premigratory Status of Hummingbirds

In August of 2013 and 2014, I detected 35 and 26 hummingbirds at the stations, respectively. Of these 61 birds, 7 hummingbirds (2013: 1 adult male and 2 adult females; 2014: 1 adult male and 3 adult females) were found to exhibit a positive relationship between daily average mass and the number of days before migration, indicating mass gain beginning 4 days prior to migration (figure 6). Of these premigratory hummingbirds, 1 adult female exhibited premigratory mass gain in both 2013 and 2014.

### 3.2 Patterns of Daily Mass Change

I assessed mass change over time for 19 of 28 possible days (7 birds x 4 premigratory days). Of these 19 days, 15 were best described by a positive linear pattern, 1 was best described by a U-shaped pattern and 3 were best represented by an inverse U-shape. A proper assessment of mass change over time could not be performed for the remaining 9 of 28 days due to insufficient data. For premigratory hummingbirds, the mean Akaike weight for the linear models of mass over time was  $0.70 \pm 0.09$  s.d. (min = 0.50; max = 0.79).

While the majority of days assessed for premigratory hummingbirds were best described by a positive linear pattern of mass change over time, non-premigratory hummingbirds exhibited both parabolic and linear patterns of mass change over time. Of the 330 days assessed from across 26 hummingbirds, 36 days were best described by an inverse U-shape pattern (adult ♀: 29; adult ♂: 6; juvenile ♂: 1), 16 days were best described by a negative linear pattern (adult ♀: 10; adult ♂: 5; juvenile ♂: 1), and 44 days were erratic as both the parabolic and linear adjusted  $R^2$  were negative. Of the remaining 234 days, 111 were best described by a U-shaped pattern (adult ♀: 40; adult ♂: 71), and 123 were best described by a positive linear pattern (adult ♀: 87; adult ♂: 33; juvenile ♂: 1; juvenile ♀: 2). The mean Akaike weight for the parabolic models of mass versus time was  $0.58 \pm 0.31$  s.d. (min = 0.19; max = 1).

### 3.3 Presence/Absence

Premigratory status and number of unique IDs as fixed effects best explained the presence of hummingbirds at the feeders during the day in 2013 and 2014 (table 4). The expected odds of premigratory hummingbirds visiting the feeders during the day are ~19 times greater than the expected odds of non-premigratory hummingbirds visiting the feeders. Similarly, as the number of unique IDs detected at the feeders increases, there is an increase in the expected odds of hummingbirds visiting the feeders.

### 3.4 Frequency of Visits

#### 3.4.1 Mornings

The frequency of morning visits is not expected to vary by premigratory status of hummingbirds, as premigratory status was not retained as a fixed effect in any of the best fitting models. In the combined analysis of 2013 and 2014 data, frequency of morning visits is expected to increase as the number of competing individuals detected at the feeders increases, while the frequency of morning visits is expected to decrease with increasing average morning temperature. The best fitting model for the 2013 data also retained number of unique IDs detected and average morning temperature as predictors with similar effects on frequency of morning visits. The best fitting model of the 2014 data indicates that frequency of morning visits is expected to decrease with increasing average morning temperature (table 5).

#### 3.4.2 Mid-day

Premigratory status was not retained as a fixed effect in the best fitting model predicting the frequency of mid-day visits for the combined analysis of 2013 and 2014 data, as well as for the analysis of the 2014 data. In the combined analysis, frequency of mid-day visits is expected to decrease with increasing total mid-day precipitation and average mid-day temperature, and is expected to increase as the number of unique IDs detected at the feeders increases. For the 2014 analysis, frequency of visits is expected to decrease only by average mid-day temperature. In contrast, the best fitting model for the 2013 data retained premigratory status and number of unique IDs detected as fixed effects, predicting an increase in the frequency of mid-day visits as

number of unique IDs detected increases, and a decrease in the frequency of mid-day visits by premigratory hummingbirds (table 5).

### 3.4.3 Evenings

In the combined analysis of 2013 and 2014 data, the frequency of evening visits is not expected to vary according to premigratory status. Rather, frequency is predicted to increase as the number of unique IDs detected increases, and decrease as average evening temperature increases.

Similarly, frequency of evening visits in 2014 is expected to be unaffected by premigratory status, as an intercept-only model was found to be the best fitting model. However, the best fitting model of the 2013 data predicts a decrease in the frequency of evening visits by premigratory hummingbirds. The frequency of evening visits is also predicted to decrease as total evening precipitation and average evening temperature increases, whereas frequency is expected to increase as the number of unique IDs detected increases (table 5).

## 3.5 Proportion of Available Time Spent at the Feeders

### 3.5.1 Mornings

The proportion of available time hummingbirds spent at the feeders during the mornings is expected not to vary as a result of premigratory status. Average morning temperature, total morning precipitation, and sex of the hummingbird were retained as fixed effects in the combined analysis of 2013 and 2014 data. Female hummingbirds are predicted to spend a greater proportion of available time at the feeders than male hummingbirds. Proportion of available time spent at the feeders is predicted to decrease with increasing average morning temperature, whereas it is predicted to increase as total morning precipitation increases. For the 2013 analysis, proportion of available time spent at the feeders is expected to decrease with increasing average morning temperature, and increase as the number of unique IDs detected increases. As in the combined analysis, males are predicted to spend a smaller proportion of available time at the feeders than females. In the best fitting model of the 2014 data, proportion of available time spent at the feeders is expected to increase as average morning temperature increases, and decrease as total precipitation increases (table 6).

### 3.5.2 Mid-day

The best fitting model for the combined analysis of 2013 and 2014 data retained premigratory status and average mid-day temperature as fixed effects. Proportion of available time spent at the feeders is expected to decrease as average mid-day temperature increases, and premigratory hummingbirds are expected to spend a greater proportion of available time at the feeders than non-premigratory hummingbirds. Premigratory status is predicted to have a similar effect in 2013; however, average mid-day temperature was replaced with number of unique IDs in the best fitting model, where proportion of available time spent at the feeders is predicted to increase as the number of unique IDs detected increases. The best fitting model of the 2014 data predicted a decrease in proportion of available time spent at the feeders with increasing average mid-day temperature (table 6).

### 3.5.3 Evenings

The proportion of available time hummingbirds spent at the feeders in the evenings is expected to be greater in premigratory birds than non-premigratory birds in the combined analysis of 2013 and 2014 data. Proportion of available time spent at the feeders is also expected to increase as the number of competing individuals increases, and decrease as average evening temperature increases. Similarly, the best fitting model for the 2014 data retained number of unique IDs and premigratory status as fixed effects; proportion of available time spent at the feeders is expected to increase as the number of unique IDs detected increases, and is expected to be greater for premigratory hummingbirds compared to non-premigratory hummingbirds. The best fitting model for the 2013 data however, did not include premigratory status. Instead, proportion of available time spent at the feeders is only expected to decrease with increasing average evening temperature (table 6).

## 3.6 Total Time Spent at the Feeders

### 3.6.1 Mornings

Premigratory status was not retained in any of the best fitting models assessing changes in the total time hummingbirds spent at the feeders. In the combined analysis of 2013 and 2014 data,

total time is expected to increase as total morning precipitation and number of unique IDs detected increase, but is expected to decrease as average morning temperature increases. Total morning precipitation and average morning temperature were retained as fixed effects in the best fitting model for the 2014 data, and are predicted to have the same effects as in the combined analysis. Analysis of the 2013 data predicted that male hummingbirds spend less total time at the feeders in the mornings than female hummingbirds. Total time is also expected to increase as the number of unique IDs detected increases, whereas it is expected to decrease as average morning temperature increases (table 7).

### 3.6.2 Mid-day

Assessment of changes in total time spent at the feeders during the mid-day was deemed unnecessary, as the defined window of time for the mid-day remained static throughout the season.

### 3.6.3 Evenings

The total amount of time hummingbirds spent at the feeders in the evenings is not expected to vary based on premigratory status. The best fitting model for the combined analysis of 2013 and 2014 data predicts a decrease in total time spent at the feeders as average evening temperature increases, whereas total time is expected to decrease as the number of unique IDs detected increases. For the 2013 data, total time spent at the feeders is expected to decrease as average evening temperature and total evening precipitation increase. The best fitting model for 2014 only predicts an increase in total time as the number of unique IDs detected increases (table 7).

## 3.7 Rate of Overnight Mass Change

A model containing age, sex, centered last mass, premigratory status and an interaction between premigratory status and sex as fixed effects best describes rate of overnight mass change.

Rates of overnight mass loss are expected to be greater as last evening mass increases. Compared to adults, juveniles are expected to exhibit reduced rates of overnight mass loss. I found that female premigratory hummingbirds are expected to exhibit a reduced rate of overnight mass loss

(52.86 mg/h) compared to non-premigratory females (82.67 mg/h). Similarly, male premigratory hummingbirds are expected to lose mass at a slower rate (47.82mg/h) than non-premigratory males (121.86 mg/h). Our analysis also revealed an effect of sex and an interaction between sex and premigratory status, where non-premigratory males lost more mass than non-premigratory females, while premigratory males lost less mass than premigratory females (table 4; figure 7).

## 4 Discussion

### 4.1 Premigratory Status

Based on the criteria outline above, analysis of available data for 61 ruby-throated hummingbirds from across 2 years revealed 7 hummingbirds that exhibited positive linear mass change over 4 consecutive days immediately prior to migration (figure 6). Hummingbirds that did not exhibit premigratory mass gain may have switched breeding habitats, departed from the breeding grounds prior to the initiation of premigratory fattening, or failed to visit the feeders often enough to permit sufficient data collection (see ‘Conclusions’ for further discussion).

For hummingbirds, weight gain can be detrimental to their ability to fly by increasing flight cost (Calder et al., 1990; Chai et al., 1996; Mahalingam and Welch, 2013) and encumbering aerial performance (Calder et al., 1990). However, seasonal mass gain, particularly in the form of fat deposition, is adaptive for periods of high energetic demand, and for periods during which there is a scarcity of resources.

### 4.2 Patterns of Daily Mass Change

I found that premigratory hummingbirds primarily exhibited a positive linear pattern of mass change over time, whereas non-premigratory hummingbirds exhibited both parabolic and linear patterns of mass change in near equal proportions. This indicates that hummingbirds prioritized energy gain differently during the premigratory period compared to the non-premigratory period (figure 8).

While a previous laboratory study (Beuchat et al., 1979) has shown that non-premigratory hummingbirds exhibit a U-shaped pattern of daily mass change, this work has found that daily mass change of hummingbirds in the wild can be characterized by other shapes as well (Hou et al., 2015). In assessing daily mass change in non-premigratory birds, I found that the U-shaped pattern was more prevalent for adult males than adult females. As male RTHU more readily engage in highly energetic territorial and courtship displays than their female counterparts (Mulvihill et al., 1992), the greater incidence of U-shaped daily mass change observed in males

lends support to the hypothesis that feeding restraint and weight control facilitates aerial performance and agility (Calder et al., 1990). In contrast, females accounted for the majority of individual-days where positive linear mass change was observed during the non-premigratory period, which may reflect the energetic requirements of life history events such as the production of eggs and provisioning of chicks. Adult female hummingbirds were also found to exhibit the majority of negative linear and inverse U-shaped patterns of daily mass change, which although suggests a negative energy balance and inadequate energy acquisition, could also reflect days on which gravid females laid eggs, and thus loss mass. Furthermore, this study could not account for individual molt status for which the loss of feathers could entail an energetic regime different from the expected U-shape. As this study assessed hummingbirds in their natural environment and across the entire breeding season, the different patterns of mass change observed may reflect life-history events, differing energetic strategies in response to a highly complex environment, or possibly measurement error; more study is needed to determine what drives these patterns of mass change.

For non-premigratory hummingbirds, the prioritization of aerial flight performance over energy acquisition can be adaptive and beneficial for fitness and survival. The high energetic cost of migration, however, necessitates a shift in priorities to that of energy acquisition in the days leading up to migration, if an individual is to survive to breed in the subsequent year (Calder et al., 1990). Male hummingbirds have been observed to forgo territorial disputes and reproductive endeavours prior to and during migration (Calder et al., 1990). The cessation of such energetically expensive activities would confer a reduction in energy expenditure, thereby facilitating premigratory fattening. Accordingly, the predominance of the positive linear pattern of mass change in premigratory hummingbirds indicates that for these individuals, establishing a positive energy balance takes precedence over other activities.

### 4.3 Frequency of Visits

As the artificial feeders provided an “unlimited” food source and a perch upon which they could sit while feeding, hummingbirds were expected to increase foraging effort at the feeders, either as frequency of visits, duration of visits, or both, as it provided a more efficient way of satisfying

the energetic requirements of premigratory fattening. Premigratory hummingbirds were expected to feed more frequently from the artificial feeders, as increased foraging frequency is a typical response to energetic stress (Hainsworth, 1978; Beuchat et al., 1979; Gass et al., 1999). Although I found that the odds of premigratory hummingbirds visiting the feeders increased many fold, contrary to my prediction, I did not find an increase in the frequency of visits to the artificial feeders by premigratory hummingbirds during any point of the day. Rather, I found a decrease in the frequency of mid-day and evening visits by premigratory hummingbirds in 2013.

#### 4.4 Proportion of Available Time and Total Time Spent at the Feeders

Although feeding frequency at the artificial feeders did not increase, premigratory hummingbirds spent a greater proportion of available time at the feeders during the mid-day in accordance with my prediction. Since time spent at the feeders increased during the mid-day but the frequency of feeder visits did not, these results taken together indicate that premigratory hummingbirds engaged in longer feeding bouts at the artificial feeders compared to non-premigratory hummingbirds. This finding supports the hypothesis that premigratory hummingbirds release the feeding restraint often exhibited during the mid-day at other points in the season. Moreover, the increase in time spent at the feeders during the mid-day supports the earlier finding where premigratory hummingbirds were more likely to exhibit a positive linear pattern of daily mass change.

For hummingbirds, it appears that mid-day foraging is an important contributor to premigratory fattening, and it may be the only time during which hummingbirds possess the behavioural flexibility to substantially increase energy intake compared to non-premigratory periods (figure 9). Premigratory hummingbirds did not increase the proportion of time spent at the feeders during the morning, and although there was an increase in proportion of time spent at the feeders in the evening, further analysis revealed that the total time premigratory hummingbirds spent at the feeders in the evening did not change. This indicated that the observed increase in proportion of time spent at the feeders was actually a result of the decrease in available evening foraging

time as days shortened. Overall, these findings suggest that hummingbirds could not increase foraging effort at the artificial feeders during these periods of the day.

For these energy-limited organisms, surviving the night and maximizing the ability to maintain overnight normothermia may require individuals to consume the maximum amount of energy allowed by their digestive physiology immediately prior to nightfall. Hummingbirds must balance changing priorities and energetic needs throughout the day. Feeding restraint, while advantageous during the mid-day in facilitating aerial agility and performance is maladaptive in the evenings as hummingbirds are incapable of nighttime foraging, and overnight energetic demands can only be met by energy accumulated within the body. Hummingbirds have been observed to exhibit hyperphagia in the evenings to increase energy gain (Calder et al., 1990); however, nectar held in the crop provides only enough energy to meet half of the overnight energy expenditure of a normothermic hummingbird (Powers, 1991), requiring the use of stored lipids to fuel the remainder. The depletion of energy reserves overnight means that hummingbirds must forage in a way that not only satisfies the immediate energetic requirements of continued foraging behaviour in the morning, but also replenishes expended energy reserves. Thus, as in the evenings, hummingbirds act to maximize energy intake in the morning regardless of time in the season. The lack of change in the foraging behaviour of premigratory hummingbirds in the mornings and evenings not only suggests that hummingbirds maximize energy consumption on a daily basis due to the energetic challenge of overnight fasting, but also suggests that the physiological upper limit to energy intake during these periods remains unchanged between the non-premigratory and premigratory states.

## 4.5 Rate of Overnight Mass Change

In addition to changes in energy intake, organisms can alter energy expenditure to establish a positive energy balance. Hummingbirds can reduce energy expenditure through the use of torpor in emergency situations when an individual is energetically stressed (Hainsworth et al., 1977). However, hummingbirds have also been observed to use torpor in situations when they are not experiencing an energy crisis, and it was postulated that this was beneficial for the conservation of fat stores in preparation for migration (Carpenter and Hixon, 1988). Without direct

observation of hummingbirds during the night, I could not determine for certain if a hummingbird descended into torpor. Moreover, calculations of theoretical mass change can be highly variable depending on the amount of time an individual spent in torpor and the ambient conditions of the surrounding microclimate. Nonetheless, the data showed that the rate of overnight mass loss was greatly reduced in premigratory hummingbirds compared to non-premigratory hummingbirds, suggesting that premigratory individuals used less energy overnight, thereby lending support to the hypothesis that hummingbirds used torpor to conserve fat stores prior to migration. While reduced overnight mass loss can also be explained by the increased insulation that fat provides, a previous study concluded that this explanation would be insufficient in accounting for the observed differences in overnight mass change by fat and lean hummingbirds (Carpenter et al., 1993). Under normothermic conditions, the difference in oxygen consumption by fat and lean hummingbirds was only 1%, whereas in the case of torpor, oxygen consumption by fat hummingbirds exceeded that of lean hummingbirds by 10% (Hiebert, 1989). For fat normothermic hummingbirds, this would mean overnight mass loss would be similar to that of lean normothermic hummingbirds, whereas for fat torpid hummingbirds, mass loss would be greater than that of lean torpid hummingbirds.

This analysis also revealed an effect of sex on rate of overnight mass loss and an interaction between sex and premigratory status. Non-premigratory males exhibited an increased rate of mass loss compared to non-premigratory females, whereas premigratory males exhibited a greater reduction in rate of mass loss than did premigratory females (figure 7). As male ruby-throated hummingbirds are smaller than females, the greater thermoregulatory challenge normothermic males face may explain the greater rate of mass loss during the non-premigratory period. The more pronounced reduction in mass loss rate suggests that premigratory males may rely more heavily on overnight torpor than their female counterparts, either by spending more time in torpor, or by allowing body temperature to fall closer to ambient temperature. However, this finding must be confirmed with additional work, as the sample size of premigratory birds was limited. While torpor provides an effective means of energy conservation, it is not without risk as torpor potentially reduces cellular function (Hainsworth et al., 1977) and individual responsiveness to predatory threats (Carpenter and Hixon, 1988). Although premigratory hummingbirds possess enough stored energy to remain normothermic overnight, their greater use

of torpor compared to non-premigratory hummingbirds is indicative of a shift in priorities in which precedence is placed on establishing and maintaining a positive energy.

## 4.6 Environmental Factors

In assessing how premigratory hummingbirds establish a positive energy balance in the wild, the analyses took into account abiotic and biotic variables that could influence behaviour and energetics. Previous studies have found that for small endothermic hummingbirds challenged by low ambient temperatures, increases in foraging frequencies allowed individuals to maintain energy balance (Beuchat et al., 1979; Gass et al., 1999). Precipitation also poses a significant challenge to hummingbirds, as storm events can reduce available foraging time (Gass and Lertzman, 1980). In best-fitting models where temperature was retained as a fixed effect, coefficient estimates consistently revealed a negative relationship between temperature and the response variables of frequency, proportion of time and total time spent at the feeders (tables 5 to 7). Thus, I find general patterns that match those revealed by other researchers using direct observation techniques (Beuchat et al., 1979; Gass et al., 1999). Coefficient estimates where precipitation was retained as a fixed effect in best fitting models revealed that frequency of visits decreases with increasing precipitation, while proportion of time and total time spent at the feeders increase (tables 5 to 7). This may reflect a greater reliance by hummingbirds on the artificial feeders during inclement weather, as the feeders are sheltered and present as a reliable and ‘infinite’ food sources.

Hummingbird foraging behaviour and energetics can also be influenced by intraspecific competition over resources. I found that in all best-fitting models where number of unique IDs detected at the feeding stations was retained as a fixed effect, coefficient estimates consistently revealed a positive relationship with the aforementioned response variables. For dominant individuals who exhibit territoriality around the feeders, greater competitive pressure means an increase in energetically expensive territorial displays and chases. Dominant individuals must therefore increase energy intake in order to meet these energetic demands. Furthermore, it has been observed that when faced with a nectar-robbing intruder, dominant hummingbirds chased away the intruder and subsequently returned to feed from the same flowers that the intruder

robbed; it was hypothesized that dominant hummingbirds exhibited this behaviour so as to further deplete recently robbed resources such that subsequent food loss due to nectar-thievery was minimized (Paton and Carpenter, 1984). For non-dominant hummingbirds, an increase in the number of competing individuals at the feeders may provide more windows of time in which nectar-robbing can occur, as dominant individuals spend more time chasing away other intruders. Furthermore, the fledging and tagging of young partway through the season, and their use of the feeders could also be driving the positive relationship observed between the number of unique individuals detected at the feeders and the aforementioned response variables.

Where I analyzed 2013 and 2014 data separately, I found that the best fitting models were often dissimilar. And while this suggests that from year to year, hummingbirds can exhibit differing strategies for establishing a positive energy balance, I would advise that this interpretation be made cautiously as a result of the disturbance caused by nectar-thieving raccoons in 2014. During this disturbance (mid-July to early-August), fewer individuals were detected at the stations despite a similar tagging effort across both years; this suggests that individuals abandoned use of the feeders during this time, thereby confounding the analyses and results of the 2014 data.

## 4.7 Conclusions

For ruby-throated hummingbirds opting to get fat prior to their departure from the breeding grounds, the mid-day is the only time during which individuals can adjust foraging behaviour by relinquishing feeding restraint. Premigratory individuals lengthen the duration of feeding visits during the mid-day in order to increase daily energy intake for fattening. For hummingbirds feeding solely on natural food sources, this finding would suggest that hummingbirds could lengthen foraging bouts by feeding at a greater number of flowers in order to meet the energetic demands of premigratory fattening. Moreover, this finding is not without relevance in the understanding of hummingbird foraging behaviour as hummingbird feeders maintained by the general public are ubiquitous across North America.

The results of this study suggest that premigratory hummingbirds are limited in their ability to increase energy intake during the day. Although relative hyperphagia during the mid-day

permitted positive linear mass gain over the course of a single day, it may be insufficient in achieving a sufficiently high rate of mass gain over 4 consecutive days prior to migration. A hummingbird's use of torpor during the premigratory period may be a consequence of the physiological inability to increase energy intake for sufficient fattening over multiple days, given the available daylight hours for foraging; without the energy conservation afforded by the use of torpor, the rate of mass gain over the course of several days would be greatly reduced (Carpenter and Hixon, 1988; Hiebert, 1993). Rapid premigratory mass gain in ruby-throated hummingbirds beginning 4 days prior to migration is achieved via a two-pronged approach of increasing mid-day foraging effort and reducing overnight energy expenditure through the use of torpor. Individuals for which premigratory fattening could not be discerned may have achieved moderate fattening through the use of one of these strategies; others may have chosen to adopt a more frequent and continuous foraging strategy along the migratory journey instead.

In contrast to previous studies that have examined hummingbird mass gain at stopover sites in which more individuals were found to exhibit rapid mass gain in the days leading up to the resumption of migration (Carpenter et al., 1983; Carpenter et al., 1993), this study examined mass gain of hummingbirds at the start of autumnal migration prior to their departure from a breeding ground. This difference in location could, in part, explain why, relatively few hummingbirds exhibited rapid mass gain prior to migration, and thus identified as 'premigratory' in this study. Having just completed a leg of the migratory journey, migratory hummingbirds at stopover sites likely experience different energetic needs, and therefore exhibit different foraging ecologies compared to individuals at breeding grounds who have yet to begin migration. Moreover, different age-sex classes may adopt different strategies for migration and, consequently, exhibit alternative premigratory fueling strategies. Although the sample size of premigratory individuals in this study is relatively low, the findings of this study are, nonetheless, fairly conclusive and implore further study to determine what alternative fueling strategies premigratory hummingbirds may employ.

## Chapter 4

### General Discussion

The implementation of a low-cost, low-power RFID reader in concert with an electronic balance in the field has permitted the automated, serial collection of both hummingbird physiological and behavioural data. Using this data, my examination of premigratory fattening in hummingbirds has provided unique insight into how energy-limited organisms achieve a positive energy balance over a 24-hour period in the wild.

For premigratory individuals, where acquiring adequate energy stores is paramount for successful migration, the lack of change in foraging behaviour during the mornings and evenings suggests that hummingbirds do not have the capacity to increase energy intake at these times. This may represent a physiological limitation where energy intake is limited by the digestive system (Diamond et al., 1986; Karasov et al., 1986), and suggests that the intrinsic limitation to a hummingbird's ability to process and accumulate energy stores in the mornings and evenings does not change with premigratory status. As digestive organs are highly metabolically active (McWilliams and Karasov, 2005), the maintenance of these organs is energetically expensive. While for some species, increasing the size of digestive organs can permit greater energy intake sufficient for fattening, this strategy may not be viable for volant organisms as larger, heavier digestive organs impose a greater energetic cost to flight.

For small endothermic organisms with high basal metabolic rates, maintaining daily energy balance can be a considerable challenge. Where organisms possess relatively little stored energy compared to their metabolic rates, regular energy consumption is essential (Wang et al., 2006), and maximum energy intake permitted by their digestive physiology during certain periods of the day may be necessary for day-to-day survival. However, in situations where mass gain is adaptive, such as for migration or hibernation, intrinsic limitations may exist that prevent additional energy gain, representing an upper limit to energy storage (Lindstrom, 1991). For such organisms lacking the phenotypic plasticity to increase energy intake, the conservation of energy can be just as important as consumption in the building of adequate energy stores. Where organisms are already exhibiting maximal foraging during certain periods of the day on a regular basis, there leaves a limited window of time during which behavioural adjustments can be made

to meet new energetic demands; hyperphagia, in which energy consumption exceeds that needed for the maintenance of a constant body mass, may be temporally constrained. Future studies investigating seasonal changes in foraging patterns should assess physiological limitations and account for potential temporal restrictions in the study design.

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## Tables

**Table 1.** Summary of the annual number of ruby-throated hummingbirds tagged (n) and number of redetections. Age of hummingbirds listed here are at the time of initial capture.

		<b>Redetections</b>		
<b>Initial Capture</b>		<b>2011</b>	<b>2012</b>	<b>2013</b>
<b>2011</b>	9 adult females	6 adult females		
	2 adult males	1 adult male	4 adult females	1 adult female
	(n = 12) 1 juvenile male	1 juvenile male		
<b>2012</b>	31 adult females		29 adult females	7 adult females
	12 adult males		9 adult males	4 adult males
	(n = 50) 2 juvenile females		1 juvenile female	1 juvenile male
<b>2013</b>	5 juvenile males		2 juvenile males	
	25 adult females			17 adult females
	9 adult males			8 adult males
(n = 56)	12 juvenile females			7 juvenile females
	10 juvenile males			6 juvenile males

**Table 2.** Models of pre-migratory mass gain in ruby-throated hummingbirds developed with average body mass between 1800 and 2100h as a function of days before migration with individual as a random factor.

Model	Equation	AIC Score	AIC Weight
Exponential	body mass = $3.60 + 1.19e^{(-0.36 \text{ [days before migration]})}$	-40.3	1.00
Linear	body mass = $-0.09[\text{days before migration}] + 4.41$	13.4	$2.18e^{-12}$

**Table 3.** Equipment cost for a miniaturized station employing a low-cost, low-power RFID reader and antenna.

Equipment	Cost
Denver MAXX digital scale (Model #: MXX-212) with YADAP-RS adapter	\$350
Notebook PC	\$240
RFID circuit board	\$150
Perky-Pet® hummingbird feeder (Model #: 220)	\$20
12V deep cycle battery	\$30
Antenna (28 gauge copper magnetic wire)	\$1

**Table 4.** Coefficient estimates, 95% confidence intervals, and marginal and conditional R<sup>2</sup> for the best fitting models describing presence/absence and rate of overnight mass change. The number of unique individuals within each premigratory status classification in the dataset used for analyses is also presented.

<i>Models</i>		Fixed effects (Random slope   Random Intercept)	Fixed effects estimates	95% CI Lower	95% CI Upper	R <sup>2</sup> Marginal	R <sup>2</sup> Conditional	n Non-Premigratory	n Premigratory
<i>Presence/Absence</i>				(Percentile)	(Percentile)				
Intercept	-3.04			-3.49	-2.94	0.16	0.68	♀ : 44	♀ : 4
Premigratory status ( <i>premig</i> )	3.00			2.05	20.68			♂ : 29	♂ : 2
Unique ID (Unique IDs   Year/ID)	0.39			0.37	0.44				
<i>Rate of overnight mass change</i>				(BCa)	(BCa)				
Intercept	-82.67			-90.58	-75.98	0.41	0.55	♀ : 24	♀ : 4
Age ( <i>juvenile</i> )	39.14			25.30	50.85			♂ : 12	♂ : 2
Sex ( <i>male</i> )	-39.19			-51.97	-22.38				
Centered last mass	-70.90			-79.67	-60.21				
Premigratory status ( <i>premig</i> )	29.81			12.49	48.58				
Premigratory status ( <i>premig</i> ) : Sex ( <i>male</i> ) (Centered last mass   ID)	44.23			17.93	77.38				

**Table 5.** Coefficient estimates, 95% confidence intervals, and marginal and conditional R<sup>2</sup> for the best fitting models describing the frequency of visits at different time periods of the day. The number of unique individuals within each premigratory status classification in the dataset used for analyses is also presented.

<i>Frequency of visits models</i>		Fixed effects estimates	95% CI Lower	95% CI Upper	n Non-premigratory	n Premigratory
Fixed effects	(Random slope   Random Intercept)					
<i>Morning</i>						
2013			(BCa)	(BCa)		
	Intercept	1.03	1.28	1.55	♀ : 23	♀ : 2
	Centered average temperature	-0.05	-0.11	-0.05	♂ : 13	♂ : 1
	Unique IDs	0.07	0.03	0.05		
	(Centered average temperature   ID)					
2014			(BCa)	(BCa)		
	Intercept	1.56	1.37	1.79	♀ : 20	♀ : 3
	Centered average temperature	-0.05	-0.07	-0.02	♂ : 16	♂ : 1
	(Unique IDs   ID)					
2013 & 2014			(Percentile)	(Percentile)		
	Intercept	1.14	0.93	1.35	♀ : 37	♀ : 4
	Centered average temperature	-0.05	-0.07	-0.03	♂ : 27	♂ : 2
	Unique IDs	0.06	0.04	0.08		
	(Centered average temperature   Year/ID)					

<i>Mid-day</i>						
<i>2013</i>			(BCa)	(BCa)		
Intercept	1.06	0.81	1.34	♀ : 26	♀ : 2	
Premigratory status ( <i>premig</i> )	-0.48	-0.74	-0.21	♂ : 12	♂ : 1	
Unique IDs	0.04	0.02	0.06			
(Centered average temperature   ID)						
<i>2014</i>			(BCa)	(BCa)		
Intercept	1.42	1.28	1.58	♀ : 18	♀ : 3	
Centered average temperature	-0.06	-0.10	-0.03	♂ : 13	♂ : 1	
(Centered average temperature   ID)						
<i>2013 &amp; 2014</i>			(Percentile)	(Percentile)		
Intercept	1.22	0.99	1.36	♀ : 38	♀ : 4	
Total precipitation	-0.03	-0.06	-0.01	♂ : 23	♂ : 2	
Unique IDs	0.03	0.01	0.05			
Centered average temperature	-0.04	-0.06	-0.02			
(Centered average temperature   Year/ID)						
<i>Evening</i>						
<i>2013</i>			(BCa)	(BCa)		
Intercept	1.43	1.19	1.79	♀ : 25	♀ : 2	
Total Precipitation	-0.03	-0.06	0.00	♂ : 11	♂ : 1	
Premigratory status ( <i>premig</i> )	-0.89	-1.32	-0.55			
Unique IDs	0.03	0.00	0.05			
Centered average temperature	-0.05	-0.07	-0.01			
(Centered average temperature   ID)						
<i>2014</i>			(BCa)	(BCa)		
Intercept	1.47	1.27	1.70	♀ : 20	♀ : 3	
(Unique IDs   ID)				♂ : 15	♂ : 1	
<i>2013 &amp; 2014</i>			(Percentile)	(Percentile)		
Intercept	1.29	0.99	1.45			
Centered average temperature	-0.03	-0.05	-0.01	♀ : 38	♀ : 4	
Unique IDs	0.04	0.02	0.06	♂ : 24	♂ : 2	
(Centered average temperature   Year/ID)						

**Table 6.** Coefficient estimates, 95% confidence intervals, and marginal and conditional R<sup>2</sup> for the best fitting models describing the proportion of available time hummingbirds spent at the feeders. The number of unique individuals within each premigratory status classification in the dataset used for analyses is also presented.

<i>Proportion of available time models</i>		Fixed effects (Random slope   Random Intercept)	Fixed effects estimates	BCa 95% CI Lower	BCa 95% CI Upper	R <sup>2</sup> Marginal	R <sup>2</sup> Conditional	n Non-premigratory	n Premigratory
<i>Morning</i>									
<i>2013</i>									
	Intercept		-6.70	-7.50	-6.11	0.15	0.58	♀ : 23	♀ : 2
	Centered average temperature		-0.07	-0.09	-0.04			♂ : 13	♂ : 1
	Sex ( <i>male</i> )		-0.99	-1.33	-0.61				
	Unique IDs (Unique IDs   ID)		0.13	0.07	0.20				
<i>2014</i>									
	Intercept		-5.71	-5.93	-5.56	0.04	0.42	♀ : 20	♀ : 3
	Centered average temperature		-0.10	-0.15	-0.06			♂ : 16	♂ : 1
	Total precipitation (Centered average temperature   ID)		0.19	0.06	0.30				
<i>2013 &amp; 2014</i>									
	Intercept		-5.48	-5.95	-5.14	0.06	0.42	♀ : 37	♀ : 4
	Centered average temperature		-0.07	-0.10	-0.05			♂ : 27	♂ : 2
	Total precipitation		0.06	0.01	0.12				
	Sex ( <i>male</i> ) (Unique IDs   Year/ID)		-0.53	-0.75	0.27				

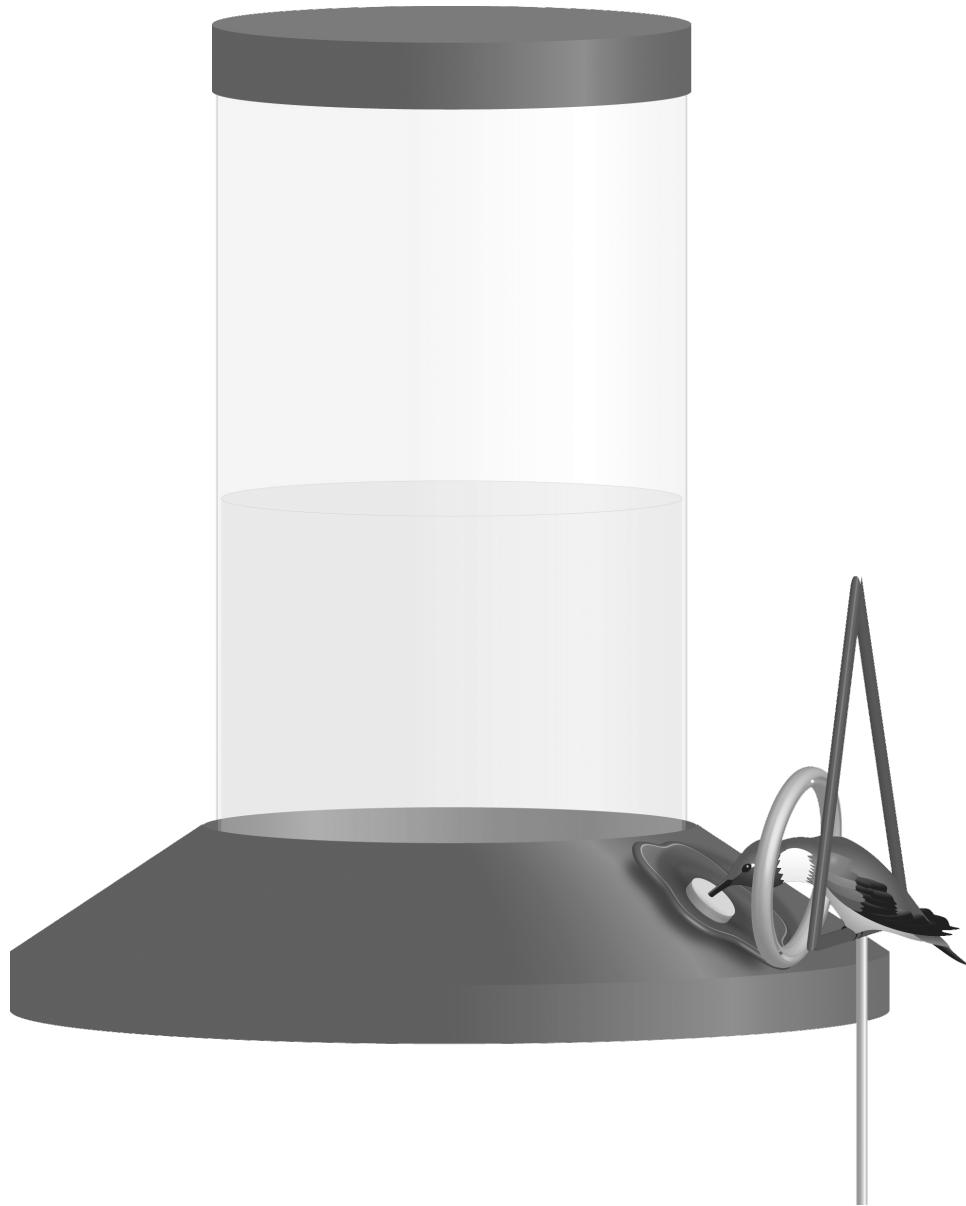
<i>Mid-day</i>							
<i>2013</i>							
Intercept	-5.98	-6.33	-5.67	0.01	0.39	♀ : 26	♀ : 2
Premigratory status ( <i>premig</i> )	0.89	0.55	1.25			♂ : 12	♂ : 1
Unique IDs (NA   ID)	0.05	0.02	0.09				
<i>2014</i>							
Intercept	-5.48	-5.61	-5.36	0.01	0.23	♀ : 18	♀ : 3
Centered average temperature (Unique IDs   ID)	-0.06	-0.10	-0.02			♂ : 13	♂ : 1
<i>2013 &amp; 2014</i>							
Intercept	-5.54	-5.69	-5.45	0.01	0.32	♀ : 38	♀ : 4
Centered average temperature	-0.02	-0.04	0.00			♂ : 23	♂ : 2
Premigratory status ( <i>premig</i> ) (Unique IDs   Year/ID)	0.53	0.17	0.98				
<i>Evening</i>							
<i>2013</i>							
Intercept	-5.53	-5.73	-5.35	0.02	0.41	♀ : 25	♀ : 2
Centered average temperature (Unique IDs   ID)	-0.05	-0.08	-0.03			♂ : 11	♂ : 1
<i>2014</i>							
Intercept	-6.21	-6.57	-5.87	0.02	0.38	♀ : 20	♀ : 3
Premigratory status ( <i>premig</i> )	0.75	0.13	1.32			♂ : 15	♂ : 1
Unique IDs (NA   ID)	0.08	0.03	0.12				
<i>2013 &amp; 2014</i>							
Intercept	-6.09	-6.43	-5.76	0.02	0.43	♀ : 38	♀ : 4
Centered average temperature	-0.04	-0.06	-0.01			♂ : 24	♂ : 2
Unique IDs	0.06	0.02	0.10				
Premigratory status ( <i>premig</i> ) (Unique IDs   Year/ID)	0.60	0.24	1.02				

**Table 7.** Coefficient estimates, 95% confidence intervals, and marginal and conditional  $R^2$  for the best fitting models describing the total time hummingbirds spent at the feeders. The number of unique individuals within each premigratory status classification in the dataset used for analyses is also presented.

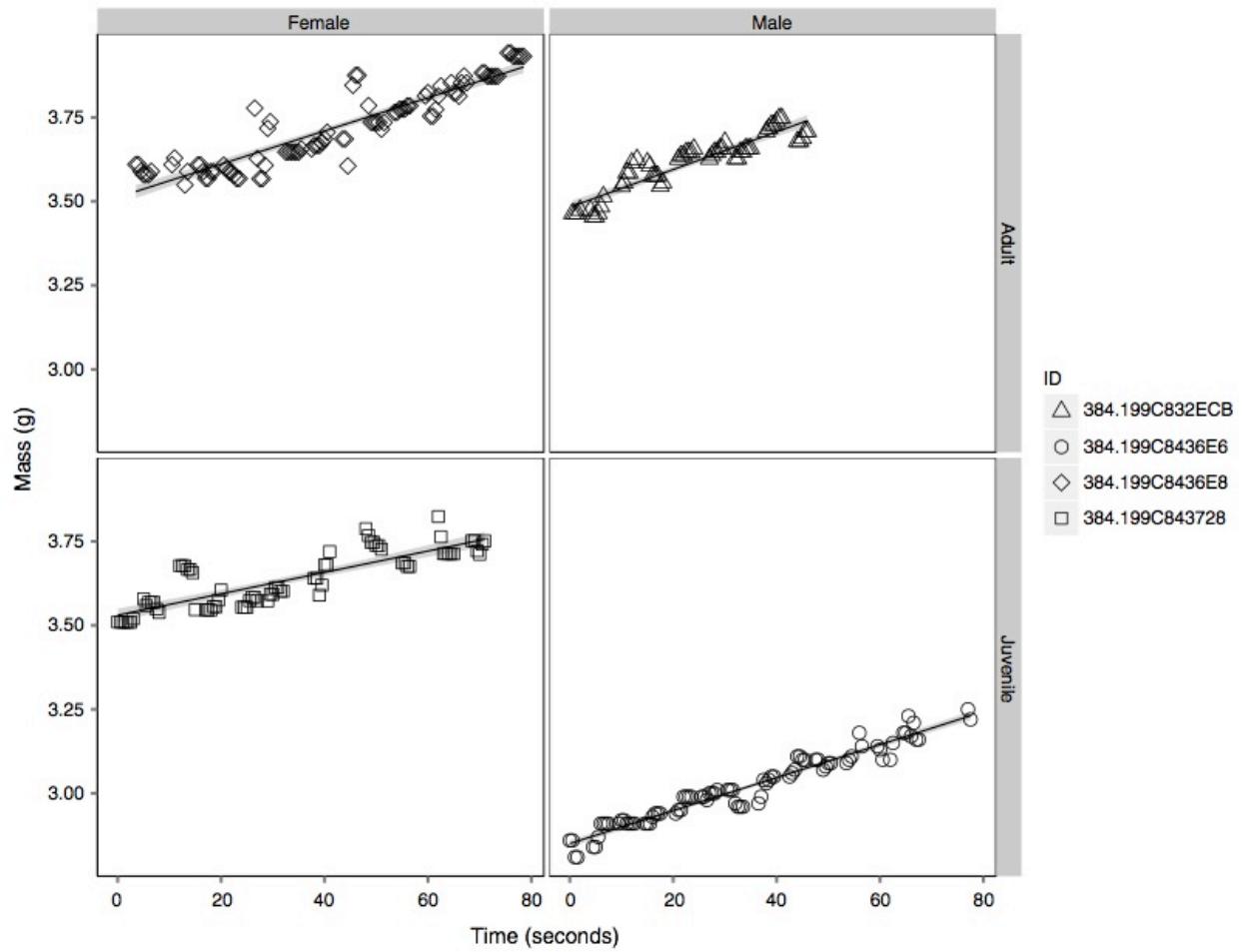
<i>Total time spent at feeders models</i>								
	Fixed effects (Random slope   Random Intercept)	Fixed effects estimates	BCa 95% CI Lower	BCa 95% CI Upper	$R^2$ Marginal	$R^2$ Conditional	n Non-premigratory	n Premigratory
<i>Morning</i>								
2013								
Intercept		3.30	2.54	3.87	0.14	0.57	♀ : 23	♀ : 2
Centered average temperature		-0.06	-0.09	-0.04			♂ : 13	♂ : 1
Sex ( <i>male</i> )		-0.95	-1.29	-0.58				
Unique IDs (Unique IDs   ID)		0.12	0.07	0.19				
2014								
Intercept		4.26	4.05	4.41	0.05	0.41	♀ : 20	♀ : 3
Centered average temperature		-0.10	-0.15	-0.06			♂ : 16	♂ : 1
Total precipitation (Centered average temperature   ID)		0.19	0.06	0.31				
2013 & 2014								
Intercept		3.43	2.97	3.78	0.05	0.51	♀ : 37	♀ : 4
Centered average temperature		-0.07	-0.09	-0.05			♂ : 27	♂ : 2
Total precipitation		0.06	0.01	0.12				
Unique IDs (Unique IDs   Year/ID)		0.08	0.04	0.13				

<i>Evening</i>							
<i>2013</i>							
Intercept	4.43	4.21	4.60	0.02	0.42	♀ : 25 ♂ : 11	♀ : 2 ♂ : 1
Centered average temperature	-0.05	-0.08	-0.03				
Total precipitation (Unique IDs   ID)	-0.04	-0.08	0.00				
<i>2014</i>							
Intercept	3.74	3.38	4.08	0.02	0.37	♀ : 20 ♂ : 15	♀ : 3 ♂ : 1
Unique IDs (NA   ID)	0.07	0.03	0.12				
<i>2013 &amp; 2014</i>							
Intercept	3.82	3.47	4.15	0.02	0.43	♀ : 38 ♂ : 24	♀ : 4 ♂ : 2
Centered average temperature	-0.04	-0.06	-0.01				
Unique IDs (Unique IDs   Year/ID)	0.06	0.02	0.10				

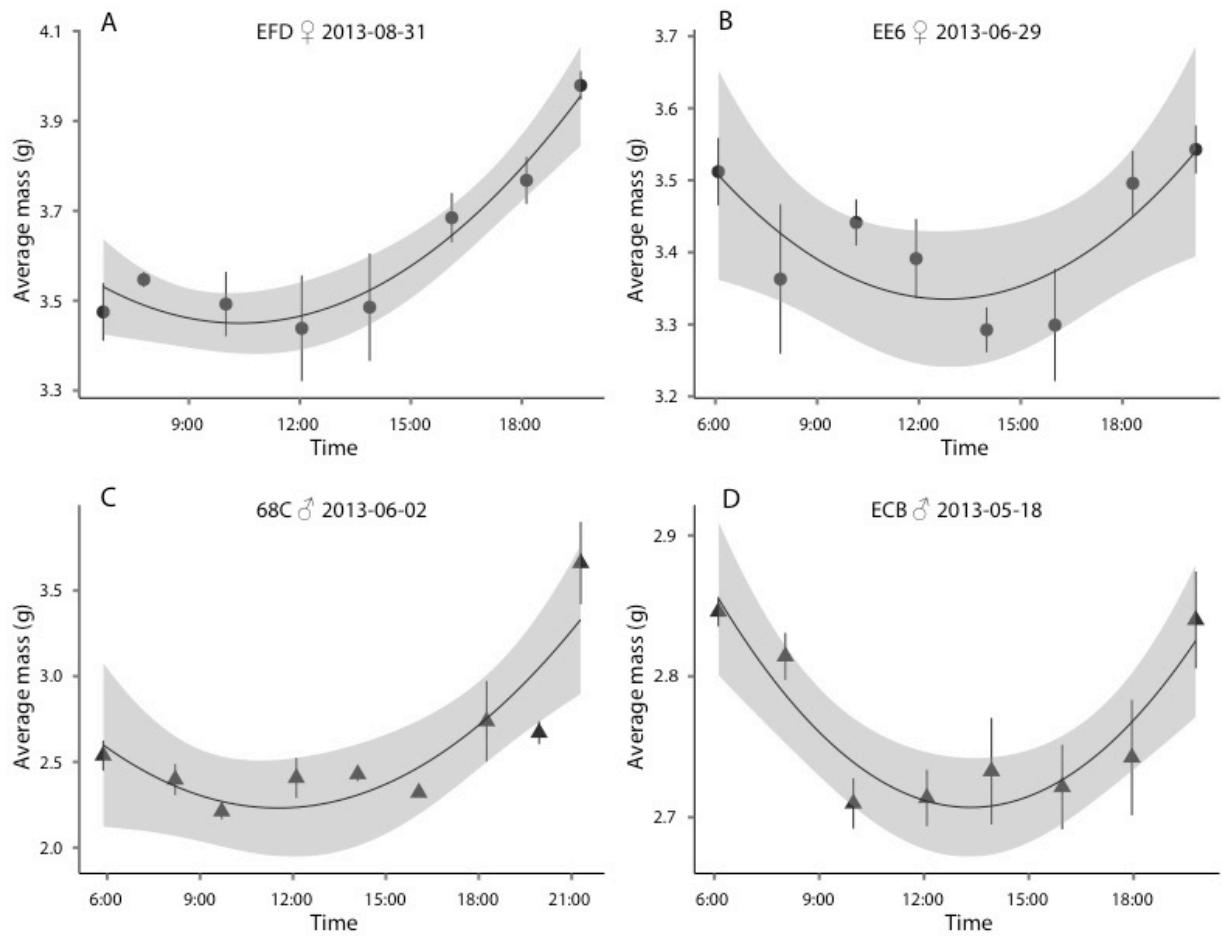
## Figures



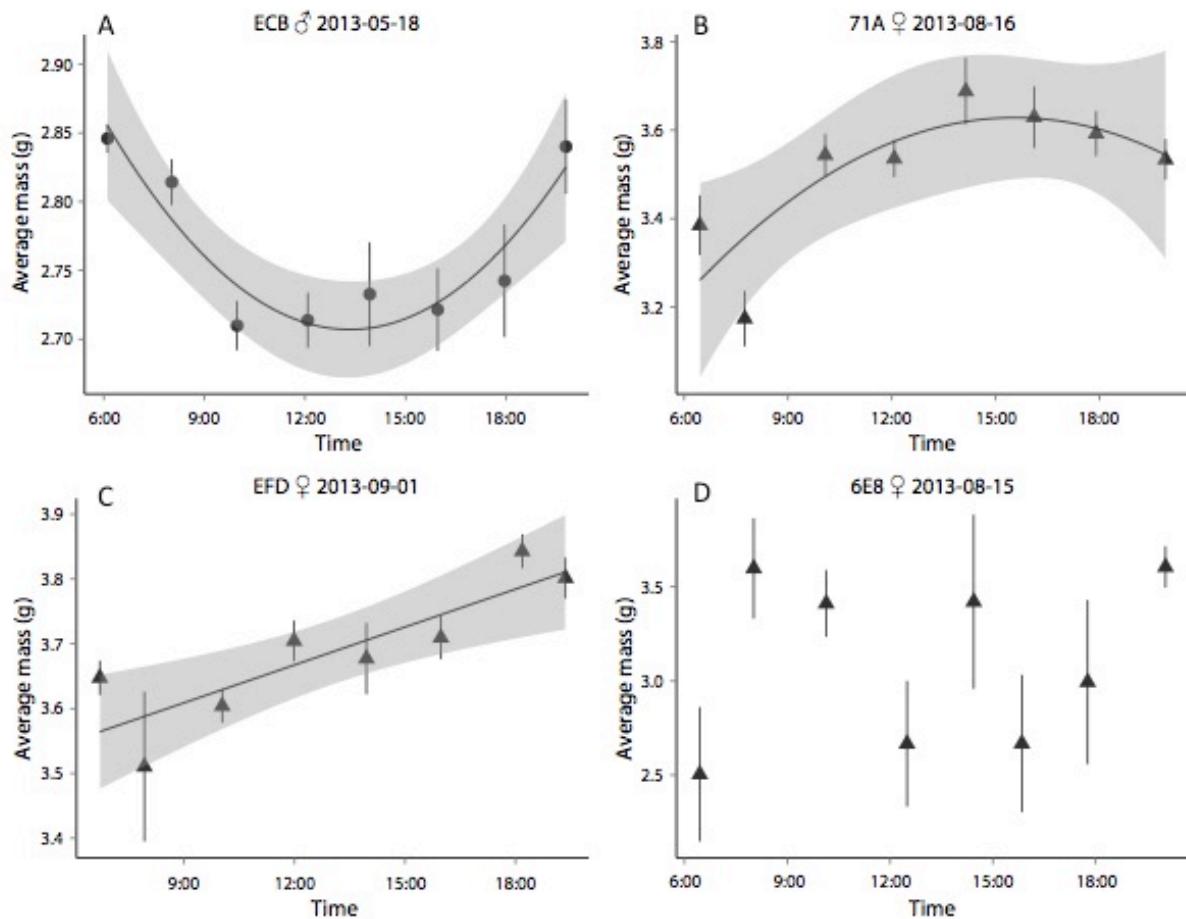
**Figure 1.** Illustration of a perched hummingbird feeding from an artificial feeder with an attached RFID antenna.



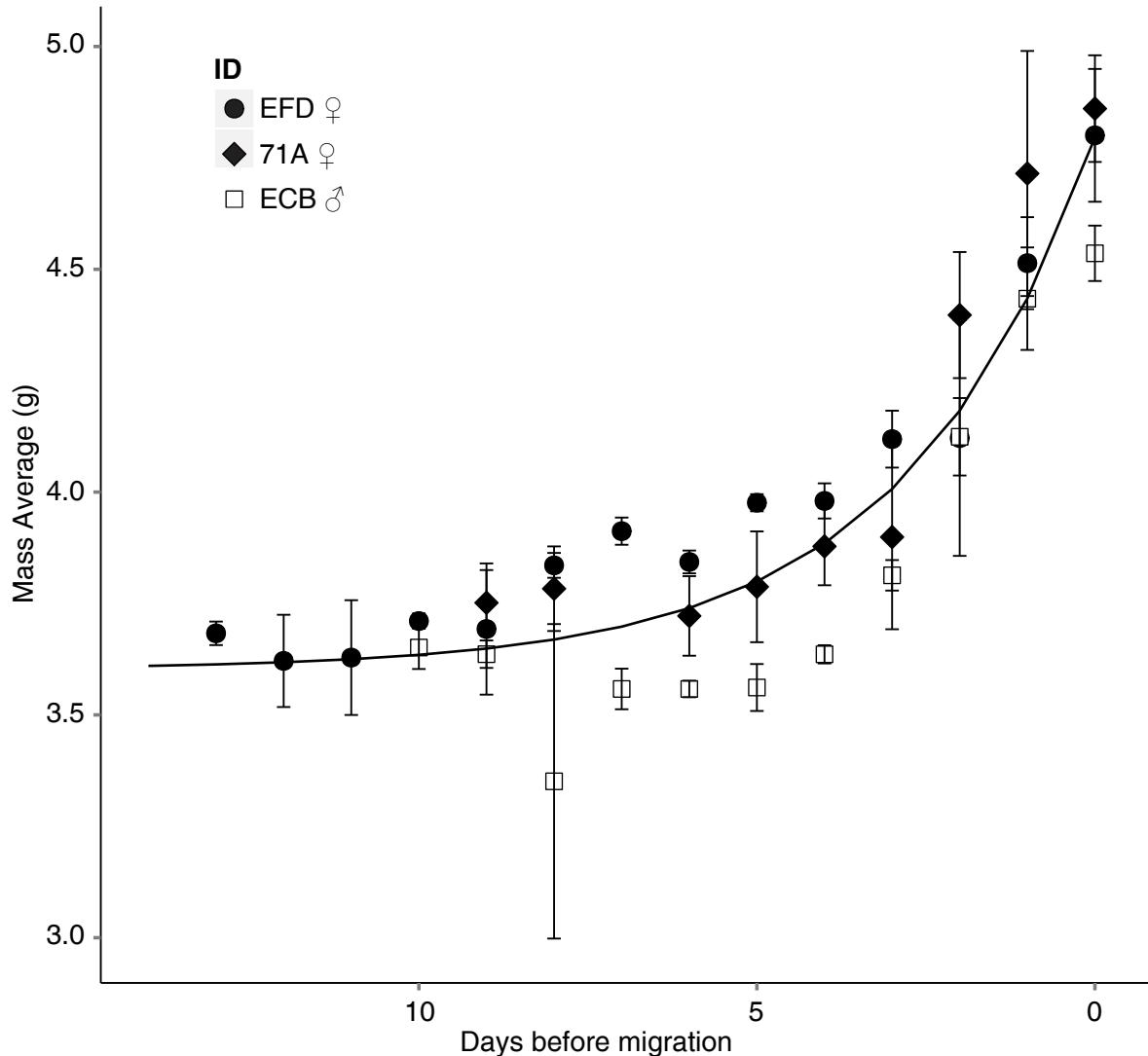
**Figure 2.** Example visitations illustrating linear mass change of ruby-throated hummingbirds. A linear least squares model with 95% confidence intervals was fitted to each visitation after the removal of influential points.



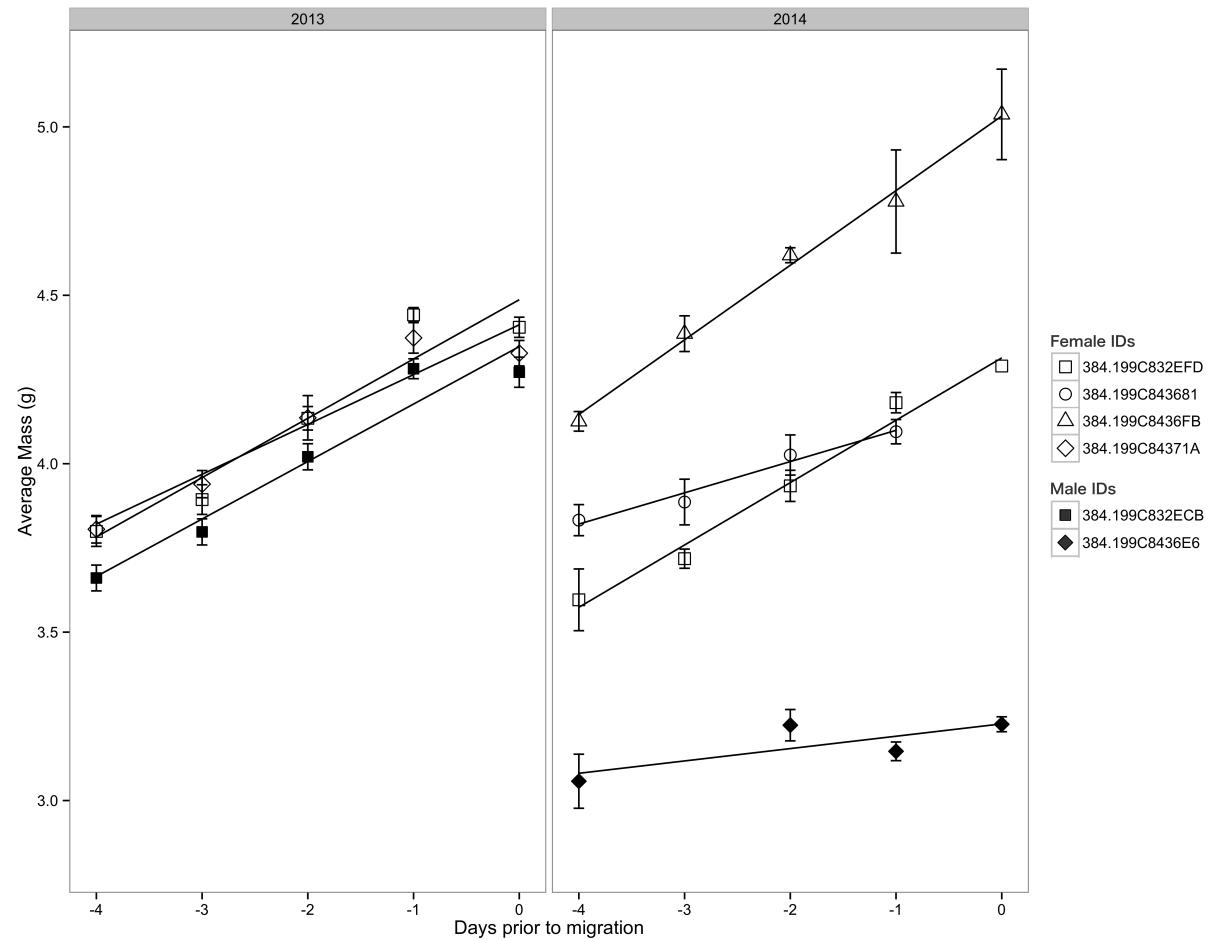
**Figure 3.** Average mass  $\pm$  standard error of (a and b) adult females, (c and d) adult males on example days fitted with a parabolic model and 95% confidence intervals. Hummingbird mass variation during the day between sunrise and sunset presents as a U-shaped curve. Mass is higher in the early morning as a result of compensatory feeding after overnight fasting. Mass then decreases and is maintained at a reduced level as the day progresses. Prior to overnight fasting, hummingbirds engage in hyperphagia resulting in mass gain. Hummingbird IDs have been abbreviated to the last three characters.



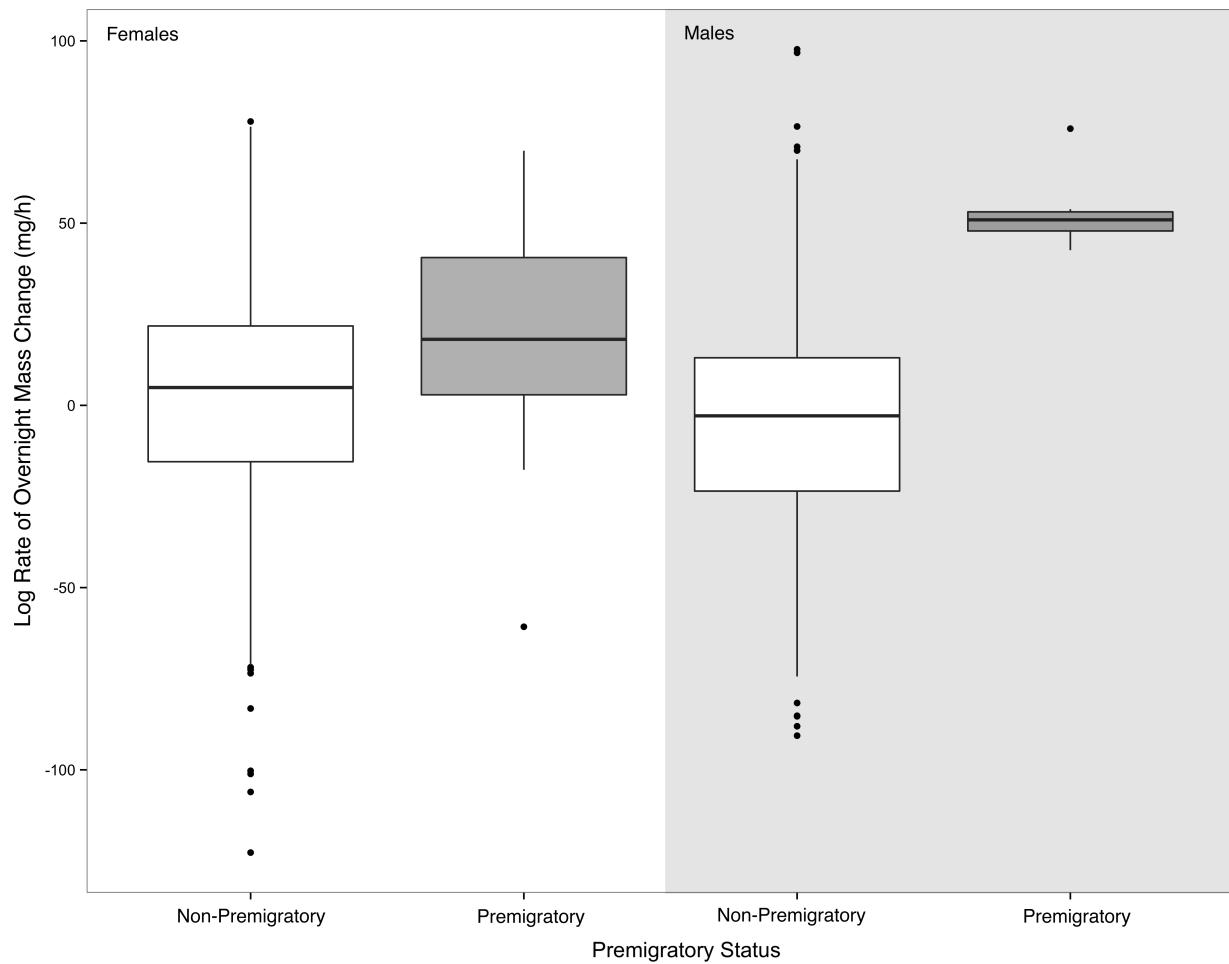
**Figure 4.** Examples of patterns of daily mass change presented as average mass  $\pm$  standard error with 95% confidence intervals. Daily mass change in hummingbirds can present as a) U-shaped curves, b) inverse U-shaped curves, c) linear, or d) erratic.



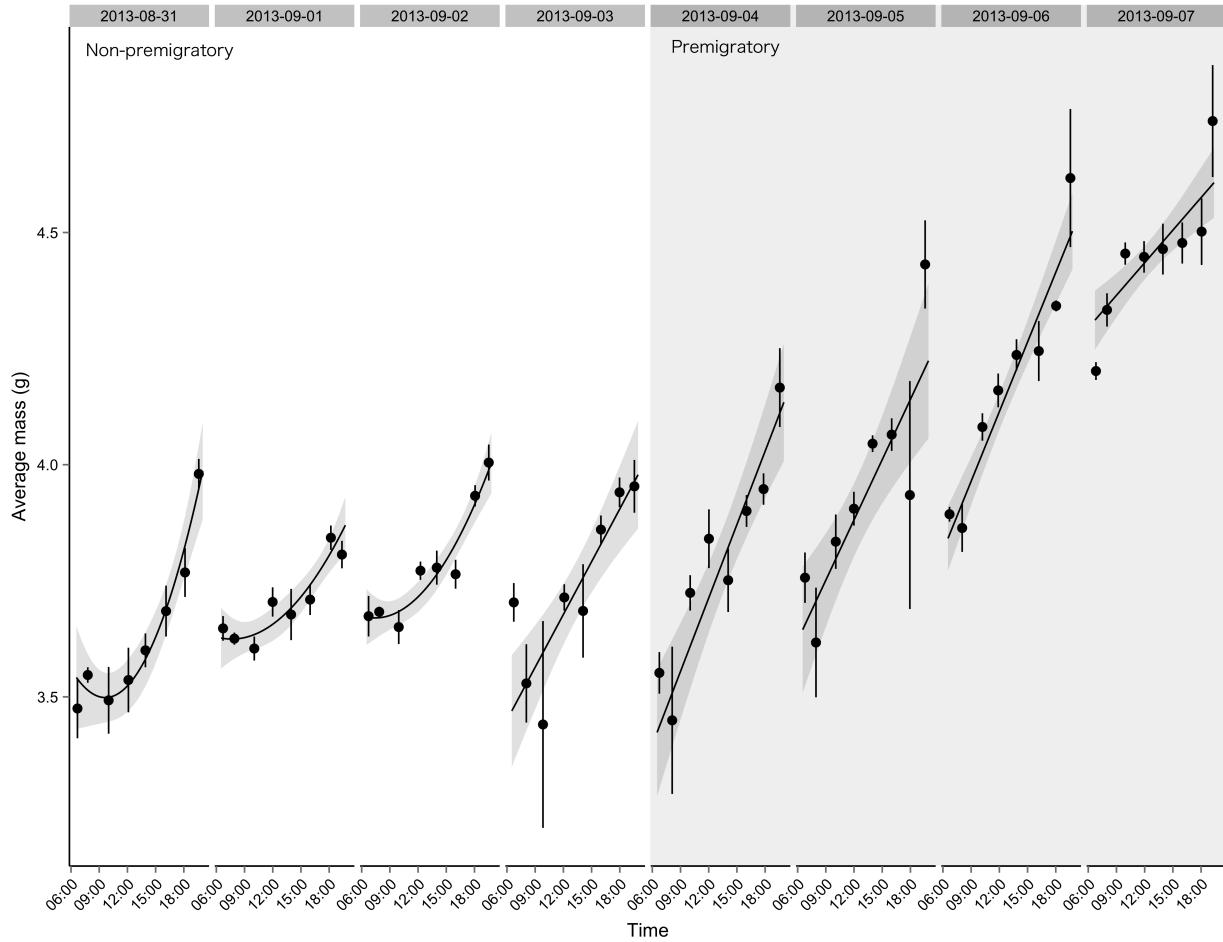
**Figure 5.** Mass change of three hummingbirds prior to migration. Day zero represents the date of last recorded mass for each individual and is the presumed date of migration. The average of median masses  $\pm$  standard error between 1800h and 2100h for each individual increases exponentially leading up to migration.



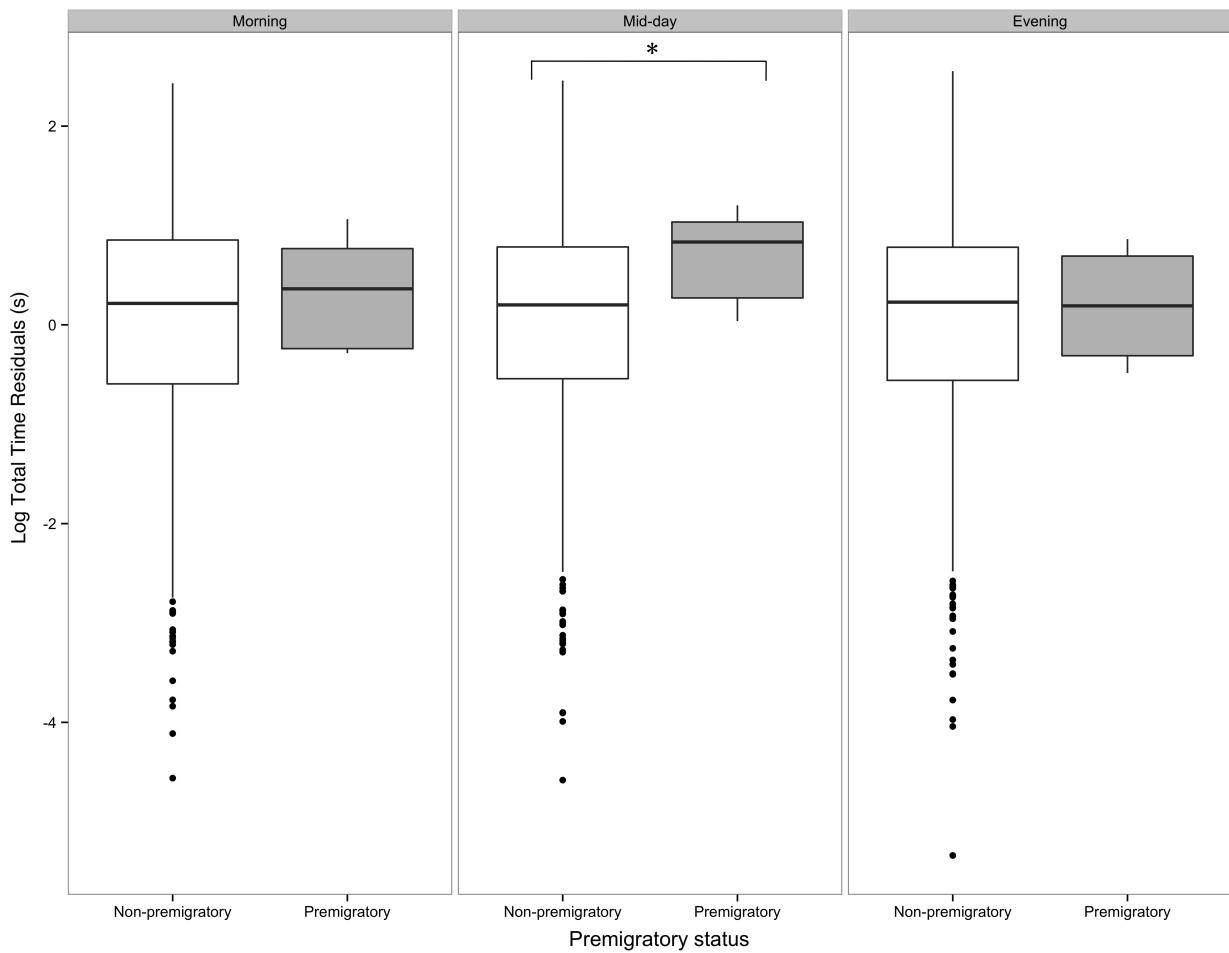
**Figure 6.** A total of 7 adult hummingbirds, 2 males (filled) and 5 females (not filled), exhibited positive linear mass change in the 4 days leading up to the presumed date of migration (day 0).



**Figure 7.** Regression of partial residuals of log rate of overnight mass change against premigratory status and sex of hummingbirds. Premigratory individuals are predicted to exhibit a reduced rate of overnight mass loss compared to their non-premigratory conspecifics, however the magnitude of this change differs by sex. Premigratory males are expected to exhibit a greater reduction in overnight mass loss compared to premigratory females, whereas non-premigratory males are expected to exhibit an increased rate of overnight mass loss compared to non-premigratory females.



**Figure 8.** An example graph illustrating the switch from a U-shape pattern of mass change during the non-premigratory period to a positive linear pattern of daily mass change during the premigratory period by an adult female RTHU (384.199C832EFD). Shown here are days ranging from -8 days (2013-08-31) to -1 days prior to migration (2013-09-07). U-shaped curves best approximated the pattern of daily mass change for days -8 to -6, as determined by the Akaike weights analysis of a linear versus parabolic regression. Using the same method, I determined that days -5 to -1 were best approximated by a positive linear pattern of mass change. For visual clarity, I plotted average mass  $\pm$  standard error for each 2-hour time bin beginning at 0500h and ending at 2300h for each day. Lines of best fit were constructed using original mass values and overlaid onto this plot.



**Figure 9.** Regression of partial residuals of log total time against premigratory status of hummingbirds for the combined analysis of 2013 and 2014 data. Analysis of the mid-day data retained premigratory status as a fixed effect in the best fitting model (\*), indicating that premigratory status is an important variable in explaining the variation in total time spent at the feeders. This, however, was not the case in the mornings and evenings, where premigratory status was dropped from the best fitting models.