ABSTRACT

SEASONAL ACCLIMATIZATION THROUGH PHYSIOLOGICAL CHANGES
IN NORTHERN CARDINALS (*CARDINALIS CARDINALIS*)

by Carrie E. Sgueo

Northern cardinals (*Cardinalis cardinalis*) are faced with seasonal challenges that must be met to ensure survival, such as thermoregulation during the winter, as well as energetically expensive breeding and parental care during the summer. Seasonal acclimatization may be accomplished by phenotypic plasticity of metabolic systems. We measured daily energy expenditure, summit metabolism, and muscle oxidative capacity in winter (December to January) and during the breeding season (May to June), to test the hypothesis that rates of energy utilization will be higher in the winter, and that acclimatization of metabolic systems will occur to meet the demands of higher energy use in winter. We found daily energy expenditure and summit metabolism are significantly greater during the winter, however there were no significant differences in muscle oxidative capacity. We conclude that winter is energetically more expensive than the breeding season, and increased maximum metabolic capacity is a physiological response for seasonal acclimatization.
SEASONAL ACCLIMATIZATION THROUGH PHYSIOLOGICAL CHANGES
IN NORTHERN CARDINALS (*CARDINALIS CARDINALIS*)

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Carrie E. Sgueo
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Advisor ________________________
(Dr. Paul J. Schaeffer)

Reader _________________________
(Dr. Richard E. Lee, Jr.)

Reader _________________________
(Dr. David E. Russell)
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Introduction

Non-migrating birds are required to withstand a wide range of seasonal challenges, from withstanding cold temperatures in the winter, to breeding in the summer. Seasonal acclimatization for non-migrating avian species may be achieved through phenotypic plasticity of physiological characteristics including changes in morphological characteristics and/or through the metabolic system. Phenotypic plasticity is defined as the capacity for change within genetically uniform organisms in response to different environmental conditions (Piersma and Lindstrom 1997). Throughout the year, each season presents a different set of energetic challenges. In the summer, birds’ primary activities are breeding and parental care. During the harsh, cold months of winter, birds undergo many adaptive energy management techniques for survival, including body fat regulation and night–time hypothermia (Brodin 2007). Due to the high energetic demands of breeding and thermoregulation, during the summer Yellow-eyed Juncos feeding fledglings can spend up to 69.3% ±2.5 of the active day foraging and walking, and during the winter this species spends up to 59.6% ±5.5 of the active day foraging and walking (Weathers and Sullivan 1993). Physiological responses to these challenges may be occurring through phenotypic acclimatization of maximum metabolic capacity, muscle oxidative capacity, or behavioral changes. Our focus is on phenotypic plasticity of energetic systems that occur in response to seasonality in a non-migratory passerine bird, the Northern Cardinal (Cardinalis cardinalis).

Currently, there are two published hypotheses that attempt to explain seasonal changes in daily field metabolic rate. The reallocation hypothesis states that there is little seasonal variation in daily energy expenditure, or the metabolic rate during an animal’s daily activities, such as foraging or flying during a given day (Masman et al. 1986). The increased demand hypothesis states that the field metabolic rate reaches an annual maximum metabolic rate during the breeding season, suggesting the breeding season is the most energetically expensive season (Masman et al. 1986). However, Cooper (2000) found the daily energy expenditure of Mountain Chickadees (Poecile gambeli) and Juniper Titmice (Baeolophus griseus) is higher in winter than summer. Thus, it is unclear which season is more energetically expensive, or why results vary between studies.

In addition to changes in daily energy expenditure, changes in the rate of energy use per hour within a day may differ between seasons, potentially due to changes in temperature, or food availability. Hourly analysis of energy use for a full twenty four hour period has not been published. However, energy expenditure was presented on an hourly basis when studied by Bisson et al. (2009), who investigated the impact of anthropogenic disturbances on the White-eyed Vireo (Vireo griseus) during the breeding season. The lack of any significant effect of human disturbance was attributed to the fast life history of that species, requiring it to respond quickly in order to allocate energy to reproduction (Bisson et al. 2009). Cardinals also have a fast life history, and frequently live during one breeding season. Therefore, cardinals need to adapt quickly to seasonal challenges in order to breed or survive, potentially through physiological changes such as differently allocating energy use on an hourly basis.

If rate of energy use changes on a seasonal basis, potential mechanisms for dealing with increased energy use are night-time hypothermia (Brodin 2007), changes in diet efficiency (Weathers and Sullivan 1993), or changes in maximum metabolic capacity, termed summit metabolism (Swanson and Garland 2009, Liknes et al. 2002). There are two published hypotheses that serve as possible explanations for variation in summit metabolism (Swanson and Garland 2009). The cold adaptation
hypothesis suggests that species wintering in cold climates will have a higher summit metabolism than those who winter in warmer climates. The flight adaptation hypothesis states that high summit metabolism may result from birds’ muscular capacity for flight, and the changes in summit metabolism are therefore associated with the ability for sustained flight. Therefore, species that migrate before the winter might have higher summit metabolism than those individuals who do not migrate (Swanson and Garland 2009). Results from Swanson and Garland (2009) supported the cold adaptation hypothesis. If summit metabolism is changing, possibly as an adaptation to either meet environmental demands based on where the bird overwinters or for migration, than summit metabolism of non-migrants may also be changing on a seasonal basis to adapt to environmental changes within a given area.

There have been varying results from studies that have directly compared summit metabolism seasonally. Liknes et al. (2002) found an increase in summit metabolism of the American Goldfinch (Carduelis tristis) during the winter compared to summer. This suggests that a bird’s metabolic capacity increases to withstand cold during winter and thus to enable survival, further suggesting that the correlation of summit metabolism and thermogenic endurance are phenotypic responses to increased cold tolerance of birds that stay north for winter (Liknes et al. 2002). However, in a review of various studies by Marsh and Dawson (1989), the difference in summit metabolism during winter and summer for avian species is relatively small in comparison to the changes in summit metabolism that occur in mammals of similar size.

In addition to changes in metabolism, changes in organ and muscle size have been observed in migrating species. Organs including heart, liver, pectoralis, and intestines change size in order to adjust to the energy demands of long distance migration (Piersma et al. 1999). If a bird is able to change the size of internal structures for long-distance migration, it may also be possible for birds to adapt internal structures to meet the demands of seasonal challenges. Therefore, we are also interested in seasonal acclimatization through changes in size or metabolic activity of muscle tissue, the primary contributor to metabolic activity. Due to the large size of the pectoralis, it can contribute to thermoregulation via shivering and is also responsible for the downward movements of the wing during take-off and flight.

Recent research has mainly focused on the changing mass of the pectoralis, demonstrating an increase in pectoralis mass during the winter season. Swanson (1991) weighed the pectoralis muscle in Dark-eyed Juncos (Junco hyemalis) during summer and winter, and observed the pectoralis mass was higher in the winter. This may allow for an increase in thermogenic abilities due to shivering, or enable increased cold tolerance for over-wintering birds (Swanson 1991). O’Connor (1995) weighed the pectoralis mass of House Finches (Carpodacus mexicanus) during the winter and late spring, the breeding season. The pectoralis increased during the winter by 20% and 33% for lean dry and wet mass, possibly to maintain proper body temperatures during cold conditions through shivering. Muscle properties, particularly understanding changes in oxidative capacities of tissue, remain unexplored.

In order to understand how birds meet the demands of seasonal metabolic challenges, we investigated rates of energy use in winter and summer, as well as structural and functional responses that occur as a means of seasonal acclimatization. We hypothesized that daily energy expenditure will be significantly higher in the winter than summer. Therefore changes in structure and function of metabolic systems will occur to meet the demands of higher energy use in winter. Specifically we asked if seasonal changes occur in 1) daily energy expenditure (DEE), 2) maximum metabolic capacity, and 3) muscle oxidative capacity, which may act as a means of seasonal acclimatization.
Materials and Methods

1. Study species and field sites
   For this study we used the Northern cardinal (*Cardinalis cardinalis*), a very abundant, non-migrant species in southern Ohio. Birds were caught with mist nets between sunrise and late afternoon, and release was always at least one hour before sunset to ensure a successful transition back to the environment following laboratory interventions. All birds were captured under Ohio Department of Natural Resources permit #11-152 and US Fish and Wildlife Service permit #MB158451-1. All animal experimentation was approved by the Institutional Animal Care and Use Committee of Miami University and complied with the “Principles of Animal Care,” publication no. 86-23, revised 1985, of the National Institutes of Health as well as the laws of the United States.

   Study sites were located near Oxford Ohio, at The Ecology Research Center (ERC) of Miami University (39°30'N and 84°45'W) and Hueston Woods Biological Station located in Hueston Woods State Park (39°34'N and 84°44'W).

2. Animal Morphometrics
   Data sets of body weight, fat score, wing chord, tarsus length, tail length, and culmen length measurements were collected between May 13th and June 18th, and from December 17th to February 28th from 2004 to 2007. For these measures, all birds were captured via mist nets and then released after data collection. Body weight measurements were taken with a digital scale at the field site upon capture. Fat score was also measured at the field site using a zero to seven score, depending on the size of the fat pouch in relation to the height of the rib cage (DeSante et al. 2009).

3. Physiology Experiments
   Northern cardinals for all other experiments described below were captured by mist nets at the ERC from December 17th to February 28th, and May 13th to June 18th in 2007 to 2009. These timelines were designed to ensure birds sampled were experiencing cold stress in the winter, and breeding stress in the summer. Upon capture, cardinals were taken to lab for one of the following procedures:

   a) Daily Energy Expenditure
      Once cardinals were brought into lab, a heart rate transmitter was attached to the back of the bird, with fine wire electrodes placed subcutaneously for detection and transmission of ECG signal as described (Butler et al. 2000; Froget et al. 2001). Transmitters (~1 gram; J.D.J.C. Corp, Fisher, IL) weighed less than 3% of the bird’s body weight, minimizing the likelihood that birds were experiencing increased metabolic effort in carrying transmitters. After transmitter attachment, and before bird release, we simultaneously measured heart rate and oxygen consumption using indirect calorimetry (Sable Systems, Las Vegas, NV) in order to generate a calibration curve as described previously (Butler et al. 2000; Froget et al. 2001). While generating the calibration curve, the bird was agitated in the chamber, raising both heart rate and metabolism to create a wide range of heart rates, representative of those that may be obtained in the field. Individual calibration curves were collected for each bird and used to determine daily energy expenditure from heart rate data collected in the field. For those animals that we were unable to generate an individual calibration curve (n=2 for winter, and n=1 for
summer) a master calibration curve for each season was produced by combining all individual curves for that season.

Once the calibration curve was generated, birds were released at the ERC. Radio telemetry was used to track each individual for 24 continuous hours, beginning at least 12 h after bird was released, enabling the bird to recover from transmitter attachment, and return to an unstressed physiological state. During the 24 h tracking period, heart rate was recorded every five min. We successfully tracked four individuals during the summer and five during the winter.

Heart rate was determined following FFT transformation and filtering of raw transmission using CoolEdit 2000, as described previously (Bisson et al. 2009). Daily energy expenditure (DEE) was then calculated using the mean heart rate from field measurements and the appropriate calibration equation to determine rate of energy use. In addition to DEE, rate of energy use was analyzed on a per hour basis to observe the pattern of energy use throughout a given day.

b) Summit Metabolism

To measure summit metabolism, a second set of birds were brought into lab and weighed. We then used indirect calorimetry to determine oxygen consumption during a maximal cold stress (following Rosenmann and Morrison 1974). Incurrent gas during summit metabolism measurements uses a mixture of helium-oxygen, where nitrogen is replaced with helium. The helium-oxygen gas mixture causes increased heat loss due to high conductance. To maximize cold stress, the metabolic chamber was placed in a 4˚C refrigerator, creating an environment that produces extreme cold stress. Summit metabolism measurements were calculated using respiration data once the bird reached a steady state plateau at maximum capacity. Summit metabolism measurements were recorded from eight individuals in both summer and winter.

c) Muscle Oxidative Capacity

Following measurements of summit metabolism, individuals were anesthetized and decapitated. The pectoralis muscle from each individual was then dissected and weighed. Additionally, a 3-5mm³ portion of tissue was removed from the center region of the pectoralis muscle, frozen in liquid nitrogen, and stored at -80°C for oxidative capacity analysis. This sample was used to measure the muscle oxidative capacity of the pectoralis muscle using a citrate synthase assay (following Chi et al. 1983).

Muscle samples were prepared by hand homogenization, with 9 volumes of homogenization medium (Tris +KCL; 0.05 M Tris, 0.15 M KCL) added, and centrifuged. Supernatant was removed, frozen in liquid nitrogen and stored at -80°C. To assay maximal citrate synthase activity, pectoralis homogenates were combined with an excess of Acetyl CoA and Oxaloacetate in 100 mM Tris buffer (Tris+OAA+AcCoA; 0.05 M Tris, 0.025 M OAA, 0.01 M AcCoA ). Rate of Acetyl-CoA disappearance was monitored at 260 nm in a temperature controlled spectrophotometer (DTX880 Multimode Detector, Beckman Coulter, Inc., Fullerton, CA) at 25°C to find rate of enzyme activity. Pectoralis samples were analyzed for 8 individuals in both the summer and winter.

4. Statistical Analysis

Pairwise comparisons of winter and summer acclimatized groups used Non-parametric t-tests for fat score and Student’s t-test for DEE, summit metabolism, muscle oxidative capacity, and both body and pectoralis weight. Hour by hour energy utilization rates were analyzed using repeated measures
ANOVA. The level of significance was set at p<0.05 in all cases, except for pairwise comparisons of individual one hour periods of rate of energy use which used a Bonferroni correction. All data are presented as means ± standard error of the mean.
Results

Morphology:
We began our study with measurements of avian morphology to understand bird composition during each season, and its impact on metabolism. We found that the average body mass in the winter was 47.2 g (± 0.9; n=22), significantly higher than the summer body mass of 41.5 (± 0.3; n=102); (p<0.05, Figure 1), suggesting changing body mass may be a means of seasonal acclimatization. There was no significant difference in wing chord, tarsus length, tail length, culmen length or pectoralis mass (p>0.05 in all cases) during the winter and summer, meaning structurally the birds did not differ between seasons (data only shown for pectoralis mass, Figure 2). Similarly, pectoralis weight per unit mass was also not significantly different during the summer and winter (p>0.05, Supplemental Figure 1). The average pectoralis weight per unit mass during the winter was 14.5 % (± 0.9; n=8), and 13.9 (± 0.4; n=8) in the summer. Thus a larger pectoralis mass is not the cause of a greater body weight during the winter, and is not a contributor to physiological adaptation during seasonal acclimatization. However, fat score, measured on a zero to seven scale, was significantly higher in the winter acclimatized birds than those in summer (Kruskal-Wallis chi-squared = 67.7383, df = 1, p-value < 0.05; Figure 3). The average fat score in the winter was 3.0 (±0.271; n=22) and 0.208 (±0.046; n=106) in the summer.

Given that body weight and fat score were significantly higher in the winter than summer (p<0.05), while wing chord, tarsus length, tail length, culmen length, and pectoralis mass were not significantly different, we concluded that the higher winter adipose tissue mass caused the greater body mass in the winter. As fat is relatively metabolically inactive, subsequent metabolic rate measurements are reported on a whole animal basis to avoid higher winter adiposity masking changes in mass specific animal metabolic measurements.

Field Metabolic Rate
To determine which season is more energetically expensive, we determined daily energy expenditure during the summer and winter using heart rate telemetry. Daily energy expenditure was significantly higher in winter acclimatized birds (Figure 4). The average daily energy expenditure in winter acclimatized birds was 5.06 LO₂·day⁻¹ (±0.22; n=5), and was 3.76 (±0.44; n=4) during summer (p<0.05). When corrected for body mass, daily energy expenditure is not significantly different between groups (Supplemental Figure 2). The average daily energy expenditure per unit mass is in the winter was 0.117 LO₂·g·day⁻¹ (±0.01; n=5), and was 0.095 (±0.01; n=4) in the summer (p>0.05), which while trending toward a higher value in winter, it is likely obscured by the higher amounts of adipose tissue in winter birds.

A significantly higher whole animal daily energy expenditure in the winter indicates that winter is more energetically expensive, and individuals may require physiological adaptations to meet these demands. Although not statistically significant, the comparison of mass specific daily energy expenditure was nearly significant (p = 0.067), suggesting that a significant increase could result with a larger sample size.
**Daily Patterns in Energy Expenditure**

We examined patterns of daily energy expenditure on an average hourly basis for each bird within a given season for a 24 h period (Figure 5). In the summer, there were two peaks of activity, the first following dawn and the second before dusk. During the heat of the day in the summer there was a decrease in energy expenditure corresponding to field observations of less activity during mid summer afternoons. In the winter, energy expenditure would plateau at elevated levels throughout the day. This indicates that cardinals are using the same amount of energy each hour during the entire day, possibly corresponding to the constant environmental challenge of low winter temperatures. During the winter, birds spent more energy during the day than night.

Repeated measures analysis of variance (ANOVA) using mixed procedure in SAS version 9.1 for Windows was used to analyze daily patterns in energy expenditure. There was a significant interaction between season and time, demonstrating the effect of hour on oxygen consumption depended on the season ($F(23, 161)= 3.60, p<0.05$). The interaction effect was analyzed further by comparing each hour of the day between seasons, creating a total of 24 comparisons. It is important to note that due to the difference in time of sunrise and sunset between summer and winter, light difference may contribute to differences for hours at sunrise and sunset. Significant differences between each hour of the day were analyzed using a Bonferroni correction to the p-values. Significant differences of oxygen consumption occurred between winter and summer for each hour, starting from the first hour of 10:00 to 11:00am, to the last hour of 6:00 to 7:00pm. This demonstrates that energy expenditure is significantly higher in the winter than summer during these hours. Findings for oxygen consumption per body weight were consistent with these results; except that no significant difference occurred between winter and summer from the hour of 10:00 to 11:00am.

**Summit Metabolism**

We measured summit metabolism to discover if changing metabolic capacity is a means for seasonal acclimatization. Whole animal summit metabolism was significantly higher in winter acclimated birds (Figure 6). The average summit metabolism in winter acclimated birds was 7.33 mLO$_2$·min$^{-1}$ ($\pm$0.34; n=9), and 6.05 ($\pm$0.26; n=8) during summer ($p<0.05$). However, mass specific summit metabolism was not significantly different between winter and summer (Supplemental Figure 3). The average mass specific summit metabolism in winter acclimatized birds was 172.08 mLO$_2$·kg$^{-1}$·min$^{-1}$ ($\pm$6.00; n=9), and 156.41 ($\pm$6.64; n=8) during summer ($p>0.05$).

The significant difference in a higher winter whole animal summit metabolism indicates that changing summit metabolism is a means for accommodating the greater demand in winter daily energy expenditure. The lack of difference in summit metabolism in mass specific summit metabolism may be due to the greater amount of adipose tissue in the winter masking the metabolic changes in the rest of the body. A p-value of 0.10 suggests that a significant statistical difference may occur with a larger sample size.

**Muscle Oxidative Capacity**

To demonstrate if changing muscle oxidative capacity is a means of seasonal acclimatization, we measured muscle oxidative capacity of the pectoralis muscle. Tissue has higher oxidative capacity when more mitochondria are present within a tissue. By measuring the relative citrate synthase activity, we
found no significant difference in the number of mitochondria in the pectoralis between the summer and winter (Figure 7, p > 0.05). This suggests that changing the oxidative capacity of the pectoralis is not used to adapt to seasonal challenges.
Discussion

We began this study with an interest in understanding avian physiological changes that occur as a means of seasonal acclimatization for Northern Cardinals. If a non-migrating bird has to endure different seasonal conditions and challenges, how does the individual meet these demands? We are interested in the possibility that phenotypically plastic physiological traits are seasonally variable. Our approach was to measure both structural and functional characteristics of the Northern cardinal to understand morphological changes that occur seasonally and have an impact on seasonal acclimatization and/or metabolism.

After analyzing our structural data, we found that body weight was significantly higher in birds examined during the winter. Wing chord, tail length, tarsus length, culmen length, and pectoralis mass did not differ between summer and winter, meaning structurally the birds were not changing. However, the fat score was significantly higher in the winter indicating that the higher winter body mass was due to a greater amount of adipose tissue. Two important issues arise from this structural data. First, the lack of change in pectoralis muscle differs from previous studies, and the second is the significance of the increase in body weight and fat score.

To address the first issue, the lack of significant difference in the pectoralis muscle mass in the summer and winter differs from a previous study by Swanson (1991) who found an increase in the pectoralis muscle mass of Dark-eyed Juncos in the winter. Swanson suggested that the increase in muscle mass may increase the capacity for shivering thermogenesis. Changes in organs are a common adaptation for many birds, particularly before migratory take off when stomach, intestines, leg muscles and liver decrease in size, while the pectoralis and heart hypertrophy (Piersma et al. 1999). If the pectoralis can change size for migration, the same could occur for thermoregulation. However, if the pectoralis is not changing in the Northern cardinals, different physiological adaptations would need to utilized to meet seasonal energetic changes that are mentioned later.

The second important topic to address from our structural data is that the higher body weight and fat score during the winter indicate that changing body composition is a means of seasonal acclimatization by increasing thermoregulatory abilities. Supporting this, Dawson et al. (1983) reported that in order to withstand the long flights of migration, birds have the ability to double their body mass due to increased adipose tissue, depending on the length of migration. Additionally, Smith (2005) found male American Redstarts that arrived at breeding grounds earlier, when temperatures were colder, had higher fat scores than those that arrived later. Therefore, if a bird can adjust its adipose tissue mass for migration, the same adaptation can occur to assist in thermoregulation, to provision fuel for inclement weather, or to withstand long winter nights.

In addition to the structural responses of the Northern cardinal, we investigated seasonal daily energy expenditure as a potentially important functional characteristic to identify which season is more energetically expensive. Daily energy expenditure was significantly higher in winter acclimated birds, indicating thermoregulation during winter is energetically more expensive than breeding. The higher daily energy expenditure in the winter differs from both the increased energy demand hypothesis, which states there is little seasonal variation in daily energy expenditure, and reallocation hypothesis that states field metabolic rate reaches an annual maximum metabolic rate during the breeding season (Masman et al. 1986). Therefore, seasonality impacts the amount of oxygen consumption within a given
day. When daily energy expenditure is expressed as a mass specific measure, there was no significant difference between seasons (p-value= 0.067). The resultant trend towards a higher daily energy expenditure in the winter is due to adipose tissue masking lean body mass effects with low power due to a small sample size.

Higher daily energy expenditure in the winter is the result of dealing with constant low temperatures, during a time when food is less abundant. In a study by Cooper (2000), daily energy expenditure was also significantly higher in the winter than summer for Mountain Chickadees (Poecile gambeli) and Juniper Titmice (Baeolophus griseus) as a result of increased thermoregulatory costs. In addition, energy expenditure is higher in the winter because individuals must survive a long cold night, in comparison to the shorter length of the night during the summer.

Another reason for the difference in energy expenditure may be due to change in diet. Weathers and Sullivan (1993) noted that Juncos switch from eating predominately seeds in the winter to insects in the summer, increasing foraging efficiency when juncos forage on insects. Northern Cardinals may be experiencing this shift in diet, and therefore the higher winter daily energy expenditure may be due to a decrease in foraging efficiency in the winter. This would be an interesting future study to investigate the impact of seasonal changes in diet on daily energy expenditure.

In addition to comparing seasonal energy use, by determining daily levels of energy expenditure we provided a compartmental break down of energy use within a given day. There was a significant interaction between season and time, demonstrating that the effect of time of day on oxygen consumption depended on the season. We further analyzed daily energy expenditure by comparing each hour of the day between seasons, creating a total of 24 comparisons. Significant differences of oxygen consumption occurred between winter and summer for each hour starting from the hour beginning at 10:00am to the hour beginning at 6:00pm, with energy expenditure being significantly higher in the winter during these hours. It is important to note that due to the difference in time of sunrise and sunset between the summer and winter, light difference may contribute to differences for hours at sunrise and sunset. This supports our data that winter is more energetically expensive than summer, and also allowed us to identify energy use patterns throughout the day.

During summer and winter, there was an increase of energy use at dawn and a decrease in energy use at dusk. However, during the summer the increase in energy use at dawn peaks, and also peaks again at dusk. This indicates that during the summer mid-day when temperatures are highest birds are using less energy compared to dawn and dusk. This coincides with field observations that individuals stayed in the shaded trees in relatively small areas during the height of the summer heat. The behavioral differences in dealing with mid-day summer heat may be possible due to the increase in food availability and quality. In a study by Weathers and Sullivan (1993) the seasonal diet change for Juncos from seeds in the winter to insects in the summer increased gross foraging efficiency. If this change in diet occurs in cardinals, the higher quality of food during the summer may allow foraging to mainly take place at dawn and dusk, as opposed to an entire day.

However, in the winter energy use plateaus throughout the entire day, without peaks, possibly due to the constant environmental cold challenge the winter season presents. No previous data compares energy use on an hourly basis between summer and winter, yet this does support data reported by Cooper (2000) which found that winter is more energetically expensive than summer for Mountain Chickadees (Poecile gambeli) and Juniper Titmice (Baeolophus griseus).
In the winter energy expenditure was higher during the day than night. In a previous study by Mayer et al. (1982), it was found that a combination of cavity roosting and night time hypothermia in Carolina Chickadees accounted for 50% savings in energy expenditure. Neither cavity roosting or night time hypothermia has been found in the Northern Cardinal, however our data suggests that a physiological change or behavioral response is occurring to create lower energy expenditure at night, when temperatures are coldest.

In order to determine if birds were changing their maximum metabolic capacity as a means to meet seasonal challenges, we measured summit metabolism. We found that whole animal summit metabolism was significantly higher in the winter than summer, indicating that changing maximum metabolic capacity is a phenotypically plastic trait for dealing with the higher winter energy demand. This agrees with results from Swanson (1990) that also found summit metabolism to be significantly higher in winter than summer of the Dark-eyed Junco (*Junco hyemalis*). Swanson suggested this increase in maximum metabolic capacity may be associated with increased shivering endurance. However, we found no significant increase in pectoralis mass, a muscle used for shivering.

Our data also agrees with results from Liknes et al. (2002) that found an increase in summit metabolism during the winter in comparison to summer of the American Goldfinch (*Carduelis tristis*). Swanson (2001) suggests the physiological adjustments that produce increased thermogenic endurance may also produce an increase in summit metabolism. Currently, the mechanism underlying increased summit metabolism remains unclear. Although we did not study thermogenic endurance in this experiment, the evidence of the correlation between summit metabolism and thermogenic endurance found in Swanson (2001) may provide a mechanism for changes in summit metabolism in the future.

There was no significant difference between mass specific summit metabolism in the winter and summer. However, similar to the results of daily energy expenditure, there is a trend towards a higher summit metabolism in the winter with a p-value of 0.10. Given the potential for greater winter adipose tissue to mask energetic differences of lean body mass, this trend supports the conclusion that summit metabolism is higher in the winter.

In order to examine whether phenotypic plasticity of skeletal muscle oxidative capacity is an important component of winter acclimatization, we observed muscle oxidative capacity of the pectoralis tissue. We found no significant difference in the citrate synthase activity of the pectoralis between the summer and winter, and conclude that changing muscle oxidative capacity is not a physiological adaptation to meet seasonal challenges. Since the pectoralis muscle does not change size between summer and winter, it would appear that seasonal acclimatization does not occur at the cellular or tissue level in this muscle.

In conclusion, winter is energetically more expensive than the summer, meaning that thermoregulation is more energetically costly than breeding. Energy expenditure during the winter midday is higher than summer, and is also higher than energy expenditure at night. Northern Cardinals have a greater maximum metabolic capacity in the winter than summer, indicating summit metabolism is a phenotypically plastic trait to deal with high winter energy demands. However, seasonal change of muscle oxidative capacity was not a means of seasonal acclimatization for the Northern cardinal.
Figure Legends
For Both Figures and Supplemental Figures

**Figure 1: Body weight of Northern cardinals is significantly higher in the winter than summer.**
In order to determine seasonal changes in morphology, we first measured body weight of Northern cardinals during winter and summer. The significant higher body weight during the winter suggests that higher body mass is a component of seasonal acclimatization, including increasing thermoregulation abilities. *= Significant difference at the p<.05 level.

**Figure 2: Pectoralis mass is not significantly different during the winter and summer.** In order to determine if changing muscle mass is a means for seasonal acclimatization, we used the pectoralis muscle to identify changes in muscle size. The lack of significant difference in the pectoralis muscle suggests that changing muscle size does not occur to facilitate seasonal acclimatization, and is also not the cause of higher body mass in the winter.

**Figure 3: Fat Score of Northern Cardinals is significantly higher in the winter than summer.** We measured fat score during summer and winter to identify if fat score is causing the higher winter body weight. Fat score was measured on a 0-7 scale. Fat score is significantly higher in the winter than summer. Therefore, it appears that higher adipose tissue during the winter is responsible for the higher body weight described in Figure 1. As adipose tissue is relatively metabolically inert, all physiological data are analyzed only as whole animal measurements. *= Significant difference at the p <.05 level.

**Figure 4: Daily Energy Expenditure is significantly higher in winter acclimatized birds.** We measured daily energy expenditure during winter and summer to find which season is more energetically expensive. DEE in free living birds was measured via heart rate telemetry. We found that DEE is significantly higher in the winter, and therefore winter months may require physiological adaptations to meet energetic demands. *= Significant difference at the p <.05 level.

**Figure 5: Rate of energy use per hour.** An average hourly energy expenditure of each bird within a given season was calculated for a twenty-four hour period to observe patterns in energy use throughout the day. Repeated measures analysis of variance (ANOVA) using mixed procedure was used to analyze hourly patterns in energy expenditure. There was a significant interaction between season and time, demonstrating that the effect of time of day on oxygen consumption depended on the season (F(23, 161)= 3.60, p<0.05). Pairwise comparisons of individual one hour periods, after Bonferroni correction indicated that significant differences of oxygen consumption occurred between winter and summer for each hour starting with the hour beginning at 10:00am to the hour beginning at 6:00pm. This indicates that the higher energy expenditure observed during the winter is due to elevated daytime energy expenditure. However, due to the difference in time of sunrise and sunset between the summer and winter, light difference may contribute to differences for these hours.
**Figure 6: Whole animal summit metabolism is significantly higher in the winter than summer.** Summit metabolism was measured in order to discover if birds are changing their maximum metabolic capacity as a means to meet seasonal challenges, as well as changes in daily energy expenditure. Summits were collected during the summer and winter by indirect calorimetry using a heliox gas mixture. The significantly higher winter summit metabolism suggests that changing summit metabolism is a physiological adaptation for seasonal acclimatization. * = Significant difference at the p<.05 level.

**Figure 7: Citrate synthase activity in pectoralis muscle is not significantly different during the winter and summer.** To demonstrate if changing muscle oxidative capacity is a means of seasonal acclimatization, we measured the relative muscle oxidative capacity of the pectoralis muscle. We found no significant difference in citrate synthase activity, a measure of mitochondrial number, comparing birds sampled in the summer and winter ( p>0.05). This suggests that changing the oxidative capacity of skeletal muscle is not an important component of seasonal acclimatization.

**Supplemental Figure 1: Pectoralis weight per unit mass is not significantly different during the summer and winter.** In order to determine if changing muscle mass is a means for seasonal acclimatization, we measured the pectoralis muscle to identify changes in muscle size. When the pectoralis weight is reported per unit body mass there is no significant difference in the winter or summer, suggesting that changing muscle size does not occur to facilitate seasonal acclimatization, and is also not the cause of higher body mass in the winter.

**Supplemental Figure 2: Mass specific daily energy expenditure is not significantly higher in winter acclimated birds.** As opposed to whole animal daily energy expenditure, mass specific daily energy expenditure was not different between groups. However, accumulation of adipose tissue may be masking whole animal differences in DEE.

**Supplemental Figure 3: Mass specific summit metabolism is not significantly different between the winter and summer.** When summit metabolism is expressed per unit body mass there is no significant difference between the winter and summer. This appears to be the result of higher adipose tissue in the winter that is diluting the mass specific summit metabolism measurements.
Figure 1

Body Mass (g)

Winter

Summer

*
Figure 2

Pectoralis Mass (g)

Winter  | Summer
---      | ---
6.0     | 5.0

Note: The graph shows a comparison of pectoralis mass between winter and summer.
Figure 3

Fat Score (0-7)

Winter  Summer

*
Figure 4

Daily Energy Expenditure (LO₂·day⁻¹)

Winter

Summer

*
Energy Expenditure (LO_2\cdot\text{hour}^{-1})

Time of Day (Hours)

Winter
Summer
Winter Night
Summer Night
Winter Day
Summer day

Figure 5
Figure 6

VO₂ Summit (mL O₂·min⁻¹)

Winter

Summer

*
Figure 7

Relative Citrate Synthase Activity (%)

- Winter
- Summer
Supplemental Figure 1

Pectoralis/Body Weight (%)

Winter
Summer
Mass Specific Daily Energy Expenditure (LO₂·g·day⁻¹)

Supplemental Figure 2

Winter

Summer
Supplemental Figure 3

VO$_2$ Summit (mL O$_2$ kg$^{-1}$ min$^{-1}$)

Winter

Summer
Literature Cited


