RESEARCH ARTICLE

Acclimatization of seasonal energetics in northern cardinals (*Cardinalis cardinalis*) through plasticity of metabolic rates and ceilings

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SUMMARY

Northern cardinals (*Cardinalis cardinalis*) are faced with energetically expensive seasonal challenges that must be met to ensure survival, including thermoregulation in winter and reproductive activities in summer. Contrary to predictions of life history theory that suggest breeding metabolic rate should be the apex of energetic effort, winter metabolism exceeds that during breeding in several temperate resident bird species. By examining whole-animal, tissue and cellular function, we ask whether seasonal acclimatization is accomplished by coordinated phenotypic plasticity of metabolic systems. We measured summit metabolism ($\dot{V}_{O_2,sum}$), daily energy expenditure (DEE) and muscle oxidative capacity under both winter (December to January) and breeding (May to June) conditions. We hypothesize that: (1) rates of energy utilization will be highest in the winter, contrary to predictions based on life history theory, and (2) acclimatization of metabolism will occur at multiple levels of organization such that birds operate with a similar metabolic ceiling during different seasons. We measured field metabolic rates using heart rate telemetry and report the first daily patterns in avian field metabolic rate. Patterns of daily energy use differed seasonally, primarily as birds maintain high metabolic rates throughout the winter daylight hours. We found that DEE and $\dot{V}_{O_2,sum}$ were significantly greater and DEE occurred at a higher fraction of maximum metabolic capacity during winter, indicating an elevation of the metabolic ceiling. Surprisingly, there were no significant differences in mass or oxidative capacity of skeletal muscle. These data, highlighting the importance of examining energetic responses to seasonal challenges at multiple levels, clearly reject life history predictions that breeding is the primary energetic challenge for temperate zone residents. Further, they indicate that metabolic ceilings are seasonally flexible as metabolic effort during winter thermoregulation exceeds that of breeding.

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Key words: seasonal acclimatization, phenotypic plasticity, daily energy expenditure, field metabolic rate, sustained metabolic rate, metabolic ceiling, summit metabolism, muscle oxidative capacity, avian life history.

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INTRODUCTION

Although birds resident in the temperate zone do not undertake migration, seasonal changes place great demands on their metabolic systems (Swanson and Garland, 2009). In the summer, the birds' primary activities are breeding and parental care. During the cold months of winter, birds face tremendous energy costs finding food under frequently difficult foraging conditions, generating sufficient metabolic heat and managing their overall energy reserves (Brodin, 2007). Responses to these seasonal challenges include alteration of numerous structural and functional components of metabolism through phenotypic plasticity – the capacity for change within genetically uniform organisms in response to different environmental conditions (Piersma and Lindström, 1997).

Migration, long-term cold exposure and reproduction are all activities requiring a sustained increase of metabolic rate. The degree to which aerobic metabolism can be increased over basal requirements is inversely related to the time over which activity can be sustained (Peterson et al., 1990; Piersma, 2011). This maximal level of sustainable activity, or metabolic ceiling (Piersma, 2011), is often reported as a multiple of the basal metabolic rate (BMR) and typically ranges from four to five times the BMR in tested avian and mammalian species (Hammond and Diamond, 1997), although

Speakman and Król (Speakman and Król, 2011) convincingly question the general applicability of this approach. The use of BMR in this context can be problematic as BMR is a measure of the energy use of numerous systems, many of which are unchanged or show decreased metabolic rate with activity (Piersma, 2002; Piersma and van Gils, 2010). Further, BMR is poorly correlated with field activity in birds (Ricklefs et al., 1996). Rather than multiples of BMR, Liknes et al. (Liknes et al., 2002) have proposed that the fraction of maximal metabolic rate that can be sustained may predict shivering endurance, as maximal metabolic rate appears closely related to the metabolic capacity of skeletal muscle. Regardless of the challenge, the metabolic ceiling of an organism is set by the availability of sufficient metabolic capacity and the ability to sustain a sufficient fraction of that capacity.

The primary life history and environmental factors considered to be important in setting metabolic ceilings are breeding status and temperature (Piersma, 2011; Speakman and Król, 2011). Based on predictions from life history theory, Masman et al. (Masman et al., 1986) proposed two hypotheses regarding seasonal energy use in birds. The 'increased demand hypothesis' predicts that energy demand should be highest during breeding, exceeding all other seasons. The 'reallocation hypothesis' predicts no difference in

seasonal energy demand as energy expenditure is shifted from activities such as wintertime thermoregulation to breeding. Both hypotheses are based on an interpretation of life history theory proposing that breeding should represent maximal metabolic effort to maximize reproductive fitness (Masman et al., 1986). However, as detailed previously (Swanson, 2010), although data on field metabolic rate (FMR; collected using doubly labeled water or time–energy budgets to calculate daily energy expenditure) appear to be in support of the increased demand hypothesis in larger birds or those that live in moderate climates, data from temperate zone resident species experiencing harsh winters led to the rejection of both hypotheses as the energy costs of winter exceeded those of other seasons (Swanson, 2010).

A proposed mechanism underlying seasonal shifts in metabolic capacity of both migratory and temperate zone resident birds is increased mass or oxidative capacity of flight muscle (Swanson, 2010). During migratory cycles, pronounced alteration of flight muscle has been observed. At 15–20% of total body mass, the pectoralis muscle is the primary tissue of activity metabolism (Lindström et al., 2000), it is responsible for the downstroke of the wing during flight, and it is a major contributor to thermoregulation *via* shivering. Increased mass of the flight muscles is frequently observed in migrating birds (Piersma, 1998; Lindström et al., 2000; Bauchinger et al., 2005), during molt (Lind and Jakobsson, 2001; Fox et al., 2008) and in response to seasonal temperature change (Swanson, 1991; O'Connor, 1995). Further, Zheng et al. (Zheng et al., 2008) found that muscle cytochrome *c* oxidase activity and state 4 respiration (resting oxygen consumption) in mitochondria isolated from skeletal muscle were both higher in Eurasian tree sparrows (*Passer montanus*) during the winter, supporting a role for flight muscle in the phenotypic response to winter acclimatization.

Our focus in this study is an integrative, multi-level approach to investigate the role of phenotypic plasticity of energy metabolic systems in seasonal acclimatization in a non-migratory, temperate zone passerine bird, the northern cardinal [*Cardinalis cardinalis* (Linnaeus 1758); hereafter 'cardinal'], within the context of life history theory. We measured the daily energy expenditure (DEE), metabolic capacity for thermogenesis ($\dot{V}_{O2,sum}$) and skeletal muscle oxidative capacity in free-living birds across the winter and summer breeding seasons. Our approach to measurement of FMR, using heart rate telemetry, permits us to partition energy use over the course of a 24-h period, thus allowing comparison of how energy use may vary within the day in relation to overall seasonal variation. We also compared the DEE of free-living cardinals with results from other species collected using doubly labeled water to assess the life history predictions of Masman et al. (Masman et al., 1986). By determining $\dot{V}_{O2,sum}$ and skeletal muscle oxidative capacity in relation to FMR during winter and summer in these animals, we ask whether these parameters are altered in a coordinated manner in support of metabolic demand with seasonal acclimatization. We hypothesize that winter acclimatization results in elevated FMR and that organismal metabolic capacity and muscle oxidative capacity both increase in response to these higher winter energy demands. Additionally, we hypothesize that metabolic rate is sustained at a similar fraction of metabolic capacity during both winter and summer breeding season, i.e. birds experience a similar metabolic ceiling during both seasons.

MATERIALS AND METHODS
Study species and field sites
We studied the northern cardinal, a non-migratory species that is abundant in southwestern Ohio. Birds were caught with mist nets

between sunrise and late afternoon, and release was always at least 1 h before sunset to ensure a successful transition back to the environment following laboratory procedures. All birds were captured under Ohio Department of Natural Resources permit no. 11-152 and US Fish and Wildlife Service permit no. MB158451-1. All animal experimentation was approved by the Institutional Animal Care and Use Committee of Miami University (protocol no. 736) and complied with the regulations of the National Institutes of Health (National Institutes of Health, 1985) as well as the laws of the United States. In all years, data were collected between 13 May and 18 June (summer) or between 17 December and 28 February (winter). These times were chosen to ensure that birds were experiencing the challenge of winter cold or breeding. Study sites were located near Oxford, Ohio, at the Ecology Research Center (ERC; 39°30′N, 84°45′W) of Miami University and Hueston Woods Biological Station (HWBS) located in Hueston Woods State Park (39°34′N, 84°44′W). Meteorological data were collected by the US EPA through their CASTNET program at the Oxford, Ohio, site from Miami University's ERC.

Animal morphometrics
Data for body mass, fat score, wing chord, tarsus length, tail length and culmen length were collected over four field seasons, from 2004 to 2007 for both breeding and over-wintering birds. All birds were captured *via* mist nets at HWBS, banded with US Fish and Wildlife bands, and then released after data collection. Body mass was measured with a digital scale (Pocket Pro, Acculab, Edgewood, NY, USA) and length was measured with a caliper or ruler. Fat score was measured using a qualitative, zero to eight score, by viewing the fat pouch in relation to the height of the rib cage (DeSante et al., 2009). All assessments of fat score were undertaken by a single individual (D.E.R.). The number of individuals for each variable is listed in Table 1.

Physiology experiments
Birds for the experiments described below were captured by mist nets at the ERC over two field seasons from 2007 to 2009 for both breeding and over-wintering birds. Upon capture, birds were taken to the Miami University laboratory for one of the following procedures: measurement of field metabolic rate, summit metabolism or muscle oxidative capacity. In all cases, measurements were made at room temperature on birds immediately after capture. In all procedures we used indirect calorimetry to determine oxygen consumption rates ($\dot{V}_{O2}$). Positive pressure gas flow (room air or

Table 1. Morphometric measurements of summer- (breeding season) and winter-caught northern cardinals

	Summer	Winter
Body mass (g)	41.5±0.3 (102)	47.2±0.9 (22)*
Wing chord (mm)	90.4±0.3 (104)	90.7±0.7 (22)
Tarsus length (mm)	25.0±0.1 (91)	24.7±0.2 (18)
Tail length (mm)	93.5±0.7 (90)	94.4±1.3 (21)
Culmen length (mm)	12.7±0.1 (91)	12.5±0.2 (18)
Fat score (0–8)	0.21±0.05 (106)	3.00±0.27 (22)*
Pectoralis mass (g)	5.39±0.2 (8)	6.26±0.5 (8)
Pectoralis mass (% body mass)	13.9±0.4 (8)	14.5±0.9 (8)

Body mass is higher in winter-acclimatized northern cardinals, primarily because of increased adiposity. No significant differences were observed in other morphometric variables.

Data are presented as means ± s.e.m. (n). *, significant difference from summer-acclimatized birds (*P*<0.05).

heliox for FMR calibration or $\dot{V}_{O2,sum}$, respectively, as detailed below) was regulated by a mass flow meter (0–5 l min^{-1}; Sierra Instruments, Monterey, CA, USA) controlled by a mass flow controller (model MFC-2; Sable Systems, Las Vegas, NV, USA), at either 1.0 l min^{-1} (for FMR) or 1.4 l min^{-1} (for $\dot{V}_{O2,sum}$). Gas passed through the chamber containing the bird (4.5 l volume for FMR or 1.2 l for $\dot{V}_{O2,sum}$) and then passed through a CO_2 analyzer (model CA-10a; Sable Systems), a Drierite (dessicant) column and an O_2 analyzer (model FC-10a; Sable Systems). Washout times were 9 and 4 min for FMR and $\dot{V}_{O2,sum}$, respectively. To reduce the equilibration time, birds were placed into chambers at room temperature and gases were equilibrated at rest before beginning experiments. Voltage outputs from the flow controller and gas analyzers were collected at a rate of one sample per second using the Expedata program (Sable Systems). $\dot{V}_{O2}$ values at each activity level were calculated from 1 min of steady-state data using the following equation from the Sable Systems user's manual:

$$\dot{V}_{O2} = STP \times f \times [(F_{iO2} - F_{eO2}) - F_{eO2} \\ \times (F_{eCO2} - F_{iCO2})] / (1 - F_{eO2}), \qquad (1)$$

where STP is standard temperature and pressure, f is flow rate, F_{iO2} and F_{iCO2} are the fractional contents of oxygen and carbon dioxide, respectively, in incurrent air, and F_{eO2} and F_{eCO2} are fractional contents of oxygen and carbon dioxide, respectively, in excurrent air.

Field metabolic rate

Once birds were brought into the laboratory, a heart rate radio-transmitter was attached to the mid-scapular region of the back of the bird with fine wire electrodes placed subcutaneously for detection and transmission of the ECG signal as described previously (Butler et al., 2000; Froget et al., 2001). Transmitters (~1 g; JDJC Corp., Fisher, IL, USA) weighed less than 3% of an average 42 g cardinal. Several studies have found little transmitter effect (e.g. Murray and Fuller, 2000); however, a meta-analysis by Barron et al. (Barron et al., 2010) suggested that transmitters of any size may cause an increase in energy expenditure. After transmitter attachment, we simultaneously measured heart rate and oxygen consumption using indirect calorimetry (as described above) to generate a calibration curve. During each measurement, which lasted from 40 to 60 min per animal, birds were held at room temperature. To generate a wide range of both heart rate and $\dot{V}_{O2}$ that reflect physiological limits, measurements were made while the bird was at rest as well as while the bird was agitated, either mildly or severely, in the metabolic chamber. Each phase of calibration extended over at least 10 min, once steady states for oxygen and carbon dioxide readouts were reached and several measurements from each phase were taken.

Individual calibration curves were collected for each bird and used to determine FMR from heart rate data collected in the field. During calibration, the range of heart rates obtained spanned those obtained in the field. For those individuals that we were unable to generate a calibration curve (N=2 for winter, N=1 for summer), a master calibration curve for each season was produced by combining all individuals from that season. This included several birds for which we generated a calibration curve, but were unsuccessful in tracking. Use of the master calibration curves for all animals produced nearly identical results. A typical, representative calibration curve demonstrating the high correlation of heart rate and oxygen consumption can be seen in supplementary material Fig. S1.

Following collection of the calibration data, birds were released at the ERC. Radio-telemetry was used to track each individual for 24 continuous hours, beginning at least 12 h after release, enabling the bird to recover from transmitter attachment and return to a normal physiological state. During the 24 h tracking period, heart rate was recorded every 5 min and was assumed to be representative for that period. We successfully collected telemetric ECG for a complete day from four individuals during the summer and five during the winter.

Heart rate was determined following fast Fourier transformation (FFT) and filtering of raw transmissions using CoolEdit 2000, as described previously (Bisson et al., 2009). The FMR was then calculated using both the mean heart rate from field measurements and the oxygen consumption rate–heart rate calibration equation obtained in the laboratory. Calculated FMR was expressed as DEE. In addition to DEE, rate of energy use was calculated on an hourly basis to observe the pattern of oxygen consumption throughout a given day.

Summit metabolism

A second set of birds was brought into the laboratory to measure metabolic capacity. After weighing, we used indirect calorimetry to determine $\dot{V}_{O2,sum}$ [oxygen consumption during a maximal acute cold stress, following Rosenmann and Morrison (Rosenmann and Morrison, 1974)]. $\dot{V}_{O2,sum}$ measurements were collected while an individual bird was placed in the metabolic chamber at a static 4°C temperature (Swanson et al., 1996) using the peak 1 min value for gas concentrations once the bird had reached a steady-state plateau. Each measurement period extended from 20 to 30 min in length. $\dot{V}_{O2,sum}$ measurements were determined for eight individuals in summer and nine in winter.

Muscle oxidative capacity

Following measurements of $\dot{V}_{O2,sum}$, the same individuals were anesthetized with inhaled isofluorane and decapitated. One pectoralis muscle from each individual was then dissected and weighed. Additionally, a 3–5 mm^3 portion of tissue was removed from the medial region of the pectoralis muscle, frozen in liquid nitrogen, and stored at –80°C for later analysis of muscle oxidative capacity using a citrate synthase assay modified from Chi et al. (Chi et al., 1983). Samples were prepared by hand homogenization with 19 volumes of homogenization medium (0.05 mol l^{-1} Tris, 0.15 mol l^{-1} KCL, pH 7.4) followed by centrifugation at 1000g. Supernatant was removed, frozen in liquid nitrogen and stored at –80°C. To assay maximal citrate synthase activity, pectoralis homogenates were added to assay reagent (0.1 mol l^{-1} Tris, pH 8.1) with an excess of acetyl CoA (0.2 mmol l^{-1}) and oxaloacetate (0.17 mmol l^{-1}). The rate of disappearance of acetyl-CoA (extinction coefficient=5.4 cm^2 μmol^{-1}) was monitored at 232 nm in a temperature-controlled spectrophotometer (DTX880 Multimode Detector, Beckman Coulter, Fullerton, CA, USA) at 25°C for 8 min to determine the rate of enzyme activity. Pectoralis samples were analyzed for seven individuals from each season.

Statistical analysis

Pairwise comparisons of winter- and summer-acclimatized groups used a non-parametric t-test for fat score and Student's t-test for DEE, $\dot{V}_{O2,sum}$, muscle oxidative capacity and all other morphometric measurements. Hour-by-hour energy utilization rates were analyzed using repeated-measures ANOVA in SAS (version 9.1 for Windows, SAS Institute, Cary, NC, USA). The level of significance was set at $P<0.05$ in all cases, except for pairwise comparisons of individual

Table 2. Temperature recordings at the field site

	Winter		Summer	
	Field seasons	Telemetry	Field seasons	Telemetry
Mean daily temperature (°C)	−1.3±0.5	−1.3±0.5	19.0±0.8	19.6±2.5
Mean minimum temperature (°C)	−5.5±0.6	−6.5±2.5	13.5±0.8	13.7±2.5
Mean maximum temperature (°C)	3.1±0.6	−1.6±1.5	24.1±0.9	24.9±2.6

Daily mean as well as mean minimum and maximum temperatures were recorded at the ERC during field seasons. Data for the complete field season did not differ from temperatures recorded during actual dates in which birds were tracked and heart rate was monitored (noted as 'telemetry').

Data are presented as means ± s.e.m.

1-h periods of rate of energy use, which used a Bonferroni correction. All data are presented as means ± s.e.m. As data for DEE and $\dot{V}_{O_2,sum}$ were not collected on the same individuals, ratios of DEE/$\dot{V}_{O_2,sum}$ were analyzed using bootstrapping (10,000 iterations) to generate a non-parametric 95% confidence interval for the true mean ratio. This analysis was performed using R (version 2.9.0, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS
Morphology
We initially measured morphological structure to determine whether body size and/or composition changed across seasons. We found that mean body mass of cardinals in the winter was 47.2±0.9 g, significantly higher than their summer body mass of 41.5±0.3 g (Table 1). There was no significant difference in wing chord, tarsus length, tail length or culmen length (*P*>0.05 in all cases), indicating that the birds did not differ structurally between seasons (Table 1). Similarly, neither pectoralis muscle mass (*P*=0.12) nor pectoralis mass per unit body mass (*P*=0.53) were significantly different between summer and winter (Table 1). Thus pectoralis muscle hypertrophy was not observed and is not the cause of a greater body mass during the winter. However, fat score was significantly higher in the winter-acclimatized birds than in those measured in summer (Table 1), thus we conclude that adiposity is primarily responsible for the greater body mass in the winter.

Field and maximal metabolic rate
During both winter and summer we determined DEE (liters O_2 consumed per day) using heart rate telemetry. We compared ambient temperature data for dates of field measurements with seasonal means and found no difference from the mean values for those seasons (Table 2). Whole-organism DEE was significantly higher (*P*<0.05) in winter- compared with summer-acclimatized cardinals (Table 3). When expressed per unit body mass, DEE was not significantly different between groups, although it was nearly so (*P*=0.067; Table 3). Low statistical power and higher amounts of adipose tissue (which is relatively metabolically inert) in winter birds likely obscured a difference in this measure. Using equations that were derived for several bird species (Bergstrom and Sherry, 2008) or for Swainson's thrushes [*Catharus ustulatus* (Seewagen, 2008)], we estimated the lean body mass of our cardinals based on fat score and other morphological measurements. Both equations generated similar outcomes and in both cases, DEE expressed per unit lean body mass was significantly higher in winter-acclimatized birds (supplementary material Table S1). These equations were derived for species that are smaller than cardinals and therefore should underestimate fat mass, giving a conservative estimate. However, as the equations may not be equally accurate when applied to cardinals, we re-calculated the lean body mass, halving the contribution of the fat score to the calculated adipose mass and still

found a significant different in DEE per unit lean body mass (data not shown). Thus the energy demands of cardinals in winter are higher than those of cardinals measured during the summer breeding season.

To assess the response of metabolic capacity to this seasonal acclimatization, we measured the $\dot{V}_{O_2,sum}$ of winter- and summer-acclimatized cardinals. Whole-animal $\dot{V}_{O_2,sum}$ was significantly higher in winter-acclimatized birds (*P*<0.05; Table 3). As with DEE, when expressed per unit body mass (Table 3), the $\dot{V}_{O_2,sum}$ of winter-acclimatized cardinals was not significantly different from that of summer-acclimatized cardinals (*P*=0.10), as greater amounts of metabolically inert adipose tissue in winter birds likely obscured a difference in this measure. As with DEE, we found that $\dot{V}_{O_2,sum}$ per unit lean body mass was significantly higher in the winter-acclimatized cardinals when we estimated lean body mass using the equations of Bergstrom and Sherry (Bergstrom and Sherry, 2008) or Seewagen (Seewagen, 2008), or our more conservative correction to those equations (supplementary material Table S1). Thus higher energy demands during winter are associated with an increase in metabolic capacity.

When DEE is expressed as a percentage of metabolic capacity (DEE/$\dot{V}_{O_2,sum}$), the relative energy expenditure of winter-acclimatized birds was observed to be 11.3% higher than that of summer birds (s.e.m.=5.5%), with a 95% bootstrap confidence interval that was 1.1–22.9% higher, indicating that the greater observed mean ratio reflects the actual distribution of mean ratios (Table 3). Higher metabolic capacity in winter directly supports, and may arise as a response to, higher daily activity and consequent

Table 3. Metabolic measurements of summer- (breeding season) and winter-acclimatized northern cardinals

	Summer	Winter
DEE ($l\,O_2\,day^{-1}$)	3.76±0.44 (4)	5.06±0.22 (5)*
Mass-specific DEE ($l\,O_2\,g^{-1}\,day^{-1}$)	0.095±0.009 (4)	0.117±0.005 (5)
$\dot{V}_{O_2,sum}$ ($ml\,O_2\,min^{-1}$)	6.05±0.26 (8)	7.33±0.34 (9)*
Mass-specific $\dot{V}_{O_2,sum}$ ($ml\,O_2\,kg^{-1}\,min^{-1}$)	156.41±6.64 (8)	172.08±6.00 (9)
DEE/$\dot{V}_{O_2,sum}$ (%)	43.84, 1.11	48.81, 1.18*
Citrate synthase activity ($\mu mol\,g^{-1}\,min^{-1}$)	123.2±5.1 (7)	122.5±5.4 (7)

Both measures of organismal metabolic rate, daily energy expenditure (DEE) and metabolic capacity ($\dot{V}_{O_2,sum}$), are higher in winter- than summer-acclimatized birds. Winter birds are operating at a higher percentage of their capacity (DEE/$\dot{V}_{O_2,sum}$, calculated using whole-animal values). Although metabolic activity is higher in winter, muscle oxidative capacity remained unchanged. DEE was determined in the field *via* heart rate telemetry. $\dot{V}_{O_2,sum}$ was determined *via* indirect calorimetry in a helium-oxygen atmosphere. Citrate synthase activity was measured from pectoralis muscle homogenates at 25°C.

Data are presented as means ± s.e.m. (n), except for DEE/$\dot{V}_{O_2,sum}$, for which data are presented as means, bootstrap standard error. *, significant difference from summer-acclimatized birds (*P*<0.05).

metabolic demand. However, the magnitude by which DEE is greater during winter is greater than the magnitude by which $\dot{V}_{O2,sum}$ is higher. Thus cardinals during winter sustain DEE at a level that is both absolutely and relatively (per fraction of $\dot{V}_{O2,sum}$) higher than that measured in summer.

Patterns in DEE

We further examined DEE by calculating hourly rates of oxygen consumption for each cardinal within a given season for a 24h period (Fig. 1). ANOVA revealed a significant interaction between season and time, demonstrating that 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, a total of 24 comparisons. It is worth noting that as sunrise and sunset occur at different times, difference in daylight may contribute to differences in pattern of metabolism for hours around sunrise and sunset. Significant differences between winter- and summer-acclimatized birds in hourly oxygen consumption were seen in daylight hours from 10:00 to 18:00h inclusive. When expressed per unit body mass, we obtained similar overall effects, except that hourly pairwise differences were seen from 11:00 to 19:00h (data not shown). Thus differences in DEE can be largely attributed to daytime energy use. In the summer there were two peaks of energy use: the first following dawn and the second before dusk. During midday, energy expenditure was similar to overnight values. In the winter, energy expenditure rose at dawn and was maintained at an even plateau throughout the day. There was no difference in night-time energy expenditures for each individual hour when comparing across seasons. When we examined the mean metabolic rate including all hours of the night, we found no significant difference ($P=0.10$) between birds in summer ($0.154\pm0.024\,\mathrm{l\,O_2\,h^{-1}}$) and winter ($0.194\pm0.006\,\mathrm{l\,O_2\,h^{-1}}$); this was due to higher variability in the summer birds (as well as low sample size). However, in addition to the trend for higher night-time metabolic rate, the winter night is also longer. Thus when we calculate the energy expenditure of the entire night, we found that during the winter night (~13h), at $2.527\pm0.075\,\mathrm{l\,O_2\,night^{-1}}$, cardinals use nearly twice the energy as required during summer nights (~9h, $1.386\pm0.213\,\mathrm{l\,O_2\,night^{-1}}$; $P<0.05$).

Muscle oxidative capacity

We measured oxidative capacity of the pectoralis muscle *via* the citrate synthase assay to examine whether this may function as a mechanism supporting whole-animal metabolic capacity. We found no significant difference in the activity of this key mitochondrial oxidative enzyme between the summer- and winter-acclimatized cardinals ($P=0.92$; Table 3), suggesting that changing oxidative capacity of this tissue does not contribute to seasonal acclimatization.

DISCUSSION

In birds that over-winter in north temperate zones, the stress of surviving extended periods of cold temperatures appears to potently drive phenotypic plasticity of metabolic capacity. In comparisons of winter- and summer-acclimatized birds, several studies have now demonstrated increased skeletal muscle mass (Swanson, 1991; O'Connor, 1995; Cooper, 2002) as well as higher basal (Piersma et al., 1995) and maximal metabolic rates ($\dot{V}_{O2,sum}$) in winter-acclimatized birds (Cooper and Swanson, 1994; Liknes and Swanson, 1996; Liknes et al., 2002; Cooper, 2002; Swanson and Garland, 2009). Both the structural and functional outcomes have been proposed as adaptive responses to the metabolic demands of winter survival (Swanson, 1991).

In accordance with these data, our findings of an approximately 20% increase in higher winter metabolic capacity in cardinals appears to be typical. Sufficient metabolic capacity to permit survival in the cold has been proposed as a constraint on avian distribution (Root, 1988; Canterbury, 2002; Forsman and Mönkkönen, 2003), although the importance of temperature as a determining factor remains equivocal (Repasky, 1991; Canterbury 2002). However, in contrast to previous reports on other species, we found no increase in either pectoralis muscle mass or muscle tissue metabolic capacity in cardinals. Flight muscle hypertrophy is a common observation in migrating birds (Piersma, 1998; Lindström et al., 2000; Bauchinger et al., 2005) and the absence of plasticity of skeletal muscle structure or function in this study contrasts with several studies examining cold acclimation (Swanson, 1991; O'Connor, 1995). However, not all birds respond to thermal challenges with muscle tissue hypertrophy (Vézina et al., 2006) suggesting that, unlike all migrants that we are aware of, flight muscle hypertrophy may not be a universal response to cold.

An additional aspect of winter survival at high latitudes is the need to provision fuel. Winter nights are long and thus energetically demanding and food may be scarce or foraging difficult because of poor weather. Thus although measures of cardinal size and shape did not differ seasonally, fat stores were found to be considerably higher during winter, similar to that seen in many other wintering species (Dawson et al., 1983). During winter, increased fat stores provide sufficient fuel to meet night-time energy demands, but not
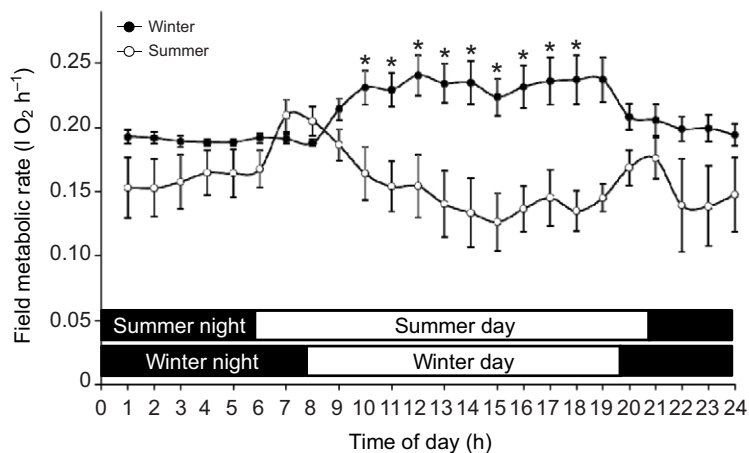

Fig. 1. An average hourly energy expenditure of birds within a given season was calculated for a 24-h period to observe patterns in energy use throughout the day. In addition to seasonal differences, there was a significant interaction between season and time of day, such that significant differences in metabolic rate occurred for each hour between 10:00 and 18:00h. This indicates that the higher field metabolic rate observed during the winter is due to elevated daytime energy expenditure. Data are presented as means ± s.e.m. *, significant difference from summer-acclimatized birds at the corresponding time of day ($P<0.05$).

more than enough to meet the demand of approximately 1 d (Dawson et al., 1983). It seems reasonable to infer that these fat stores carry some cost, as cardinals typically do not have comparable adiposity in the summer.

Earlier studies of avian reproductive life history proposed that natural selection has favored maximal reproductive output and therefore breeding should be the most energetically demanding activity in the animals' annual cycle (Lack, 1968; Drent and Daan, 1980). Supporting this view, reproduction or feeding of young rank among the activities with the highest sustainable metabolic rates measured in both avian and mammalian species (Hammond and Diamond, 1997). This observation suggests two alternative hypotheses regarding daily energy use. The first, the increased demand hypothesis, proposed that breeding should be the most metabolically demanding season, during which daily energy use exceeds that of other periods. The second, the reallocation hypothesis, proposed that the breeding season is metabolically equivalent to other seasons, such that although energy demands of specific challenges may vary from season to season, overall demand is constant (Masman et al., 1986). The hypothesis that breeding must be accompanied by maximal metabolic effort has been rejected for several species of small temperate zone residents [summarized in Swanson (Swanson, 2010)]. Our results for cardinals also led us to reject both the increased demand and the reallocation hypotheses. Instead, winter was the time of highest DEE, likely because of a combination of increased thermoregulatory costs due to nearly constant low temperatures, and increased foraging costs. A similar scenario was uncovered by Weathers and Sullivan for two species of juncos (Weathers and Sullivan, 1993).

By measuring both FMR and $\dot{V}_{O2,sum}$, we were able to further assess the relative effort of birds in each season. Birds may increase FMR by both increasing capacity and maintaining constant percent effort, or by increasing the fraction of capacity that is sustained without increasing capacity (Liknes et al., 2002). We determined that cardinals have a higher metabolic capacity during the winter and, as their metabolic demand (DEE) increases more than their metabolic capacity ($\dot{V}_{O2,sum}$), they must operate at a higher percentage of that capacity, sustaining higher relative effort than summer birds. So why did breeding cardinals not work harder? Weathers et al. (Weathers et al., 1999) asked the same question of breeding white-crowned sparrows (*Zonotrichia leucophrys*), whose DEE was higher in winter than during breeding. One explanation may be that the higher intensity of metabolic effort during winter incurs costs that impact adult survival, as has been demonstrated for European kestrels (*Falco tinnunculus*) following brood manipulation (Daan et al., 1996). These data suggest a novel perspective for the consideration of sustained metabolic rate. Rates of sustainable energy use during any challenge may be better understood as a fraction of capacity (Marsh and Dawson, 1989) than as a multiple of basal metabolic rate, as is commonly reported (Drent and Daan, 1980; Hammond and Diamond, 1997). This perspective suggests that as increasing metabolic capacity becomes limiting, an organism is required to extend its sustainable metabolic ceiling (Piersma, 2011), with potentially significant effects on survival.

Previous studies of seasonal differences in DEE measured metabolic rates using either time–activity budgets or doubly labeled water. In contrast, we determined metabolic rates *via* heart rate telemetry. Heart rate telemetry has been used to accurately determine FMR in a number of bird species with very low error (Froget et al., 2001; Bisson et al., 2009) and compares well with the doubly labeled water method (Nolet et al., 1992; Bevan et al., 1995). Using heart rate to estimate DEE yielded conclusions in cardinals similar to those found in several other bird species using other approaches (Swanson, 2010). An advantage of this technique is that we were able to dissect DEE into smaller time increments and examine a compartmental breakdown of energy use within a given day (reviewed in Green, 2011), similar to that done with wandering albatrosses (Weimerskirch et al., 2002). These data demonstrated the strength of the heart rate technique for estimating FMR and permitted identification of the periods in which FMR was at its daily peak. The ability to parse out energy use rates during specific periods was used by Bisson et al. (Bisson et al., 2009), who examined acute responses to anthropogenic disturbances in the white-eyed vireo (*Vireo griseus*) during the breeding season. By examining metabolic rates in the period subsequent to experimental disturbance, they concluded that those birds quickly determined the lack of significant threat and were thus able to continue to allocate energy to reproduction. In the present study, the primary difference in patterns of DEE was the elevation in metabolic rate throughout the winter day. In both summer and winter, metabolic rate increased at dawn and decreased at night. However, during the summer, FMR peaked at dawn and dusk whereas mid-day metabolic rates were lower, similar to those observed at night. This coincides with field observations that individuals stayed in the shaded trees in relatively small areas during the height of the summer heat. In the winter, metabolic rate remained high throughout the entire day, possibly because of the constant environmental cold challenge the winter season presents as well as the need for more frequent foraging because of both the shorter day length and increased difficulty of finding food. The overnight metabolic rate did not differ between seasons (although the winter birds experienced the coldest temperatures of the day), suggesting that cardinals may use night-time hypothermia as an energy-saving mechanism. Previously, Mayer et al. (Mayer et al., 1982) found that a combination of cavity roosting and night-time hypothermia in Carolina chickadees (*Poecile carolinensis*) accounted for 50% savings in energy expenditure. Cardinals are not cavity roosters; however, the actual role of hypothermia as an overnight energy-saving mechanism remains unexplored.

We began this study with an interest in understanding coordinate physiological changes that occur as a means of seasonal acclimatization for cardinals. If a temperate zone resident bird has to endure different seasonal conditions and challenges, how does the individual meet these demands? We found that winter is energetically more expensive than the summer, meaning that thermoregulation is more energetically costly than breeding for these animals. Energy expenditure during the winter mid-day is higher than that in summer, and is also higher than energy expenditure at night. In support of increased DEE, cardinals have a higher $\dot{V}_{O2,sum}$ in the winter than summer. The fraction of metabolic capacity used was also greater in winter birds, indicating that the metabolic ceiling is extended, clearly arguing against a primary role of reproduction in setting ceilings for sustained metabolic performance. Unlike the response of migrants and some temperate residents, seasonal change of muscle oxidative capacity was not a means of seasonal acclimatization for these cardinals. Thus tissue and cellular mechanisms of physiological change remain unclear.

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