# ABSTRACT

## SEASONAL AND GEOPGRAPHICAL VARIATION IN DOWNY WOODPECKER (*PICOIDES PUBESCENS*) METABOLISM AND THERMOREGULATION

by

Christopher J. Cousineau

Downy woodpeckers (*Picoides pubescens*) are small, non-migratory, non-passerine birds. Non-migratory birds that overwinter in cold temperate zones are exposed to energetically costly conditions during winter. These conditions include decreased daytime for foraging, long nights requiring increased periods of fasting, and decreased food availability. These birds will also be exposed to extremely cold temperatures in winter months. To survive these conditions birds must go through a process of season acclimatization. In birds, seasonal acclimatization has been found to mainly focus on adjustments in metabolic rates such as basal metabolic rates (BMR) and summit metabolic rates ($M_{sum}$) when under cold stress. Another possible factor in seasonal acclimatization is the substitution of heat produced from locomotive muscle activity for thermoregulatory heat. Despite the impact that metabolic rates have been shown to have on seasonal acclimatization, there has been little research into geographic variation in metabolic rates and ventilation of birds. The purpose of this study was to observe seasonal variation in metabolic rates and ventilation variables between Wisconsin Downy woodpeckers and South Dakota Downy woodpeckers. A secondary goal of this study was to observe seasonal variation in thermoregulatory substitution in Wisconsin Downy woodpeckers.

Open-circuit respirometry was used to measure oxygen consumption (VO2) and ventilation in seasonally acclimatized Downy woodpeckers. Metabolic rates and ventilation were measured under cold stress using a helox (~79% helium and ~21% oxygen) gas mixture and at thermoneutral zone (TNZ) conditions. Foraging activity was also measured using an infrared camera and a closed-circuit television monitor. During foraging trials activity was measured by time spent foraging on a peanut feeder placed into the metabolic chamber. Heat of activity was measured using a thermocoupler when birds were foraging and when feeders had been removed and birds were at rest.

Wisconsin Downy woodpeckers showed a significantly higher BMR and $M_{sum}$ in winter compared to summer. South Dakota Downy woodpeckers had a significantly higher $M_{sum}$ in both summer and winter compared to Wisconsin Downy woodpeckers. Wisconsin Downy woodpeckers had significantly higher $EO_2$ in the winter compared to summer during both BMR and $M_{sum}$ testing. Thermoregulatory substitution was observed in Wisconsin Downy woodpeckers during summer.

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by

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A Thesis Submitted
In Partial Fulfillment of the Requirements
For the Degree of

Master of Science – Biology

Biology

at

The University of Wisconsin Oshkosh
Oshkosh WI 54901-8621

October 2012

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ACKNOWLEDGMENTS

I would like to thank my parents, Tony and Patti Cousineau for their continuous and unwavering support. I would also like to thank my fiance, Liz Kiracofe for her constant support and encouragement. I would like to thank Dr. Sheldon Cooper for introducing me to the fields of avian energetics and ecological physiology. Without Dr. Cooper's constant support, patience, and understanding this project could not have been completed. I would finally like to thank Dr. Dana K. Merriman and Dr. David Dilkes for their time and advice in reviewing my thesis.

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# Chapter I - Introduction

## Energy Metabolism and Thermoregulation

There are four major components to energy metabolism: (1) basal metabolism (BMR), (2) thermoregulatory metabolism, (3) activity metabolism, and (4) heat increment of feeding or specific dynamic action (Gessman, 1987). Basal metabolism is minimum metabolism required by an organism for maintenance. Thermoregulatory metabolism is metabolism used to compensate for heat gain or loss to the environment. Activity metabolism is metabolism related to energy expenditures from locomotor activities, such as foraging and flying. Heat increment of feeding or specific dynamic action is the energy cost related to digestion and absorption of a meal (Gessman, 1987).

Birds are commonly considered to be homeothermic endotherms. Homeothermic means that the animal is able to regulate its internal body temperature, regardless of ambient temperature. An endotherm is an animal that primarily uses the heat generated from metabolic activities to regulate its body temperature. Birds maintain their body temperatures within a narrow range from $38.59 \pm 0.96^{o}C$ when resting to $43.85 \pm 0.94^{o}C$ when active (Prizinger et. al., 1991). The hypothalamus, spinal cord, and deep-body thermosensors outside of the central nervous system are the primary sites responsible for temperature control in birds (Marsh and Dawson, 1989). Resting birds primarily rely on shivering thermogenesis as a source of heat production (Marsh and Dawson, 1989). Shivering thermogenesis is a process in which heat is produced through isometric,

antagonistic muscle contractions. The principle sites of shivering thermogenesis in birds are their flight muscles, such as the M. pectoralis and M. supracoracoideus (Marsh and Dawson, 1989).

An understanding of the Scholander-Irving model of thermoregulation in endotherms is essential to understand avian thermoregulation and homeothermic maintenance (Scholander et. al., 1950) (Figure 1). The thermoneutral zone (TNZ) is the range of ambient temperatures where the rate of heat production equals the rate of heat gain or loss to the environment. Within the TNZ, metabolically inexpensive adjustments such as postural and insulatory adjustments allow birds to thermoregulate. These conditions allow for the measurement of the basal metabolic rate (BMR). At the lower end of the TNZ is the lower critical temperature ($T_{lc}$) and at the upper end is the upper critical temperature ($T_{uc}$). Metabolism must increase below the $T_{lc}$ to account for increased heat loss to the environment. Above the $T_{uc}$, metabolism must increase to counter increased heat gain from the environment. Metabolism increases above the $T_{uc}$ due to activities that increase active dissipation of heat through evaporative cooling, such as panting and gular flutter.

**Seasonal Acclimatization**

Throughout the year, birds undergo several changes in energy metabolism and thermoregulation. These changes are cumulatively known as seasonal acclimatization and occur via various mechanisms such as use of torpor. Torpor is a controlled decreased

in metabolism and body temperature in response to decreased ambient temperature (review: McKechnie and Lovegrove, 2002). Using torpor at reduced ambient temperature reduces the energy costs of thermoregulation. Birds that use nighttime torpor do not follow the Scholander-Irving model as tightly as birds that do not use torpor. While most birds conform to the Scholander-Irving model of thermoregulation, there is variation in the $T_{lc}$, $T_{uc}$, and TNZ for each species. Metabolic rates increase linearly with decreasing temperatures below the $T_{lc}$ until a maximum metabolic rate is reached, this is summit metabolism ($M_{sum}$). In the past, measurement of $M_{sum}$ has been difficult due to problems with generating ambient air temperatures that were sufficient to elicit maximum thermogenesis. At temperatures that were able to produce maximum thermogenesis, birds were likely to receive freezing injuries, such as frostbite. Furthermore, maximum thermogenesis at low temperatures was traditionally indicated at the point of the bird's death (Hart, 1962). Recently, a helium-oxygen gas mixture (helox) consisting of ~79% helium and ~21% oxygen has been used for cold stress studies (Marsh and Dawson, 1989; Swanson, 1990). The higher thermal conductivity of helox compared to nitrogen in air facilitates heat loss and allows ($M_{sum}$) to be recorded at relatively moderate air temperatures and with a reduced risk of injury (Rosenmann and Morrison, 1974).

**Thermoregulatory Substitution**

Another possible cause for seasonal variation in thermoregulation and birds deviating from the Scholander-Irving thermoregulation model is from the use of thermoregulatory substitution. Some birds are able to substitute the heat produced from locomotor muscles during foraging activity for heat required for thermoregulation. Replacing activity thermogenesis for thermoregulatory heat production from shivering is known as substitution. Substitution allows for the reduction of metabolic rates at temperature below $T_{lc}$ by reducing the amount of heat production required for thermoregulation (Bruinzeel and Piersma, 1998; Webster and Weathers, 1990).

**Metabolic Rate Measurement**

Either direct or indirect calorimetry can be used to measure metabolic rates. Direct calorimetry uses measurement of energy released as heat over a given time period to determine metabolic rates. Commonly, a pre-determined mass of water is used to trap the heat released from an animal, and the temperature increase in the water reflects the heat loss from the animal. It can be difficult to measure low metabolic rates using direct calorimetry (Randal et. al., 2002). For these reasons, indirect calorimetry is generally used to measure metabolism. The oxidation of food molecules and their products releases energy. The amount of oxygen consumed by this oxidation directly relates to the amount of heat produced in aerobic oxidation. This relationship is very useful due to the fact that aerobic oxidation accounts for 95% of metabolism in vertebrates (Schmidt-

Neilson, 1997). This in combination with the fact that ratio of heat produced to oxygen consumed is fairly constant regardless of the substrate being catabolized, means metabolic rates can be calculated by measuring oxygen consumption.

One of the most common used indirect techniques for measuring metabolic rates is open-circuit respirometry (Gessman, 1987). Open-circuit respirometry is a technique where air is passed through a metabolic chamber containing a bird (Figure 2). Traditionally, a pump is placed upstream of the metabolic chamber and pushes dry, $CO_2$-free air from a gas scrubber column, through the open-circuit system. The metabolic chamber must be air-tight prior to taking measurements to prevent respired gases from leaking out of the system. Therefore, before taking measurements, efflux gas flow rates are monitored to check for leaks in the system. Flow rates of air in the system are monitored and controlled by a flowmeter. A differential pressure transducer is placed in the metabolic chamber to measure pressure differences from warming and wetting of air by respiration and to determine ventilation. Efflux gases from the metabolic chamber then pass through a second gas scrubber column to remove water and $CO_2$ before the gases are pumped through an $O_2$ analyzer. Standard equations for determining O2 consumption rates require dry, CO2-free air. $O_2$ consumption rates are then recorded by the $O_2$ analyzer and send to the data collection system.

**Primary Goals of This Project**

There are two behavioral responses for small birds that live in temperate-zone climates, like Wisconsin. These birds can either migrate to more tolerable conditions or remain as permanent residents. Remaining year-round in temperate-zones means birds will be exposed to the energetically expensive conditions of winter. These conditions include longer nights requiring increased fasting, shorter days for foraging, and decreased food availability compared to summer conditions. Additionally, birds are also exposed to extremely cold temperatures in winter as compared to summer. The large surface-to-volume ratio of small birds allows for rapid heat loss to the environment and requires strict management of energy reserves (Kleiber, 1961).

Seasonal acclimatization in birds appears to be primarily driven by metabolic adjustments (Dawson and Marsh, 1989; Dawson and O'Connor, 1996). Winter birds may have significant increases in summit metabolism ($M_{sum}$) and basal metabolic rates (BMR) relative to their summer counterparts (Arens and Cooper, 2005a; Cooper and Swanson, 1994; Liknes and Swanson, 1996; Swanson, 1990). These adjustments in metabolic rates facilitate increased cold tolerance (Barnett, 1970; Pohl and West, 1973) and elevated thermogenic endurance (Dawson et al., 1983; O'Connor, 1995; Swanson, 1990) in winter birds compared to summer birds. Elevated $M_{sum}$ is correlated with elevated cold tolerance in many avian species (Swanson, 2001). Adjustments in $O_2$ transport are also required in correspondence to changes in $O_2$ consumption rates. Variation in breathing frequency (*f*), tidal volume or breath volume ($V_T$), and percent oxygen absorbed or oxygen extraction efficiency ($EO_2$) can vary to accommodate

changes in $O_2$ consumption rates ($VO_2$) and metabolic adjustments. Changes to any one, all, or any combination of these ventilation variables may affect $VO_2$ (Chappell and Dawson, 1994).

Downy woodpeckers are small, non-migratory non-passerine birds. The majority of avian metabolic studies have been performed on passerine species and little is known of the differences between passerine and non-passerine species. Downy woodpeckers have many characteristics that make them ecologically comparable to previously studied passerine bird species. Previous seasonal acclimatization studies have focused on changes in metabolic rates such as $M_{sum}$ and BMR. There is lack of studies looking into the seasonal changes in ventilation that correspond to metabolic adjustments. In addition to the lack of information on seasonal changes in Downy woodpeckers, there is a lack in studies looking at geographical variation in Downy woodpeckers. Another area of seasonal acclimatization that has been over looked is the process of thermoregulatory substitution during foraging. My first goal of this study was to observe the seasonal variation in summit and basal metabolic rates as well as ventilation variables in seasonally acclimatized Wisconsin Downy woodpeckers. My second goal of this study was to examine differences in metabolic rates between seasonally acclimatized Wisconsin Downy woodpeckers and South Dakota Downy woodpeckers. My final goal was to observe differences in use of thermoregulatory substitution in seasonally acclimatized Wisconsin Downy woodpeckers.

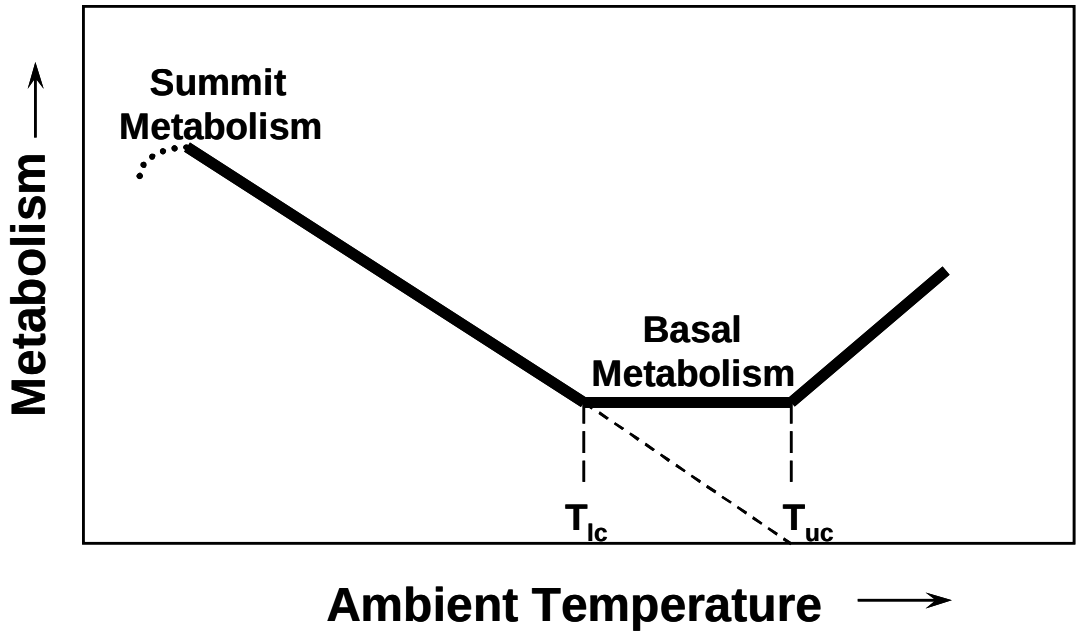


*Figure 1.* The Scholander-Irving model of thermoregulation.

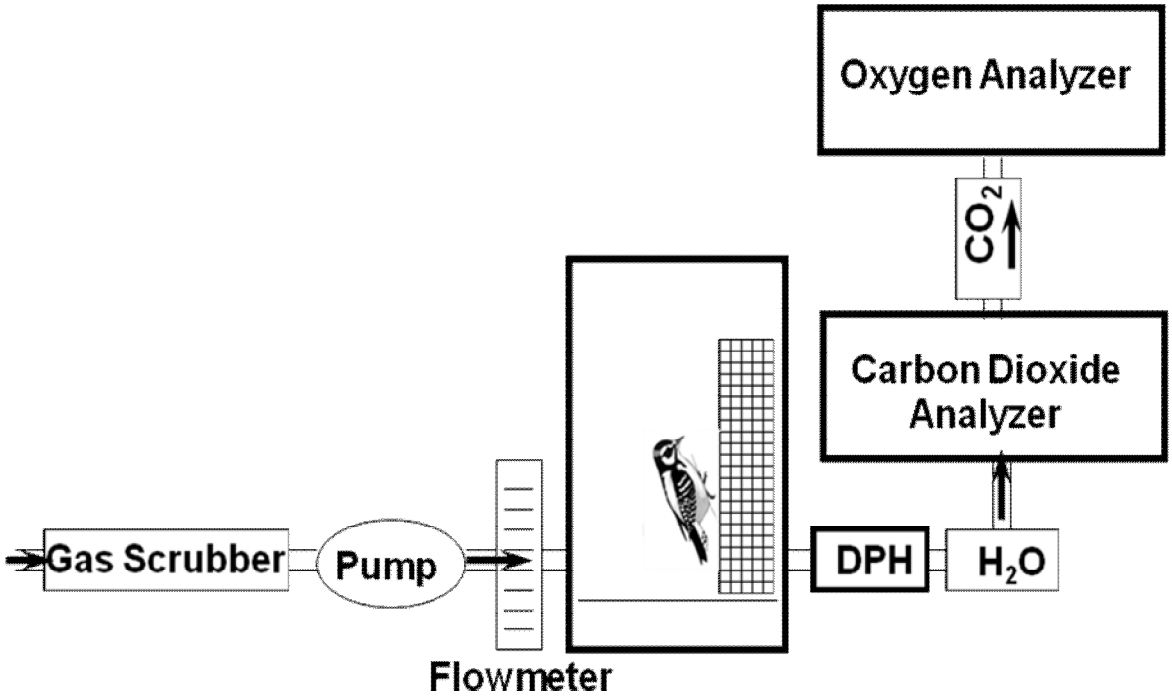


*Figure 2.* A schematic of an open-circuit respirometry system

## Chapter II - Seasonal And Geographic Variation In Metabolism And Ventilation In Downy Woodpeckers

### Introduction

Small birds that remain in cold temperate regions year-round require increased rates of energy expenditure for thermoregulation in winter. In order for birds to survive the cold air temperatures and short days of winter, they must undergo a process of seasonal acclimatization. Seasonal acclimatization in birds appears to be primarily a process of metabolic adjustments (Dawson and Marsh, 1989; Dawson and O'Connor, 1996; Swanson, 2010). These adjustments allow for increased cold tolerance (Barnett, 1970; Pohl and West, 1973) and elevated thermogenic endurance (Dawson et al., 1983; Swanson, 1990; O'Connor 1995) in winter birds compared to summer birds. In addition, winter birds have significant increases in summit metabolism ($M_{sum}$) and basal metabolic rates (BMR) relative to their summer counterparts (Arens and Cooper, 2005b; Swanson, 1990; Cooper and Swanson, 1994; Liknes and Swanson, 1996; Swanson, 1990). Elevated $M_{sum}$ is correlated with elevated cold tolerance in many avian species (Swanson, 2001). Geographic variation in BMR, $M_{sum}$ and cold tolerance has been documented primarily in passerine species (Liknes, Scott, and Swanson, 2002; Olson et al., 2010; Swanson, 1993). Few studies have looked at the variation in metabolic rates across a species throughout large geographical ranges (Olson et al., 2010; Swanson, 1993).

Increased understanding of geographical differences in metabolic adjustments may lead to increased understanding of species distribution or habitat preference.

To date, the effect of metabolic rates on the geographic distribution of an avian species is not clear. Root (1988) suggested that the distribution of wintering birds in North America is limited to regions where metabolic rate does not exceed 2.5 times that of their basal metabolic rate. However, the extent of the impact of metabolic rates on distribution of birds is still controversial (Castro, 1989; Repasky, 1991). Black-capped and Carolina chickadees have been found to have a relatively constant basal metabolic rate over a large geographical distribution (Olson et al., 2010). Swanson found no significant geographical variation in Msum between South Dakota and Oregon wintering juncos (Swanson, 1993). Further comparisons of basal and summit metabolic rates across populations of a species from differing geographical locations may provide further insight into the impact of metabolic rate on the distribution of a species.

The relative lack of information on geographic variation in metabolic rates in seasonally acclimatized birds is accompanied by a lack of data on possible seasonal ventilatory adjustments to cold stress. Adjustments in metabolism during seasonal acclimatization should require changes in oxygen ($O_2$) transport. Variation in ventilation variables such as breathing frequency ($f$), tidal volume or breath volume ($V_T$), and percent oxygen absorbed or oxygen extraction efficiency ($EO_2$) can vary to accommodate changes in metabolism. Adjustments in ventilation to accommodate changes in oxygen requirements may occur in one, all, or any combination of these components (Chappell and Dawson, 1994).

The majority of birds studied thus far have shown significant changes in minute volume ($V_I$ =[$V_T$ x *f*]) and $EO_2$ in response to increased oxygen consumption ($VO_2$) at temperatures below the thermoneutral zone. The changes in minute volume seem primarily due to increases in tidal volume rather than breathing frequency (Morgan et al., 1992). Several birds such as parrots (Bucher, 1981; Bucher, 1985; Bucher and Morgan, 1989), chukars (Chappell and Bucher, 1987), prairie falcons (Kaiser and Bucher, 1985), storm petrels, kelp gulls, skuas (Morgan et al., 1992), and tawny frogmouths (Bech and Nichol, 1999) have demonstrated an increase in $V_I$ rather than $EO_2$ in response to changes in metabolic rate below thermoneutrality. Other birds such as European coots (Brent et al., 1984), pekin ducks (Bech et al., 1984), kittiwakes (Brent et al., 1983), and giant petrels (Morgan et al., 1992) exhibit sustained or decreased $V_I$ along with increased $EO_2$. Both the rosy finch and house finch, two passerine birds have shown significant adjustments in $V_I$ due to increases in *f* and $V_T$, but no change in $EO_2$ (Clemens, 1988). The black-capped chickadee, another passerine species has been shown to increase $EO_2$ to maintain metabolism under severe (helox) cold stress (Cooper and Same, 2000). House sparrows have been shown to increase *f*, $V_T$, $V_I$, and $EO_2$ to maintain increased metabolic rates under (helox) cold stress and increased basal metabolic rate in winter birds when compared to summer birds (Arens and Cooper, 2005a). While there is little ventilatory data on passerine species of birds, there is even less on small, non-passerine species that undergo seasonal acclimatization and it is not possible to form any patterns of ventilatory accommodation in response to changes in oxygen demand.

In this study, I examined seasonal metabolic and ventilatory acclimatization to cold stress in downy woodpeckers. Downy woodpeckers are small, nonmigratory non-passerine birds. The primary objective of this study was to examine the effects of cold stress on metabolism and ventilation in seasonally acclimatized downy woodpeckers in Wisconsin. The second objective of this study was to examine geographical variation in metabolism between seasonally acclimatized downy woodpeckers from Wisconsin and South Dakota. I examined $M_{sum}$, BMR, and ventilation parameters ($f$, $V_T$, $V_I$, and $EO_2$) in seasonally acclimatized downy woodpeckers under thermoneutral and cold stressed (helox) conditions.

## Methods

### Birds.

Downy woodpeckers were captured with mist nets at Heckrodt Wetland Reserve, Winnebago County, Wisconsin. Birds captured from June 19, 2006 to August 7, 2006 and on June 4, 2007 were considered summer birds. Birds captured from January 20, 2007 to March 5, 2007 were considered winter birds. Birds were trapped under state (SCP.NER.131) and federal (MB003340-1) collecting permits. Body mass was determined to the nearest 0.1g immediately upon capture using an Ohaus Scout II portable electronic balance. Fat scores were visually determined using a 0-5 scale (Helms and Drury, 1960) and wing chord and tail lengths were recorded. Age of birds was determined through skull ossification and breeding characteristics (Pyle et al., 1997). Adult birds were transported to the laboratory in individual cages (21 x 27 x 31 cm) and

held at room temperature (20-25$^{o}$C). Caged birds were provided water, mealworms, peanuts, and suet ad libitum. All metabolic tests were performed on the day of capture to avoid the effects of captivity on metabolic rates (Warkentin and West, 1990). After testing, birds were banded with U.S. Fish and Wildlife Service aluminum bands (federal banding permit 22934) and released at site of capture. All testing was performed under the University of Wisconsin – Oshkosh Institutional Animal Care and Use Committees (protocol number 26-000126-10-02-01). Data for downy woodpeckers from South Dakota was collected from 1992-1994 (Liknes and Swanson, 1996).

**Cold Stress.**

Maximum cold-induced thermogenesis (summit metabolism) was achieved using a 79% helium and 21% oxygen gas mixture (helox). The higher thermal conductivity of helox compared to air facilitates heat loss and allows ($M_{sum}$) to be recorded at relatively moderate air temperatures (Rosenmann and Morrison, 1974). Although there are physical differences between nitrogen and helium, helox has not been found to affect measurement of respiratory variables in resting animals (Brice and Welch, 1983). In addition, helox was not found to have significant ventilatory effects on house sparrows (*Passer domesticus*) (Arens and Cooper, 2005b).

$M_{sum}$ of individual woodpeckers was elicited by using a sliding cold exposure technique (Swanson et al., 1996). Summer birds were exposed to helox temperatures of 8$^{o}$C, 5$^{o}$C, and 2$^{o}$C and winter birds were tested at -6$^{o}$C, -9$^{o}$C, -12$^{o}$C, and -15$^{o}$C. The sliding temperature method was required to elicit hypothermia in all birds (indicated by a decrease in oxygen consumption ($VO_2$) over 3 min) at each season in relatively similar

times in the test periods. Birds were allowed to feed for two hours prior to cold-stress testing. All cold stress testing was completed between 1000 and 1700 (CST). Birds were placed in a 1 L glass metabolic chamber (equipped with a vertical hardware cloth perch) placed inside a Hotpak incubator (model 352602, Wisconsin birds) or immersing chambers into a bath of water and ethylene glycol (South Dakota birds) in which temperature was controlled within $\pm$ 0.1$^{o}$C. Metabolic chamber temperature was recorded ($\pm$1$^{o}$C) with a Sable Systems TC1000 thermocouple meter. Birds were weighed immediately before and after each test. Constant mass loss throughout each test was assumed to determine body mass for all metabolic trials. Birds were allowed to equilibrate for 15min then readings were recorded for 10min at the first temperature, then 25min at the second temperature and 25min at the third temperature, or until hypothermic. Birds were removed from metabolic chambers when hypothermia was indicated by steady decline in $VO_2$. Once removed from the metabolic chamber, body temperature ($T_b$) was measured ($\pm$0.1$^{o}$C) with a Cole-Parmer thermocouple thermometer and 30-gauge copper-constant thermocouple. Body temperature was taken within 30s and the thermocouple was inserted into the cloaca to a depth where further insertion did not alter the reading. Body temperatures of <37$^{o}$C were considered hypothermic.

**Summit Metabolic Rate.**

Once a bird was placed in the metabolic chamber, $VO_2$ (ml $O_2$ $min^{-1}$) was measured during helox cold-stress using open-circuit respirometry. A Cole-Parmer precision rotameter was used upstream of the chamber to regulate flow rates of dry, $CO_2$-free helox between 1107 - 1111 ml $min^{-1}$ $\pm$1%. Rotameters were calibrated with helox to $\pm$1%

accuracy with a soap bubble meter. These flow rates allowed changes in oxygen content between influx and efflux gas of ~0.4-0.8% and maintained efflux oxygen content above 20.1%. A Sable Systems FC-1B oxygen analyzer (Las Vegas, NV) (Wisconsin birds) or a Ametek S-3A oxygen analyzer (South Dakota birds) was used to resolve fractional concentration of oxygen in dry, $CO_2$-free efflux gas. Measurements of dry, CO2-free efflux gas were recorded every second on a computer using Warthog Systems software (Riverside, CA) and a Sable Systems U12 A/D converter. The first fifteen minutes of each test were considered periods of oxygen concentration equilibration and were not used in $M_{sum}$ calculations. Oxygen consumption rates were calculated as instantaneous rates (Bartholomew et al., 1981). $M_{sum}$ values were obtained by averaging $VO_2$ levels over consecutive 10-minute periods (Dawson and Smith, 1986; Swanson, 1990). The maximum 10-minute average of $VO_2$ was determined to be the $M_{sum}$ of a given test temperature. Warthog System LabHelper and LabAnalyst (Riverside, CA) were used respectively to record and analyze $VO_2$ measurements.

**Basal Metabolic Rate.**

Measurement of basal metabolic rates (BMR) used similar methods for $M_{sum}$ measurements. Instead of helox, air was used during BMR testing and two birds were generally tested at the same time. A Sable Systems multiplexer (model TR-RM4) controlled switching of airstreams between two individual birds in separate metabolic chambers. Switching occurred between chambers every fifteen minutes. Prior to BMR testing birds were fasted for at least six hours to assure birds were postabsorptive. Once birds were placed in metabolic chambers, an hour was used for equilibration. After the

equilibration period, 30 minutes of data were used to determine BMR. Metabolic chambers were checked for gas leaks prior to all metabolic tests by momentarily checking efflux gas rates with a flow rotameter. No leaks occurred in the chambers. Flow rates between 360.1-426.8 ml $min^{-1}$ $\pm$1% were regulated upstream of chambers using two separate Omega mass flow controllers (model FMA-A2048). The oxygen analyzer was referenced against incurrent gas prior to and following all test periods. Chambers were stabilized at a temperature of 30$^{o}$C $\pm$0.1$^{o}$C for all BMR testing which is within the thermoneutral zone for downy woodpeckers (Liknes and Swanson, 1996). BMR testing was completed between 2100 and 0100(CST) for summer birds and between 2200 and 0200(CST) for winter birds. BMR data are reported as 10-minute minimum averages of the last 30 minutes of a trial. Oxygen consumption was calculated as steady state $VO_2$ and corrected for STP (standard temperature and pressure) (Hill 1972, eq. [2]).

**Ventilation Measurements.**

Ventilation was measured using whole-body plethysmography in the open-circuit respirometry system (Bucher, 1981; Malan, 1973). Constant flow rate was maintained to metabolic chambers for all tests. Differential pressure transducers PT-100B (Sable Systems) were used to measure pressure changes within the chambers due to warming and wetting of inspired air. Warthog LabHelper software was used to record data every 0.05sec for BMR tests and 0.03sec for $M_{sum}$ tests. Reference injections of 1ml of air were introduced to the chambers (10-15 times) both prior to and after completion of testing. In summer, dew point ($\pm$0.1C) was constantly monitored using a Sable Systems

Rh-100 humidity meter. In winter, relative humidity was continuously recorded with the Rh-100 humidity meter in place of dew point. Comparisons between reference injections and ventilation deflections were used to calculate $V_T$ (Malan, 1973; eq. [6]).

Whole-body plethysmography has been extensively used because it facilitates noninvasive measurement of ventilation in unrestrained animals (review: Mortola and Frappell, 1998). Time-decay of pressure signal is an important potential source of error of plethysmography in open-flow respirometry systems (Szewczak and Powell, 2003). Comparing deflections kinetics of calibration injections with those from ventilation is commonly used to account for pressure signal time-decay (Bucher, 1981; Malan, 1973). Ventilatory effort can vary for each breath and this comparison may not wholly compensate for signal decay (Szewczak and Powell, 2003). Using guidelines from Szewczak and Powell (2003), we characterized the decay of our plethysmography system. No significant pressure vs. frequency response was found in our system and thus, no correction factor was needed (Arens and Cooper, 2005a).

Both frequency ($min^{-1}$) and VT ($ml\ min^{-1}$) were recorded in unison with $VO_2$ measurements. Frequency was computed using the periodicity of ventilation deflections. $EO_2$ was calculated as $EO_2 = VO_2 / (FEO_2 \times V_I) \times 100$, where the fractional oxygen concentration of effluent air from the metabolic chamber is $FEO_2$. All ventilation data were calculated as BTPS (body temperature pressure saturated) and STPD (standard temperature pressure dry).

**Statistical Analyses.**

Statistical values are given as means $\pm$ SD. Given that, body mass did not significantly vary between sex ($F_{1,33} = 1.81$, $P = 0.19$) and season ($F_{1,33} = 0.16$, P = 0.69), all data is provided on a whole organism level. Whole organism data may be more useful when making seasonal comparisons (Dawson and Smith, 1986; Swanson, 1991), and it removes possible misleading effects of mass-specific data, which are ratios (Packard and Boardman, 1999). Two-way analysis of variance (ANOVA) with season and gender as independent variables was used to test the effects of ambient temperature ($T_a$) on body temperature ($T_b$), metabolic rate, and thermal conductance (C). Two-way ANOVA was used to analyze the effect of $VO_2$ on tidal volume, breathing frequency, minute volume, and oxygen extraction efficiency. Pairwise comparisons of significant effects were performed using *t*-tests. For two-way comparisons that had unequal variances we followed Ruxton's (2006) approach and performed unequal variance t-test. We reported the calculated *t'* and *P*-value with these data. Geographic comparisons between South Dakota and Wisconsin birds were made using *t*-tests for morphometric characteristics (body mass, fat scores, and wing chord), BMR, and $M_{sum}$. All statistics were computed using SPSS 13.0 (SPSS, Chicago). Statistical significance was accepted at $P < 0.05$.

## Results

### Morphometrics.

Analysis of Wisconsin Downy woodpecker at capture morphometric data by two-way ANOVA showed significant seasonal differences in wing chord ($F_{1,33} = 12.345$, $P < 0.01$) but no significant gender differences ($F_{1,33} = 1.54$, $P = 0.23$). There were no significant differences in visual abdominal or furcular fat scores between seasons or genders (Table 1). There were no significant gender ($F_{1,33} = 1.81$, $P = 0.19$) or seasonal ($F_{1,33} = 0.16$, P = 0.69) differences in body mass. South Dakota Downy woodpeckers had significantly greater body mass and wing chord in winter compared to summer woodpeckers (Liknes and Swanson, 1996).

Body mass and wing chords were significantly higher in summer downy woodpeckers from Wisconsin compared to summer South Dakota woodpeckers (mass: $t = 3.16$, $P < 0.01$; wing chord: $t = 3.10$, $P < 0.01$). There was no significant difference in body mass between Wisconsin and South Dakota winter woodpeckers ($t'_{25.922} = -0.13$, $P = 0.90$). Wisconsin Downy woodpeckers had significantly larger wing chords than South Dakota woodpeckers in winter ($t = -4.17$, $P < 0.01$). Furcular fat scores were significantly higher in South Dakota birds in summer ($t = 3.80$, $P < 0.001$) and in winter ($t'_{27.270} = 5.75$, $P < 0.001$) compared to Wisconsin birds. There was no significant difference in abdominal fat between Wisconsin and South Dakota woodpeckers in summer ($t = 0.86$, $P = 0.39$) or in winter ($t = 1.24$, $P = 0.22$, ).

**Cold Tolerance and Summit Metabolic Rate.**

Winter woodpeckers from Wisconsin were able to withstand colder helox temperatures than summer birds before becoming hypothermic. The temperature at which birds became hypothermic ($T_{cl}$) was significantly lower in winter birds than in summer birds ($t'_{17.22} = -8.83$, $P < 0.001$). Mean $T_{cl}$ was -7.2 $\pm$ 4.3 $^{o}$C for winter birds and 3.7 $\pm$ 1.6 $^{o}$C for summer birds (Figure 3). Mean $T_b$ was not significantly different after $M_{sum}$ tests than after BMR tests in summer birds ($n = 22$, $t = 0.51$, $P = 0.62$). Mean $T_b$ was significantly lower after $M_{sum}$ tests than after BMR tests in winter birds ($t'_{16.84} = 5.38$, $P < 0.001$). Mean $T_b$ in summer birds was 35.9$^{o}$ $\pm$ 1.5$^{o}$C after $M_{sum}$ tests and 36.2$^{o}$ $\pm$ 1.6$^{o}$C after BMR tests. Mean $T_b$ in winter birds was 34.8$^{o}$ $\pm$ 2.1$^{o}$C after $M_{sum}$ tests and 38.3$^{o}$ $\pm$ 1.0$^{o}$C after BMR tests. Changes in seasonal cold tolerance could be effected by seasonal changes in thermal conductance. Therefore, thermal conductance ($C = M_{sum} / [T_b - T_a]$; Scholander et al., 1950) was calculated and compared between seasons and genders. Conductance showed no significant variation between seasons for $M_{sum}$ ($F_{1,21} = 0.12$, $P = 0.74$, Table 2). There was no significant difference in conductance between genders for $M_{sum}$ ($F_{1, 21} = 0.29$, $P = 0.59$).

In winter woodpeckers, $M_{sum}$ was significantly higher than in summer birds ($F_{1,24} = 24.45$, $P < 0.001$), but there were no significant differences between genders ($F_{1,24} = 0.27$, $P = 0.56$, Table 2). Comparison of $M_{sum}$ data between Wisconsin and South Dakota downy woodpeckers using t-tests showed significantly higher summit metabolic rates in South Dakota birds during both summer ($t = 2.97$, $P < 0.01$) and winter ($t = 5.36$, $P < 0.001$).

**Basal Metabolic Rate.**

Winter woodpeckers from Wisconsin also had significantly higher BMR than summer birds ($F_{1,25} = 7.15$, $P = 0.01$), with no significant difference between genders ($F_{1,25} = 2.61$, $P = 0.12$, Table 2). Conductance showed no significant seasonal differences during BMR ($F_{1, 24} = 0.29$, $P = 0.60$) trials. There were no significant differences in conductance between genders for BMR ($F_{1,24} = 0.74$, $P = 0.40$) tests. There was no significant within season geographical difference in BMR between Wisconsin and South Dakota downy woodpeckers during summer ($t = 1.92$, $P = 0.08$) or winter ($t = 0.39$, $P = 0.703$).

**Ventilation.**

Ventilation measurements were only recorded for woodpeckers from Wisconsin. Breathing frequency was significantly higher during cold stress than within the thermal neutral zone for both summer ($t'_{11.0} = -9.60$, $P < 0.001$) and winter woodpeckers ($n = 27$, $t = -12.33$, $P < 0.001$, Table 3). Breathing frequency during BMR tests showed no significant difference between seasons ($F_{1, 24} = 0.49$, $P = 0.49$) or genders ($F_{1,24} = 2.52$, $P = 0.13$). Breathing frequency did not vary significantly between seasons ($F_{1,19} = 1.90$, $P = 0.18$, Figure 4) or genders ($F_{1,19} = 0.95$, $P = 0.34$).

Tidal volume was significantly higher during $M_{sum}$ trials when compared to that that BMR trials for both summer ($t'_{6.2} = -8.84$, $P < 0.001$) and winter ($t'_{12.5} = -13.14$, $P < 0.001$, Table 3). There were no significant seasonal or gender differences in tidal volume during BMR ($F_{1,24} = 0.32$, $P = 0.58$; $F_{1,24} = 0.60$, $P = 0.45$) or $M_{sum}$ trials ($F_{1,19} = 0.76$, $P = 0.47$; $F_{1,19} = 0.30$, $P = 0.59$, Figure 4).

Minute volume was also significantly higher during $M_{sum}$ trials as compared to BMR trials for summer ($t'_{6.0} = -8.70$, $P < 0.001$) and winter ($t'_{12.2} = -15.75$, $P < 0.001$, Table 3). Minute volume was not significantly different between seasons ($F_{1,24} = 0.53$, $P = 0.47$, Figure 4) or genders ($F_{1,24} = 0.00$, $P = 0.984$) during BMR trials. There were also no significant differences between seasons ($F_{1,19} = 0.25$, $P = 0.64$) or genders ($F_{1,19} = 0.05$, $P = 0.83$) during $M_{sum}$ trials.

Oxygen extraction was significantly higher during BMR trials when compared to $M_{sum}$ trials for both summer ($n = 18$, $t = 7.00$, $P < 0.001$) and winter ($t'_{15.3} = 12.61$, $P < 0.001$, Table 3). Oxygen extraction was significantly higher in winter as compared to summer for both BMR ($F_{1,24} = 10.24$, $P < 0.01$) and $M_{sum}$ trials ($F_{1,19} = 7.66$, $P = 0.01$, Figure 4). Oxygen extraction had no significant gender differences during BMR ($F_{1, 24} = 0.28$, $P = 0.60$) or $M_{sum}$ trials ($F_{1,19} = 0.35$, $P = 0.56$).

## Discussion

### Morphometrics.

Although winter wing chords were significantly greater than summer wing chords, they were both similar to the average range of Wisconsin downy woodpecker wing chords recorded by Ouellet (1977) (male: 96.7 $\pm$ 0.6, female: 97.3 $\pm$ 0.7). While there was a significant increase in wing chord from summer birds to winter birds, there was no significant difference in body mass between summer and winter woodpeckers. It is possible that some hatch year (HY) birds were tested during the summer. Plumage and

skull ossification were used to help determine the age of captured birds. Downy woodpeckers that have molted their juvenile plumage can be difficult to age correctly due to skull obfuscation from the tongue (Liknes and Swanson, 1996). The tongue of Downy woodpeckers wraps around the skull and can obscure the skull ossification. More mature birds would have an increased wing chord. Another possible source of this variation may come from social hierarchy structure in downy woodpeckers. Our birds were trapped at a feeder station and more dominant birds may have been present at the feeder during winter than during the summer (personal observation). Decreased food abundance in winter may lead to an increased role of social dominance at feeder stations. More dominant birds may be larger than less dominant birds and may have increased wing chord. Wisconsin summer birds also had significantly higher wing chords and masses than South Dakota summer birds. This geographical variation may be the result of differences in measurement techniques for wing chord. We used a wing chord rule to measure the length or wing chords in our birds. South Dakota bird wing chords were measured with calipers. Differences in the method of wing chord measurement could lead to significant differences in the data. The use of a wing chord rule could lead to compression of feathers at the tip of the wing, causing shortened measurements of wing chord (Yunick, 1986). South Dakota woodpeckers had significantly higher furcular fat scores in both summer and winter compares to Wisconsin birds. This geographic variation in fat scores may be a result of inherent variation in fat scoring methods. Significant variation has been recorded in both inter- and intraobserver fat scoring (Krementz and Pendleton, 1990). The lack of seasonal variation in body mass and fat

scores of the woodpeckers in this study is typical of other tree foraging species such as mountain chickadees and juniper titmice (Cooper, 2007).

**Cold Tolerance and Thermal Conductance**.

Winter birds in Wisconsin were able to withstand colder temperatures than summer birds before becoming hypothermic. The mean $T_{cl}$ was -7.2 $\pm$ 4.3 $^{o}$C for winter birds and 3.7 $\pm$ 1.6 $^{o}$C for summer birds. The mean $T_b$ after $M_{sum}$ tests was 34.8 $\pm$ 2.1 $^{o}$C for winter birds and 35.9 $\pm$ 1.5 $^{o}$C for summer birds. However, a higher percentage of birds became hypothermic in winter (85.7%, 12 out of 14 birds tested), than in summer (58.3%, 7 out of 12 birds tested). There was no significant seasonal variation in thermal conductance, indicating that Wisconsin downy woodpeckers do not rely on insulatory improvements to enhance cold tolerance. South Dakota downy woodpeckers required lower temperature ranges to elicit hypothermia. South Dakota Downy woodpeckers became hypothermic from ˉ9 to ˉ15 in winter and from 0 to ˉ12$^{o}$C in summer. The temperatures required for hypothermia in Wisconsin Downy woodpeckers were higher in both winter and summer than those from South Dakota. This suggests the $T_{cl}$ of South Dakota woodpeckers may be lower than the $T_{cl}$ of Wisconsin woodpeckers. The geographic variation in cold tolerance between Wisconsin and South Dakota Downy woodpeckers is not a result of geographic variation in body mass. A source of the difference in cold tolerance may come from a variation in shivering endurance or other thermoregulatory activities.

**Summit and Basal Metabolic Rates.**

Summer woodpeckers from Wisconsin displayed nocturnal hypothermia during BMR tests (5 out of 12 birds tested, 41.7%) winter birds did not. Several species of birds such as Great tits, Common Redpolls, and Willow tits have shown relationships between energy reserves and degree of hypothermia (Reinertsen and Haftorn, 1983, 1986). While no seasonal difference in mass or fat scores were found among our birds, a seasonal difference in energy reserves may have been undetected and may account for the use of nocturnal hypothermia by only summer woodpeckers. Wisconsin downy woodpeckers had increased $M_{sum}$ during winter compared to summer birds. This finding is similar to those found for many species of birds (Cooper, 2002; Cooper and Swanson, 1994; Liknes and Swanson, 1996). The increase in $M_{sum}$ and decreased in $T_{cl}$ during winter suggests there may be a correlation between the two. This finding is consistent with correlations found with many species of birds (Arens and Cooper, 2005b; Swanson, 2001). Arens and Cooper found a 64% increase in $M_{sum}$ in winter house sparrows compared to summer house sparrows along with a decrease in $T_{cl}$ from $5^{o}C$ in summer to $^{-}11^{o}C$ in winter (Arens and Cooper, 2005b). South Dakota downy woodpeckers had higher $M_{sum}$ in both summer and winter than Wisconsin birds. South Dakota birds required lower temperatures to elicit hypothermia during $M_{sum}$ testing. This may represent a lower $T_{cl}$ in South Dakota woodpeckers and may account for some of the geographic variation in metabolic rates. South Dakota birds forage in areas of less dense cover than Wisconsin birds. South Dakota birds are exposed to increased wind and disruption of feathers (D. Swanson, per. comm.). This indicates a possible variation in thermal conductance

between South Dakota and Wisconsin birds. The mean conductance of South Dakota Downy woodpeckers was 2.68 $mW \cdot g^{-1} \cdot {}^{o}C^{-1}$ in winter and 2.84 $mW \cdot g^{-1} \cdot {}^{o}C^{-1}$ in the summer (Liknes and Swanson, 1996). The thermal conductance of Wisconsin Downy woodpeckers was 16% lower than the thermal conductance of South Dakota Downy woodpeckers in the winter and 15% lower in the summer. The variation in thermal conductance may account for the differences in metabolic rates between South Dakota and Wisconsin birds. Mean $M_{sum}$ of Wisconsin winter birds was 8.7% lower than allometrically ($VO_{2Msum} = 0.785(mass)^{0.716}$; Dutenhoffer and Swanson, 1996) predicted values. Mean $M_{sum}$ of Wisconsin summer birds was 27.9% lower than predicted values. $M_{sum}$ of South Dakota woodpeckers was 64.4% higher than predicted in winter and 12.7% higher than predicted in summer (Liknes and Swanson, 1996).

BMR in Wisconsin winter Downy woodpeckers was approximately 1.22 times higher than in summer birds. This variation in metabolic rates is consistent with many species of birds (Arens and Cooper, 2005b; Cooper, 2002; Liknes et al., 2002). Swanson suggested that this increase in metabolic rate could be due to increased metabolic costs from thermogenesis in winter (Swanson, 1991). It is possible that this increased metabolic cost is due to maintenance of increased pectoralis muscle mass. Increased pectoralis muscle mass accommodates increased shivering thermogenesis in winter. Winter pectoralis muscle mass has been found to increase in bird species such as dark-eyed juncos, house finches, mountain chickadees, and juniper titmice (Copper, 2002; O'Connor, 1995; Swanson, 1991). Mean BMR of winter birds was 60.3% higher than allometrically predicted values (W; $\log BMR = -1.461 + 0.669 \log M_b$; McKechnie and

Wolf, 2004). Mean BMR of summer birds was 40.4% higher than predicted values. BMR of South Dakota woodpeckers was 106.6% higher than predicted in winter and 45.2% higher than predicted in summer (Liknes and Swanson, 1996). Wisconsin Downy woodpeckers had a metabolic expansibility of approximately 5.0 during winter and 4.7 during summer. South Dakota woodpeckers had a metabolic expansibility of approximately 6.8 during winter and 6.8 during summer.

South Dakota Downy woodpeckers had an increased $M_{sum}$ compared to Wisconsin woodpeckers in both winter and summer, but no geographical variation in BMR. A possible source for the geographical variation in $M_{sum}$ is geographical variation in proximate air temperatures. However, variation in proximate air temperatures would also lead to variation in BMR. It is more likely the geographical variation in metabolic rates is due to variation in wind exposure and wind speeds and roosting and feeding sites. Wolf and Walsberg demonstrated an increase in wind speed from 0.4m/s to 3.0 m/s could elicit a 14% increase in metabolic rates of verdins. Typical wind speeds in woodlots in Wisconsin where woodpeckers were captured do not exceed 2.0 m/s (Cooper unpubl. Data) but may typically exceed 5.0 m/s in fragmented woodlots.

**Ventilation.**

Wisconsin downy woodpeckers had increased oxygen extraction during BMR tests for both summer and winter compared to $M_{sum}$ tests. Winter birds had increased oxygen extraction for both BMR tests and $M_{sum}$ tests compared to summer birds. This may be a component of seasonal acclimatization in Downy woodpeckers. However, this variation in oxygen extraction rates has only been found in downy woodpeckers and

black-capped chickadees and is not necessarily a universal phenomenon (Cooper and Same, 2000). Both winter and summer Downy woodpeckers in Wisconsin had increased breath frequency, tidal volume and minute volume in low $T_a$. House finches and rosy finches show the same pattern (Clemens, 1988). Mean breath frequency was 4.2% higher than predicted values in summer and was as expected in winter. Mean tidal volume was 31.2% lower than predicted values in summer and 36.9% lower in winter. Mean minute volume was 29.2% lower than predicted values in summer and 37.5% lower in winter

Table 1. *Mean (± SD) morphometric values of downy woodpeckers during summer and winter in Wisconsin and South Dakota.*

| Season/ Geography | Body Mass (g) | Fat Score | | Wing Chord (mm) |
|---|---|---|---|---|
| | | Furcula | Abdomen | |
| Summer: | | | | |
| Wisconsin | 27.806 ± 2.127 | 0.18 ± 0.393 | 0.47 ± 0.624 | 92.324 ± 3.107 |
| South Dakota | 26.263 ± 1.419[a] | 0.71 ± 0.515[a] | 0.61 ± 0.495 | 89.776 ± 2.663[a] |
| Winter: | | | | |
| Wisconsin | 27.935 ± 1.826 | 0.13 ± 0.342 | 0.38 ± 0.500 | 95.906 ± 2.718[*] |
| South Dakota | 27.870 ± 1.137[*] | 1.16 ± 0.688[a] | 0.68 ± 0.885 | 92.632 ± 1.963[a,*] |

[*] Indicates significant differences between seasons within a geographical location ($P < 0.05$)

[a] Indicates significant differences between geographic locations within a season ($P < 0.05$).

Table 2. *Mean (± SD) metabolic rates of downy woodpeckers during summer and winter in Wisconsin and South Dakota.*

| Season/ Geography | Msum ($ml\ O_2\ min^{-1}$) | BMR ($ml\ O_2\ min^{-1}$) | Conductance Msum ($ml\ O_2\ min^{-1} \cdot {}^oC^{-1}$) | Conductance BMR ($ml\ O_2\ min^{-1} \cdot {}^oC^{-1}$) |
|---|---|---|---|---|
| Wisconsin: | | | | |
| Summer | 5.857 ± 0.625 | 1.249 ± 0.262 | 0.200 ± 0.041 | 0.178 ± 0.016 |
| Winter | 7.653 ± 1.008* | 1.529 ± 0.307* | 0.188 ± 0.073 | 0.180 ± 0.019 |
| South Dakota: | | | | |
| Summer | 6.623 ± 0.723[a] | 0.984 ± 0.117 | --- | --- |
| Winter | 10.037 ± 1.277[a] | 1.467 ± 0.073 | --- | --- |

* Indicates significant differences between seasons within a geographical location ($P < 0.05$)

[a] Indicates significant differences between geographical locations within a season ($P < 0.05$)

Table 3. *Mean (± SD) ventilation parameters of downy woodpeckers in summer and winter under helox cold stress and thermal neutral conditions.*

| Season/ Condition | *N* | Freq. (Hz) | $V_T$ (BTPS) (ml $O_2$) | $V_T$ (STPD) (ml $O_2$) | $V_i$ (BTPS) (ml $O_2$ * $S^{-1}$) | $V_i$ (STPD) (ml $O_2$ * $S^{-1}$) | $EO_2$ (STPD) (%) |
|---|---|---|---|---|---|---|---|
| Summer: | | | | | | | |
| BMR | 11 | 62.34 ± 7.70 | 0.92 ± 0.29 | 0.31 ± 0.11 | 55.67 ± 15.05 | 19.52 ± 6.32 | 32.96 ± 8.64 |
| Msum | 9 | 107.30 ± 28.66* | 2.47 ± 1.04 | 2.12 ± 1.20* | 274.29 ± 147.41 | 247.76 ± 152.47* | 18.83 ± 18.87* |
| Winter: | | | | | | | |
| BMR | 14 | 59.80 ± 9.94 | 0.96 ± 0.29 | 0.29 ± 0.09 | 57.17 ± 20.48 | 17.30 ± 6.22 | 45.26 ± 9.14 |
| Msum | 15 | 119.37 ± 30.41* | 1.86 ± 0.61 | 2.05 ± 0.88* | 226.93 ± 88.91 | 257.56 ± 114.17* | 18.29 ± 13.88* |

* indicates significant differences between testing conditions within a season ($P < 0.05$)

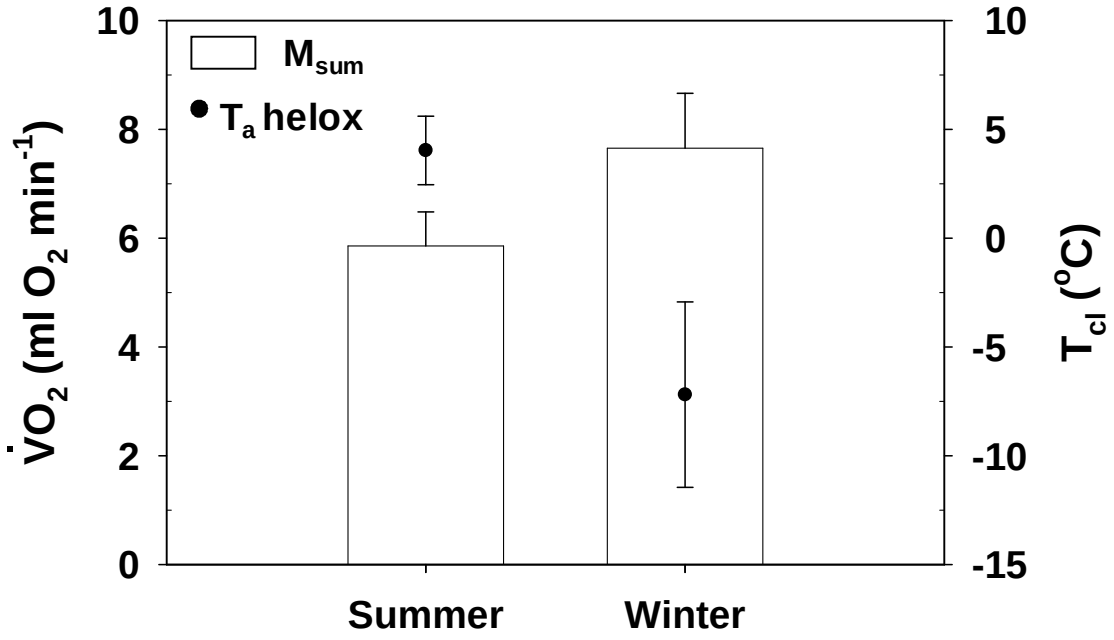


*Figure 3.* Seasonal variation in $M_{sum}$ and cold tolerance ($T_{cl}$) for Summer and Winter acclimatized Wisconsin downy woodpeckers.

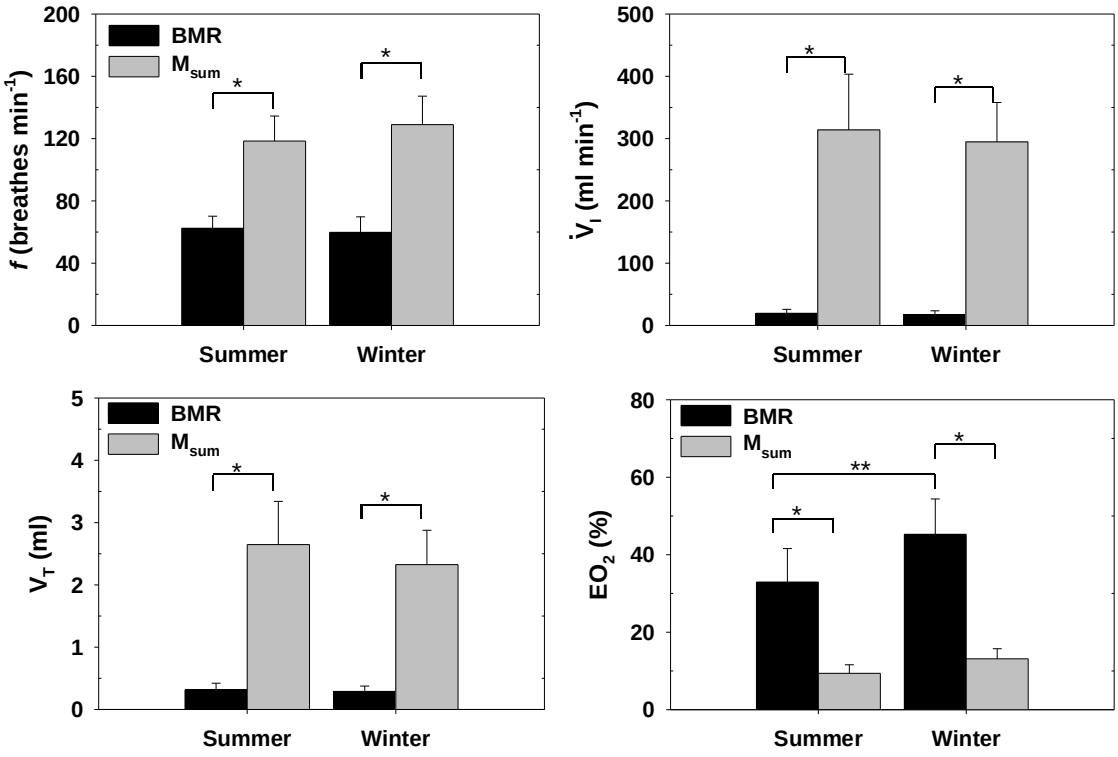


*Figure 4.* Mean breathing frequency (f), minute volume ($V_I$), tidal volume ($V_T$), and oxygen extraction efficiency ($EO_2$) during helox cold stress (Msum) and thermal neutral (BMR) conditions in Summer and Winter Wisconsin downy woodpeckers.

* indicated significant differences between testing conditions ($P < 0.05$)

** indicates significant differences between seasons ($P < 0.05$)

## Chapter III - Foraging-generated Heat Production Contributes To Thermoregulation In Seasonally Acclimatized Downy Woodpeckers (*Picoides pubescens*)

### Introduction

Small birds that overwinter in cold temperate regions require high rates of energy expenditure for thermoregulation. Thermoregulatory costs for winter birds typically range from 20% to 40% of daily energy expenditure (Weathers et al., 1984; Weathers and Sullivan, 1989) and may reach nearly 60% of the daily energy budget (Weathers and Sullivan, 1993). In order for birds to meet these high thermoregulatory demands, small birds must forage for much of the day. The time spent foraging during the day in winter for small birds ranges from 50% in mountain chickadees (*Poecile gambeli*) and juniper titmice (*Baeolophus ridgwayi*; Cooper, 2000) to 90% in Verdins (*Auriparus flaviceps*; Austin, 1978). In general, birds produce heat for thermoregulation through shivering (Marsh and Dawson, 1989; Swanson, 2010). However, another possible source of energy for thermoregulation in birds is from the heat produced from locomotor muscles during foraging activity or activity thermogenesis. The replacement of thermoregulatory heat production by activity thermogenesis is called substitution. Thus, the heat generated while foraging may replace heat that would otherwise be generated by shivering. Substitution allows for the reduction of the net energy cost of foraging (Webster and Weathers, 1990).

Substitution has been reported for a number of ground-foraging birds. Typically, heat production has been measured as oxygen consumption while at rest and during terrestrial locomotion, usually while a bird is walking or running on a treadmill (Bruinzeel and Piersma, 1998). In addition, combining doubly labeled water measurements with laboratory measurements of metabolism and field time-budgets has shown that foraging activity substitutes for thermoregulation in both Verdins (Webster and Weathers, 1990) and Yellow-eyed Juncos (*Junco phaeonotus*; Weathers and Sullivan, 1993). For tree-foraging birds, substitution has been recorded using laboratory metabolic rates of daytime resting compared to daytime foraging in mountain chickadees, juniper titmice and black-capped chickadees (*Poecile atricapillus*; Cooper, 2000; Cooper and Sonsthagen, 2007). All tree-foraging birds tested to date have been passerines. Birds other than passerines (non-passerines) have not been tested to our knowledge.

Woodpeckers are an interesting tree-foraging group compared to chickadees, titmice, and verdins. Chickadees, titmice, and verdins, primarily forage using glean-and hang maneuvers (Robinson and Holmes, 1982). Downy woodpeckers (*Picoides pubescens*) foraging behavior includes three primary types: percussion, scaling, and peering and poking (Jackson, 1970). Percussion refers to rapid continuous series of blows along a limb, scaling is the movement of a bird along a limb, and peering and poking includes poking into fissures in the bark of trees (Jackson, 1970). For small tree-foraging species such as woodpeckers, that store very little body fat and thus have fewer

energy reserves compared to ground-foraging birds (Cousineau and Cooper, 2012; Rogers, 1987) substitution of thermoregulatory costs from activity thermogenesis may be critically important in maintaining energy balance, especially during the nocturnal fast.

To test if substitution occurs in a typical woodpecker (a non-passerine species), we measured and compared metabolic rates of seasonally acclimatized downy woodpeckers (*Picoides pubescens*) while perching compared to foraging during the normal active phase of the daily cycle. Foraging behavior in downy woodpeckers involves vigorous vertical movements along feeding surfaces. In addition, seasonal variation in metabolic response to temperature was also recorded in woodpeckers in order to determine if seasonal variation in thermal conductance occurs and if this impacts substitution in small birds.

## Methods

### Birds.

Downy woodpeckers were trapped using mist nets in Winnebago County, Wisconsin. Summer birds were trapped from June 19, 2006 to August 7, 2006 and on June 4, 2007. Winter birds were trapped from January 20, 2007 to March 5, 2007. Birds were trapped under state (SCP.NER.131) and federal (MB003340-1) collecting permits. An Ohaus Scout II portable electronic balance (Ohaus Corporation, Parsippany, NJ) was used to measure body mass at time of capture. Skull ossification and breeding characteristics were used to determine age of birds (Pyle et al., 1997). Adult birds were transported to the laboratory and held at room temperature (20-25$^{o}$C) in individual cages

(21 x 27 x 31 cm). Water, mealworms (*Tenebrio* sp.), peanuts, and suet were provided to caged birds *ad libitum*. All birds maintained body mass while in captivity. Daytime metabolic tests were performed on birds after allowing them to feed for a minimum of 2 hours. Birds were banded with U.S. Fish and Wildlife Service aluminum bands (federal banding permit 22934) and released at site of capture after testing. All testing was performed under Institutional Animal Care and Use Committees protocol number 26-000126-10-02-01.

**Foraging Trials.**

We measured metabolic heat production of birds during the active phase of the daily cycle between 0900 and 1700 CST in summer and between 0900 and 1500 in winter. Measurements were made on individual birds placed in 5-L glass metabolic chambers. The chamber had a wire mesh perch placed along the side of the chamber. Each metabolic measurement lasted 90 min, with the first 30 min used for equilibration. Perching heat production was then measured from 30 – 60 min in the chamber and foraging heat production was measured from 60 – 90 min inside the metabolic chamber. Woodpeckers foraged on a peanut feeder (23 cm height x 8 cm diameter) placed inside the chamber after the first 60-min of heat production measurement. Perching birds rested in the metabolic chamber without food or lighting. Originally it was intended that the perching group would have lighting. However, even without food, woodpeckers exhibited significant hopping activity. Thus, they could not be considered to be perching. Foraging birds foraged on peanuts from a feeder inside the metabolic chamber. Lighting was provided by fluorescent lamps that provided illumination similar to natural early

morning or late afternoon light but did not provide significant thermal heating (< 3 W $m^{-2}$). Foraging activity in woodpeckers was observed using an outdoor infrared black and white CCD camera and black and white CCTV monitor (Model CEM0902, CBC Corp.). The infrared camera was placed near the metabolic chamber and allowed us to view all movements made by foraging woodpeckers without disturbing them. Foraging activity was visually observed for 100 seconds over three separate random time periods, one within the first fifteen minutes of foraging and two within the last fifteen minutes of foraging. All movements other than perching were considered foraging activity. The number of stops woodpeckers made while foraging was also recorded. Activity during foraging is reported as the average of the three time periods as seconds of activity per 100 seconds following Grubb (1978).

Activity was not recorded on perching birds since they only appeared to make minor postural adjustments during random visual observations. In order to verify that birds had were not moving while perching, ventilation records were recorded using whole-body plethysmography (Mortola and Frappell, 1998). The pressure transducers (Sable Systems, PT-100B) used to measure ventilation show large recording spikes when a bird becomes active in the metabolic chamber (Arens and Cooper, 2005a). No spikes larger than those from ventilation in resting birds were observed in the ventilation records of perching birds. The metabolic chamber was placed inside a temperature-controlled incubator (Model 352602, Hotpack, Philadelphia, PA) capable of controlling temperature within 0.1$^{o}$C. Air temperature ($T_a$) inside the chamber was monitored continuously

throughout each test with a Sable Systems thermocouple thermometer (TC-1000, Las Vegas, NV) using a 30-gauge copper-constantan thermocouple.

**Measurement of Metabolic Heat Production.**

Metabolic rates were determined by simultaneous measurements of oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) by open-circuit respirometry. Metabolic measurements were made at $T_a$ between -18°C and 15°C to ensure that all metabolic rates were measured below the lower critical temperature ($T_{lc}$) for downy woodpeckers based on allometric predictions of Calder and King (1974). The $T_a$ must be below the $T_{lc}$ because substitution does not occur within the thermal neutral zone where there are no thermoregulatory costs (Bruinzeel and Piersma, 1998).

Each metabolic measurement lasted 90 min. Birds were weighed to the nearest 0.1 g before and after each metabolic test. Constant mass loss or gain was assumed in order to calculate body mass during the metabolic trials. At the end of each trial, birds were removed from the metabolic chamber and body temperature ($T_b$) was recorded ($\pm$0.1°C) by inserting a 30-gauge copper-constantan thermocouple into the birds' cloaca at a depth where further insertion did not alter the reading. $T_b$ was obtained within 30 sec of removing the bird from the metabolic chamber. $T_b$ was measured for perching and foraging woodpeckers.

Inlet air was dried with indicating Drierite (anhydrous $CaSO_4$, W.A. Hammond Co., Xenia, Ohio) and scrubbed of $CO_2$ with Ascarite before being pushed through the metabolic chamber with a diaphragm pump. Inlet flow rates were maintained at 1380 - 1400 mL $min^{-1}$ by a Cole-Parmer precision rotameter calibrated with air to ± 1%

accuracy by a soap bubble meter. These flow rates yielded changes in oxygen and carbon dioxide content between influx and efflux gas of 0.4% and 0.8%, respectively, and maintained oxygen content of efflux gas above 20.1%. Excurrent air from the metabolism chambers was directed into a manifold to bleed the excess volume of air so that a subsample could be pulled through the respirometry system at a flowrate of 100 mL $min^{-1}$. The subsample of air first passed through a Sable Systems dew point humidity meter (model RH-200), then through a drying tube containing magnesium perchlorate. The air stream was next directed into a Sable Systems carbon dioxide analyzer (model CA-2A). Lastly, the air stream was then directed through a tube of Ascarite and Drierite and a Sable Systems oxygen analyzer (model FC-1B). Low permeability Bev-a-line tubing was used throughout the system. The carbon dioxide analyzer was calibrated using both nitrogen and a calibration gas of 1% $CO_2$. Measurements of the excurrent gas were recorded every 1 sec on a computer using LabHelper data collection software and analyzed with LabAnalyst software (Warthog Systems, Riverside, California). To compensate for the washout characteristics of the respirometry system, oxygen consumption and carbon dioxide production values were calculated as instantaneous rates (Bartholomew et al., 1981). We used the individual respiratory quotients (RQ) to estimate total heat production for each metabolic trial using thermal equivalents from Brody (1945).

Measurements of heat production during nocturnal metabolic response to varying ambient temperatures were performed similarly to daytime perching tests. All metabolic responses to temperature trials were conducted between 2100 and 0200 CST. Birds were

at least 4 hour postabsorptive before trials began. Birds were placed in 1-L glass metabolic chambers equipped with a wire mesh perch placed along the side of the chamber. Flow rates between 360.1-426.8 ml $min^{-1}$ $\pm$1% were regulated upstream of chambers using two separate Omega mass flow controllers (model FMA-A2048). Two individual birds were recorded at the same time in separate metabolic chambers using a Sable Systems multiplexer (model TR-RM4). Switching occurred between chambers every fifteen minutes. Air temperature within the chamber ranged between $-10^{o}$ – $10^{o}$C for metabolic response to temperature trials.

**Statistical Analyses.**

Data are presented as means $\pm$ SD. For an individual trial, mean metabolic heat production ($H_m$) was calculated as the maximum 10 min value within the 30 min trial period during the perching and foraging portion of the metabolic trials. We used maximum values since foraging birds were very active and we wanted to compare the highest $H_m$ while foraging. For metabolic response to temperature 10 min mean values from the last 30 min of the trial were used. Data were analyzed for normality (Shapiro-Wilks test) and for homogeneity (Levene's test) prior to parametric analyses. We compared means using independent two-tailed *t*-tests. Regression lines were fitted by the method of least squares. Slopes and intercepts of regression lines were compared using analysis of covariance (ANCOVA; Zar, 1996). Two-way analysis of variance (ANOVA) was used to test the effects of ambient temperature ($T_a$) on metabolic rate (W). Pair wise comparisons of active phases within seasons and between seasons were performed using Tukey's tests or *t*-tests. Statistical significance was accepted at $P < 0.05$. All statistics

were computed with SPSS 13 (SPSS, Chicago). All values are presented on a whole-organism basis to avoid the possible confounding effects of ratios (Packard and Boardman, 1999).

## Results

### Body Mass.

There were no significant difference in body mass due to season, time of day, ($F_{3,36}$ = 2.76, $P$ = 0.06) or gender ($t$ = 0.95, $P$ = 0.35). Mean body mass during metabolic tests of woodpeckers for each season and time of day were as follows: summer daytime, 25.4 $\pm$ 1.9 g ($n$ = 13); summer nighttime, 24.8 $\pm$ 1.7 g ($n$ = 7); winter daytime, 26.4 $\pm$ 1.6 g ($n$ = 12); and winter nighttime, 25.9 $\pm$ 1.3 g ($n$ = 8). Since there were no significant gender differences in body mass, all other data were pooled without regard to gender for statistical analyses.

### Activity Levels.

Summer woodpeckers spent 44.3 sec./min foraging ($n$ = 12), which did not differ significantly from winter birds (46.6 sec./min; $n$ = 12, $t$ = 0.93, $P$ = 0.36). Summer woodpeckers had 1.9 stops/min while foraging ($n$ = 12) which did not differ significantly from winter birds (1.5 stops/min; $n$ = 12, $t$ = -1.30, $P$ = 0.21). Using winter foraging data for downy woodpeckers from Grubb (1978), we calculated predicted foraging activity given the $T_a$ of our metabolic trials. Since summer and winter woodpeckers had no significant differences in foraging values we pooled them to compare to predicted values. Predicted foraging time for woodpeckers from South Dakota was 26.5 sec./min, which is

significantly lower than Wisconsin woodpeckers ($n$ = 24, $t$ = -12.20, $P$ < 0.001). Predicted stops during foraging were 8.4 stops/min, which is significantly lower than Wisconsin woodpeckers ($n$ = 24, $t$ = -16.93, $P$ < 0.001).

**Metabolic Heat Production.**

Metabolic heat production of woodpeckers for each season and state of activity increased with decreasing $T_a$ (Fig. 5). Analysis of metabolic data showed the following relationships between metabolic heat production ($H_m$) and $T_a$.

Summer perching woodpeckers ($n$ = 12, $r^2$ = 0.53, $P$ < 0.01):

$$H_m\ (W) = 1.19 - 0.031T_a. \quad (1)$$

Summer foraging woodpeckers ($n$ = 12, $r^2$ = 0.45, $P$ = 0.02):

$$H_m\ (W) = 1.02 - 0.028T_a. \quad (2)$$

Winter perching woodpeckers ($n$ = 12, $r^2$ = 0.48, $P$ = 0.01):

$$H_m\ (W) = 1.53 - 0.039T_a. \quad (3)$$

Winter foraging woodpeckers ($n$ = 12, $r^2$ = 0.36, $P$ = 0.04):

$$H_m\ (W) = 1.22 - 0.023T_a. \quad (4)$$

The regression equations relating $H_m$ to $T_a$ for perching summer birds compared to foraging summer birds were not significantly different in slope ($F_{1, 20}$ = 0.05, $P$ = 0.83) or in intercept ($F_{1, 21}$ = 2.60, $P$ = 0.12). The equations relating $H_m$ to $T_a$ for perching winter birds compared to foraging winter birds did not differ significantly in slope ($F_{1, 20}$ = 0.92, $P$ = 0.35), but were significantly different in intercept ($F_{1, 21}$ = 8.44, $P$ = 0.008). Regression equations relating $H_m$ to $T_a$ for perching summer birds compared to perching winter birds were not significantly different in slope ($F_{1, 20}$ = 0.11, $P$ = 0.745) or in

intercept ($F_{1, 21} = 4.02$, $P = 0.058$). The equations relating $H_m$ to $T_a$ for foraging summer birds compared to foraging winter birds were not significantly different in slope ($F_{1, 20} = 0.26$, $P = 0.614$), but were significantly different in intercept ($F_{1, 21} = 7.14$, $P = 0.014$).

Mean body temperature ($T_b$) at the end of metabolic trials was $39.1 \pm 1.2^oC$ ($n$ =12) for winter foraging woodpeckers which did not differ significantly from the mean summer foraging woodpecker $T_b$ of $38.3 \pm 1.1$ ($n = 12$, $t = 1.62$, $P = 0.12$). To examine possible differences in heat loss between foraging and perching birds we calculated thermal conductance ($C = H_m/(T_b - T_a)$. Thermal conductance did not vary with $T_a$. There was no significant relationship between conductance and $T_a$ for non-foraging summer birds ($n = 12$, $r^2 = 0.038$, $P = 0.546$) or foraging summer birds ($n = 13$, $r^2 = 0.12$, $P = 0.275$). There was no significant relationship between conductance and $T_a$ for non-foraging winter birds ($n = 10$, $r^2 = 0.28$, $P = 0.120$) or foraging winter birds ($n = 10$, $r^2 = 0.049$, $P = 0.538$). Analysis through T-tests showed no significant difference in conductance between non-foraging summer and non-foraging winter birds ($n = 22$, t = 1.40, $P = 0.177$, Table 4). Conductance was significantly different between foraging summer and foraging winter birds ($n = 23$, t = 2.20, $P = 0.039$). There was no significant difference in conductance between non-foraging summer and foraging summer birds ($n = 25$, t = -1.42, $P = 0.168$). There was a significant difference in conductance between non-foraging winter and foraging winter birds ($n = 20$, t = -2.62, $P = 0.018$).

**Metabolic Response to Temperature and Thermal Conductance.**

Metabolic heat production of woodpeckers during nocturnal metabolic response to temperature trials increased with decreasing $T_a$ (Figure 6). Analysis of metabolic data showed the following relationships between metabolic heat production (W) and $T_a$. Summer nighttime woodpeckers ($n$ = 7, $r^2$ = 0.79, $P$ < 0.01):

$$H_m\ (W) = 1.01 - 0.026T_a. \quad (5)$$

Winter nighttime woodpeckers ($n$ = 8, $r^2$ = 0.66, $P$ = 0.015):

$$H_m\ (W) = 0.97 - 0.046T_a. \quad (6)$$

The equations relating metabolic heat production to $T_a$ for summer nighttime birds compared to winter nighttime birds were not significantly different in slope ($F_{1, 11}$ = 2.05, $P$ = 0.18) or in intercept ($F_{1, 12}$ = 0.34, $P$ = 0.57, Table 5). The regressions relating metabolic heat production to $T_a$ for summer perching birds compared to summer nighttime birds were not significantly different in slope ($F_{1, 15}$ = 0.02, $P$ = 0.90) or in intercept ($F_{1, 16}$ = 0.38, $P$ = 0.55). The regressions relating metabolic heat production to $T_a$ for winter perching birds compared to winter nighttime birds were not significantly different in slope ($F_{1, 16}$ = 1.78, $P$ = 0.20) but were significantly different in intercept ($F_{1, 17}$ = 5.12, $P$ = 0.04).

Mean thermal conductance below the lower critical temperature in downy woodpeckers was 0.029 $\pm$ 0.008 $W\,^oC^{-1}$ during summer daytime, which did not differ significantly from 0.029 $\pm$ 0.006 $W\,^oC^{-1}$ ($n$ = 22, t = 1.40, $P$ = 0.18) during summer nighttime (Table 5). The thermal conductance during winter daytime was 0.032 $\pm$ 0.007 $W\,^oC^{-1}$, which did not differ significantly from 0.027 $\pm$ 0.005 $W\,^oC^{-1}$ ($n$ = 16, t = 1.00, $P$ =

0.33) during winter nighttime. There was no significant relationship between conductance and $T_a$ for summer birds during daytime ($n$ = 12, $r^2$ = 0.038, $P$ = 0.546) or nighttime (n = 7, $r^2$ = 0.142, $P$ = 0.404). There was no significant relationship between conductance and $T_a$ for winter birds during daytime ($n$ = 10, $r^2$ = 0.275, $P$ = 0.120) or nighttime ($n$ = 9, $r^2$ = 0.052, $P$ = 0.557).

## Discussion

### Foraging.

Foraging woodpeckers in this study had significantly higher activity levels than woodpeckers foraging in their natural outdoor environment recorded by Grubb (1978). Woodpeckers foraging inside metabolic chambers were 71% more active (s/min) than their naturally foraging counterparts. However, we were unable to accurately record the rate of distance traveled while foraging as in outdoor foraging woodpeckers. Grubb (1978) recorded for downy woodpeckers an average of 11.9 m traveled/min while foraging. Given the height of the peanut feeder, this is equivalent to woodpeckers moving up and down the feeder 26 times in the 100 s observation period. Perching woodpeckers in this study only had minor perch adjustments while in the chamber as detected by our pressure transducers. Thus, any substitution recorded in this study can be considered a conservative estimate of substitution.

The heat production of perching and foraging woodpeckers did not differ significantly in summer birds. Thus, the heat produced during foraging activity, or activity thermogenesis, substitutes for thermoregulatory requirements and indicates that

foraging behavior in downy woodpeckers incurs no significant additional energetic costs across a fairly broad range of temperatures. Heat production for winter woodpeckers during foraging is significantly less than while perching. How foraging birds can possibly have lower metabolism than perching birds is unclear. One possible explanation is that perching woodpeckers became stressed when they were deprived of food during their normal period of foraging activity. Laboratory foraging mountain chickadees also have significantly lower heat production than perching chickadees in winter (Cooper, 2000). The stress of not being allowed to forage may be higher in winter relative to summer given the shorter length of day in winter. Summer woodpeckers foraging in their natural environment would have approximately 15 h available to forage while winter birds would have only approximately 9 h of daylight for foraging. Thus, one hour inside a metabolic chamber with no food available makes up much greater percentage of potential foraging time in winter compared to summer.

Substitution of heat from foraging activity for thermoregulatory heat allows for more energy to be used for other activities such as mating, growth, care for young, and competition for resources. Winter downy woodpeckers did not demonstrate substitution during foraging. In winter, non-foraging was more energetically costly than foraging. Similar results have been found in winter mountain chickadees (Cooper, 2000). Increased stress from not being able to forage during an active phase of the day may be responsible for the increase in energetic cost while the birds were not foraging. There was no direct measurement of activity during the non-foraging trials. However, there were records of disturbance in the ventilation records from activity during these periods.

These activity disturbances in the ventilation records were much more frequent in winter non-foraging trials than in summer non-foraging trials. This indicates that the winter birds were highly active even during non-foraging trials. This increase in activity may be the result of not being able to forage during a phase of the day when winter birds would normally be foraging. At decreased air temperatures and increased wind speeds, Ohio Downy woodpeckers have been found to increase the number of stops in their foraging activity or to move more slowly (Grubb, 1978). This data suggests that birds exposed to colder temperatures in winter would be less active than summer birds exposed to warmer temperatures. When comparing our birds to the Ohio birds, Ohio woodpeckers spent more time stationary in seconds per minute than Wisconsin woodpeckers ($t = -12.196$, $P < 0.001$). Ohio woodpeckers also average more stops in activity per minute than Wisconsin woodpeckers ($t = -16.929$, $P < 0.001$). Our birds were exposed to similar temperatures and air flow rates during both winter and summer non-foraging and foraging trials. The increase in activity in our winter birds is likely to be a result of increased stress to forage in winter birds than in summer birds, rather than a result of temperature differences.

Thermal conductance did not vary significantly between perching and foraging woodpeckers in summer or in winter. This shows that insulation is similar for perching and foraging woodpeckers, despite potential differences in posture between perching and foraging birds. Postural differences during foraging can lead to plumage disruption in some species of birds (Cooper and Sonsthagen, 2007). Conductance was significantly higher in foraging winter birds than in non-foraging winter birds. The lower conductance

in non-foraging winter birds could explain some of the change in metabolic rate during non-foraging trials. It is possible the increased movement during winter non-foraging tests was able to disrupt plumage and in turn decrease thermal conductance. Stress may also be responsible for some of the variation in conductance between non-foraging and foraging winter birds.

**Metabolic Response to Temperature.**

In this study, summer birds showed no significant circadian difference in metabolic rate. While this finding is contrary to some studies, recent studies have shown no strong correlation between time of measurement (being during active or inactive phases of the day) and variation in metabolic rates (McNab, 2009). There was an increase in metabolic rate in daytime winter birds when compared to nighttime winter birds. This increase in winter daytime metabolic rate is as expected with increased pressure to forage during the winter daytime.

There was no significant difference in thermal conductance between daytime and nighttime summer birds. There was also no significant difference in conductance between daytime and nighttime winter birds. These findings suggest the circadian differences in metabolic rates between winter daytime and nighttime birds are not due to differences in conductance or plumage disturbances.

Table 4. *Mean (±SD) values for Ta, metabolic heat, and conductance during winter and summer for non-foraging and foraging trials.*

| Season/ Activity | *n* | Ta ($^{o}$C) | Metabolic heat (watts) | Conductance (W $^{o}C^{-1}$) |
|---|---|---|---|---|
| Winter: | | | | |
| Non-foraging | 12 | -1.31 ± 6.95 | 1.25 ± 0.27 | 0.03 ± 0.01 [a] |
| Foraging | 12 | 1.48 ± 6.51 | 1.48 ± 0.37 | 0.04 ± 0.01 [b] |
| Summer: | | | | |
| Non-foraging | 13 | 8.12 ± 9.23 | 0.88 ± 0.30 | 0.03 ± 0.01 |
| Foraging | 14 | 10.21 ± 9.14 | 0.98 ± 0.32 | 0.04 ± 0.03 |

[a] Indicates significant differences between trial conditions within a season ($P < 0.05$)

[b] Indicates significant differences between seasons within a trial condition ($P < 0.05$)

Table 5. *Mean (±SD) values for Ta, metabolic heat, and conductance during winter and summer for daytime and nighttime trials.*

| Season/ Time | *n* | Ta ($^{o}$C) | Metabolic heat (watts) | Conductance (W $^{o}C^{-1}$) |
|---|---|---|---|---|
| Winter: | | | | |
| Daytime | 12 | -1.31 ± 6.95 | 1.25 ± 0.27 | 0.032 ± 0.01 |
| Nighttime | 10 | 0.05 ± 7.27 | 0.99 ± 0.31 | 0.027 ± 0.01 |
| Summer: | | | | |
| Daytime | 12 | 6.55 ± 7.63 | 0.89 ± 0.31 | 0.029 ± 0.01 |
| Nighttime | 9 | 3.94 ± 8.55 | 0.73 ± 0.39 | 0.029 ± 0.01 |

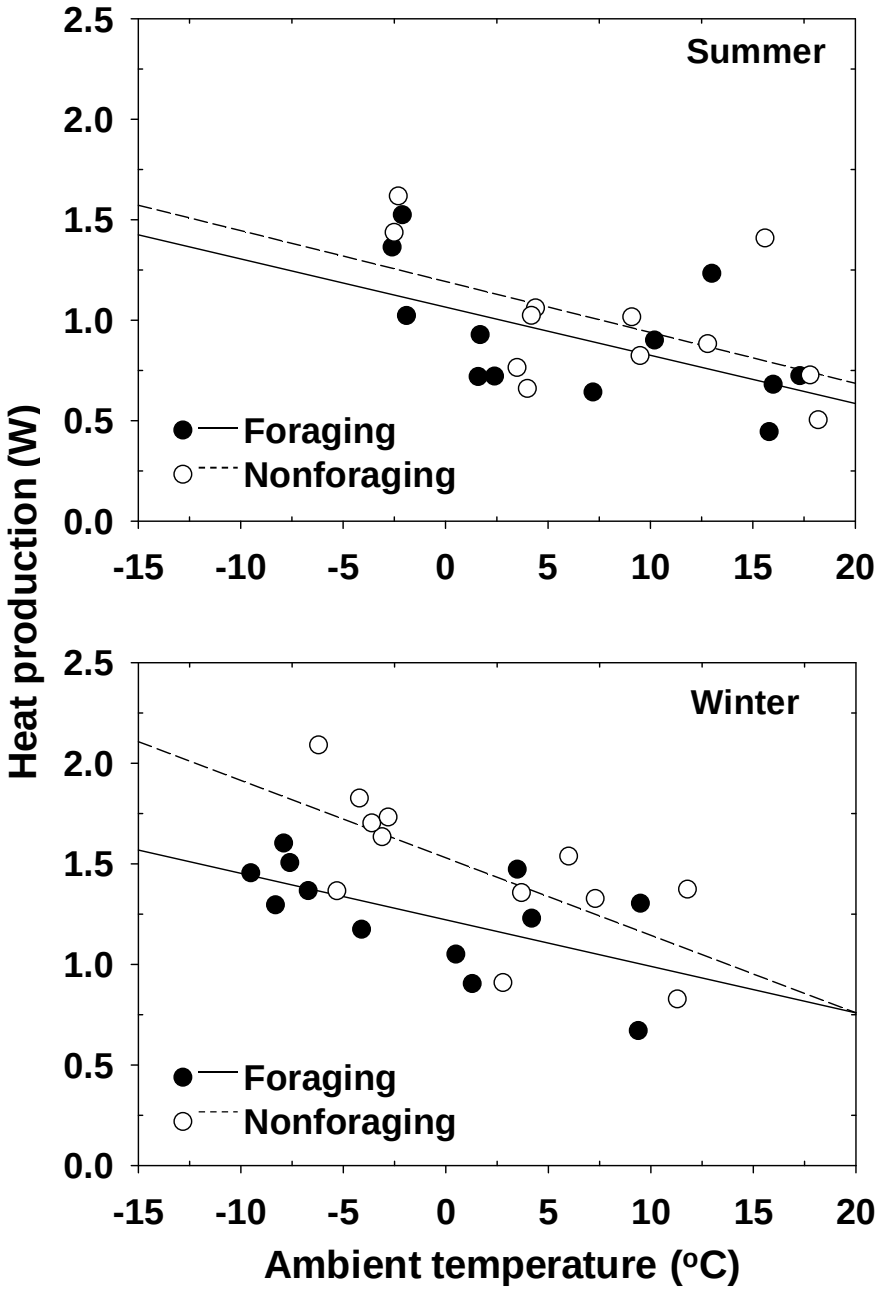


*Figure 5*. The relationship between metabolic heat production and activity level in Summer and Winter in Wisconsin downy woodpeckers.

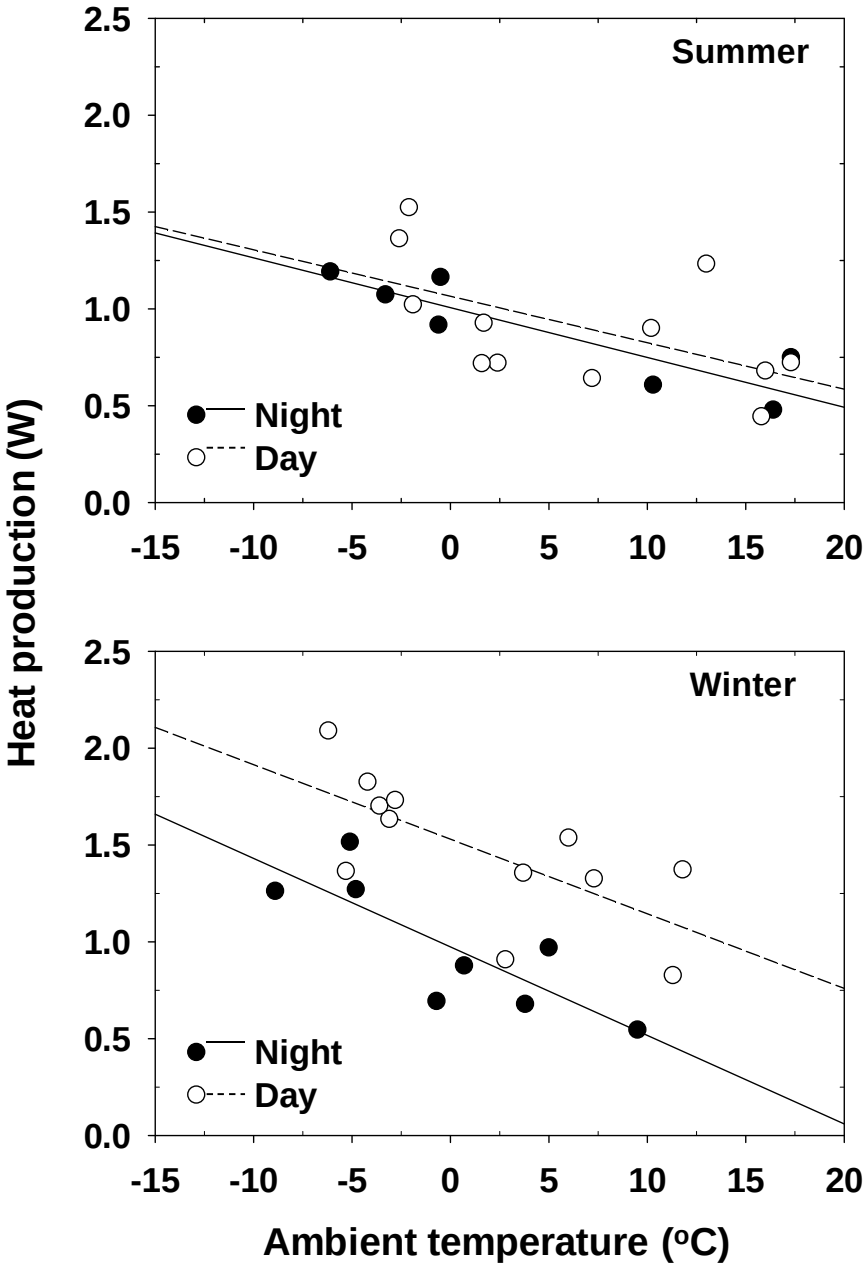


*Figure 6.* The relationship between metabolic heat production and circadian phase in Summer and Winter Wisconsin downy woodpeckers.

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