AN ABSTRACT OF THE THESIS OF

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Winters in north-temperate climates impose high thermogenic demands upon small birds which are met by seasonal acclimatization. This thesis investigates the extent and mechanisms of seasonal acclimatization in dark-eyed juncos (Junco hyemalis) inhabiting western Oregon.

Although insulation is significantly increased in winter, acclimatization is primarily metabolic. Increased metabolism is required to offset heat loss even at moderate temperatures at both seasons. At average daily temperatures, thermoregulation in January requires 1.8 times the energy expended for thermoregulation in July and August. Standard metabolic rate (SMR) is significantly elevated in winter (3.45 $mLO_2[gXhr]^{-1}$) compared to summer (3.16 $mLO_2[gXhr]^{-1}$), suggesting elevated metabolic capacities. Furthermore, winter birds, but not summer birds, were capable of modifying thermal conductance at low temperatures, allowing additional heat conservation.

Helium/oxygen cold stress demonstrated that cold tolerance was improved in winter juncos relative to summer birds. Maximal thermogenic capacity also increased significantly in winter birds (7.39 mLO_2/min to 5.78 mlO_2/min). These values exceed SMR by 7.2 times in winter and 6.6 times in summer.

Oxygen dissociation curves were generated on whole blood by saponin/potassium ferricyanide dissociation and were similar to those for other passerines. The curves did not vary significantly between summer and winter (P_{50} = 54 torr). Hematocrit and oxygen carrying capacity were significantly increased in winter. Apparently increased oxygen demands in winter juncos are met, in part, by increased oxygen carrying capacity, but not by decreased oxygen affinity.

Plasma and tissue metabolites were assayed after differing levels of cold stress to analyze seasonal variation in carbohydrate and lipid metabolism. Pectoralis muscle glycogen was significantly greater after severe cold stress in winter, although thermoneutral levels did not vary significantly. Furthermore, plasma levels of free fatty acids were significantly increased and plasma glucose was significantly decreased under severe cold. These data suggest reduced reliance upon carbohydrate for shivering thermogenesis in winter juncos and subsequent preservation of muscle glycogen stores. This may contribute to enhanced shivering endurance and cold tolerance in winter birds.

SEASONAL THERMOREGULATION IN THE DARK-EYED JUNCO

(Passeriformes: *Junco hyemalis*)

by

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CHAPTER 1

GENERAL INTRODUCTION

Thermoregulation is an energetically expensive process for endothermic vertebrates and, over a 24 hr period, may require as much as 30 times the energy expenditure by equal-sized ectotherms (Bennett and Nagy, 1977). Thermoregulatory costs for endotherms are influenced by a variety of factors. Important among these are body temperature (T_b), external environmental conditions, insulatory capacity, body size, and basal metabolic rate. Birds generally maintain the highest T_b of any vertebrate (about 40°C-42°C [Calder and King, 1974]) which often results in large gradients for heat loss to the environment and high levels of maintenance metabolism.

Thermoregulatory costs are greatest in small birds which necessarily have a relatively great surface area to volume ratio. In addition, capacity for insulation is reduced in all small birds because they cannot maintain the thick plumage typical of many large birds (Schmidt-Nielson, 1984). Insulation is also limited in birds generally due to flight constraints. Additionally, avian subcutaneous fat is stored primarily in furcular and abdominal deposits and contributes little to increasing insulatory capacity. Accordingly, small birds have only limited insulative capacity and restricted ability to

modify heat flux in response to changing environmental conditions (Dawson et al. 1983a).

In north-temperate climates, resources (food, cover, etc.) are abundant and environmental conditions are generally favorable during spring and summer but are less amenable in winter (Perrins and Birkhead, 1983). Temperate birds exhibit two very different responses to heterogenous resources and climates, migration and permanent residency. Long distance migration is often energetically demanding, but allows avoidance of season-long hazards associated with regionally declining resources and ambient temperatures. Shorter distance migrations to more equable temperate environs include local and/or altitudinal migrations, and irruptive movements. Even so, many short-distance migrants, as well as permanent residents in temperate climates, must endure relatively harsh winter conditions.

Thermoregulatory problems confronting small temperate-wintering birds are exacerbated by the forced fast of long winter nights when ambient temperatures are coldest, especially when diurnal foraging has been limited due to inclement weather (e.g., snow or ice cover). Thus, small birds that overwinter in north temperate environments must frequently deal with high thermoregulatory energy expenditures for prolonged periods coupled with relatively scarce food availability.

Utilization of favorable microclimates offers limited relief from energetic costs of overwintering. Passerines

generally roost overnight in dense vegetation, although tree cavities and subnivean spaces may also be utilized (Webb and Rogers, 1988). These roost sites may decrease energetic costs by providing shelter from wind and precipitation, reducing radiative heat loss, and/or elevating local air temperatures. Although, reduced convective and radiative heat loss account for some thermal benefit, variation in microclimate temperature appears unimportant in most cases (Buttemer, 1985; Walsberg, 1986; Webb and Rogers, 1988; but see Yom-Tov et al. 1977).

Thermogenesis in birds appears to be primarily or exclusively dependent on shivering (West, 1965; Dawson et al 1983a). Birds apparently do not possess brown fat (Johnston, 1971; Olson et al. 1988; Saarela et al. 1989), or any other well-developed capacities for non-shivering thermogenesis similar to those of mammals (Dawson et al. 1983a). Since small birds have limited insulatory capacity, they must increase metabolic rate above basal levels to support shivering thermogenesis, even at relatively moderate temperatures, for maintenance of normothermia (Dawson and Carey, 1976). Enhanced thermogenesis therefore appears necessary to meet elevated winter thermoregulatory demands. As a result, small passerines undergo a process of seasonal metabolic acclimatization which facilitates enhanced shivering thermogenesis and maintenance of thermoregulatory homeostasis under adverse winter conditions (Hart, 1962;

Barnett, 1970; Pohl and West, 1973; Dawson and Carey, 1976; Dawson et al. 1983b).

The mechanistic basis underlying winter enhancement of shivering thermogenesis is not well understood. It could involve seasonal changes in the mass-specific aerobic capacity of the muscles involved in shivering and/or seasonal changes in the capacities for prolonged endurance in these muscles, presumably through changes in substrate metabolism that might facilitate fatigue-avoidance. Such changes might be associated with preservation of muscle fuel reserves, decreased formation or increased clearance of waste products, or increased tolerance of waste products.

The flight muscles (pectoralis and supracoracoideus) are the largest muscles in the avian body, comprising >15% of body mass, and predominate in shivering thermogenesis (West, 1965; Calder and King, 1974; Carey et al. 1978; George, 1984). There are three basic histochemical types of avian muscle: 1) slow oxidative (tonic muscle with primarily oxidative enzymes), 2) fast glycolytic (few mitochondria, predominantly glycolytic enzymes), and 3) fast oxidative-glycolytic (many mitochondria, both glycolytic and oxidative enzymes) (Phillips et al. 1985). The flight muscles of small passerines, especially the pectoralis, are composed primarily of fast oxidative-glycolytic fibers (Carey et al. 1978; Marsh, 1984). Studies of seasonal aerobic capacity and endurance in

passerine muscle have therefore focused on the pectoralis and fast oxidative-glycolytic fibers. However, available evidence suggests inter- and intraseasonal stability of mass-specific aerobic capacity in pectoralis muscle of migratory and temperate-wintering passerines (Carey et al. 1978; Marsh, 1981; Marsh and Dawson, 1982; Yacoe and Dawson, 1983).

Seasonal changes in fat and carbohydrate metabolism have been documented for some passerines which exhibit seasonal variation in cold tolerance (Marsh and Dawson, 1982), but not in others (Marsh et al. 1984). Furthermore, the significance of the seasonal variation in substrate metabolism is not clear (Yacoe and Dawson, 1983).

Consequently, this thesis was undertaken to investigate seasonal thermoregulatory adjustments in the Dark-eyed Junco with a view toward better definition of how small birds adjust for season-long cold. The subject of this study is the Dark-eyed Junco (Emberizidae: Junco hyemalis), a relatively small passerine (mean body mass = 18.6g) characteristic of boreal climates in North America. The junco's range extends into some areas of relatively severe winters (AOU Checklist 1983) and it is present year round near Corvallis, Oregon. The breeding range of the race nesting around Corvallis extends from southwestern British Columbia south to about 43^{o} N latitude, chiefly west of the Cascade Divide. It winters at low elevations throughout the breeding range, south into California, and

sparsely southeast to northern Idaho, Utah, Colorado, southern Arizona and New Mexico, into Mexico (Chihuahua) and western Texas (AOU Checklist 1957,1983). Wintering junco populations near Corvallis are supplemented by more northerly breeding birds (Gabrielson and Jewett, 1940; AOU Checklist 1957,1983), but Bird Banding Laboratory returns indicate that there are substantial numbers of juncos that winter on or near breeding grounds in western Oregon. Other life history features that make the junco an excellent study organism include their abundance and ready response to seed feeders, predisposing them to easy capture.

Winter climate in western Oregon is dominated by marine weather systems and is relatively mild. Nevertheless, there is marked seasonal variation in climate (OSU Climatic Research Institute). Average daily maximum, minimum, and mean temperatures are given in Figure 1. The mean daily low temperature for the winter period (Dec.-Feb.) is 1.2^{o}C. The mean daily low temperature in summer (June-Sept.) is 9.6^{o}C. The seasonal difference in extreme minimum temperatures is more pronounced. Mean extreme minimum temperature is -5.4^{o}C in winter and 4.7^{o}C in summer, but the extreme minimum temperature for the winter period is -26^{o}C, whereas in summer it is only 0^{o}C. Due to wind and humidity, true environmental minimum temperatures are probably somewhat less than cited. Precipitation is far greater in winter than in summer. Mean precipitation

for the winter period is 44.4 cm, but is only 8.4 cm in summer. Monthly averages for days with measureable precipitation are 21 in Dec., 20 in Jan., and 18 in Feb. but only 7 in June, 2 in July, 4 in Aug., and 7 in Sept. Mean annual snowfall from Dec.-Feb. is 10.8 cm.

Seasonal extreme minimum temperatures or mean low temperatures appear to be the ultimate, or evolutionarily-significant, factors controlling winter fattening in American Goldfinches (Dawson and Marsh, 1986). It is likely that they are also important in maintenance of cold tolerance capabilities in passerine birds. Natural selection presumably favors individuals with the capacity to withstand the most extreme conditions encountered during a given season. In this regard, seasonal differences in effective extreme minimum temperatures would appear most important to establishment of cold tolerance capacities. Therefore the climate of western Oregon would seem well-suited for studies of seasonal acclimatization in passerine birds.

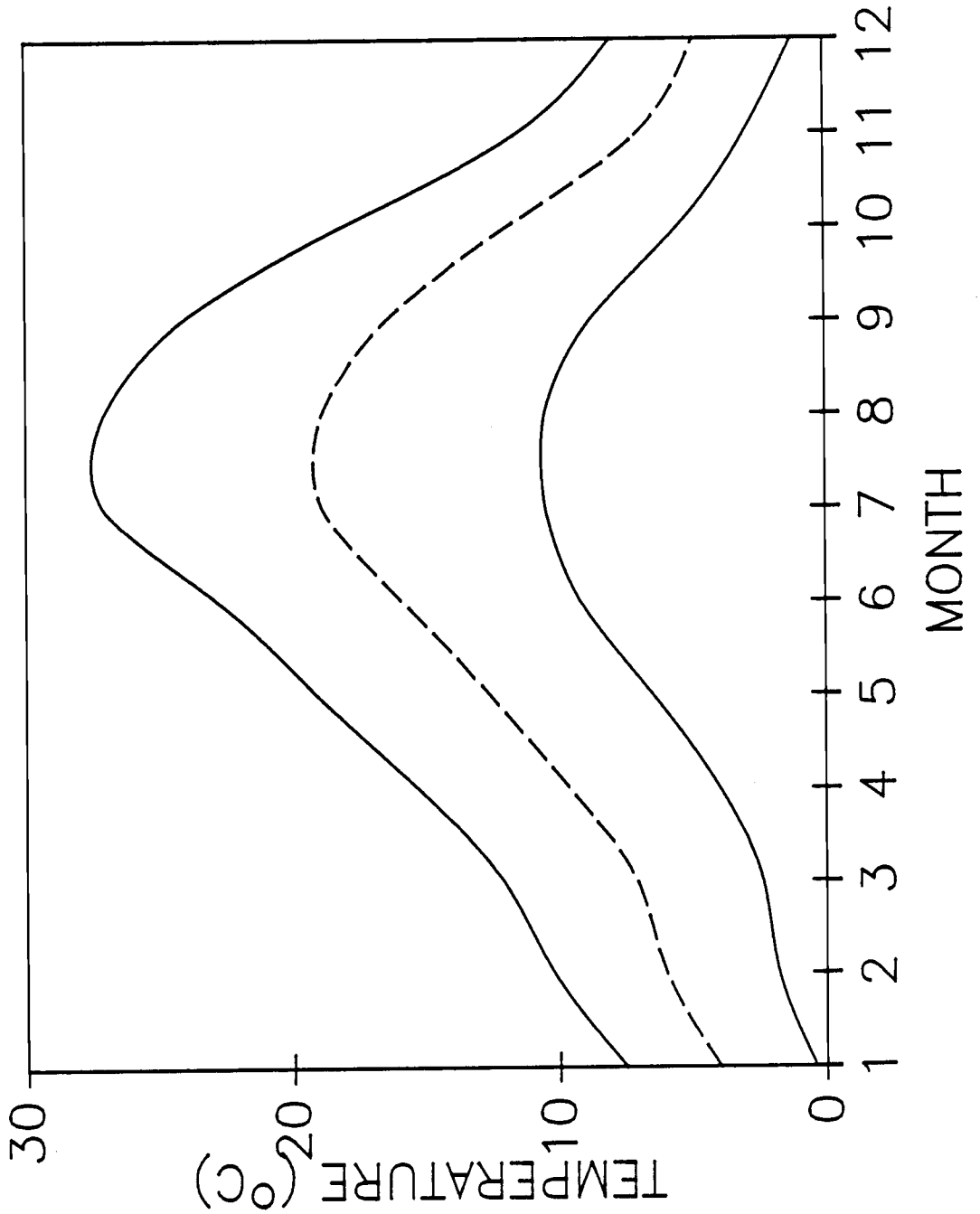
In summary, temperate-wintering results in substantial cold stresses for small passerine birds. Physiological response is primarily through metabolic, rather than insulatory, alterations. However, mechanisms underlying this physiological response are poorly understood.

This thesis involves two main goals: 1) establishment of background information on physiological acclimatization in the junco, including the extent of acclimatization (e.g.

cold tolerance) and metabolic and insulatory contributions to acclimatization, and 2) elucidation of physiological mechanisms underlying metabolic acclimatization. The first two manuscripts (Chapters II and III) deal with the first goal. Chapter IV and V examine mechanistic bases underlying acclimatization. Specifically, Chapter II deals with seasonal measurements of body mass and composition, plumage mass, body temperature in relation to ambient temperature and circadian rhythms, and oxygen consumption in relation to ambient temperature. Chapter III examines seasonal variation in cold tolerance and peak, cold-induced oxygen consumption rates. Seasonal variation in blood physiology relative to elevated oxygen demands in winter is the subject of Chapter IV. Chapter V investigates regulation of substrate metabolism under cold stress in plasma, muscle, and liver of seasonally-acclimatized juncos.

Figure 1: Average maximum, minimum, and mean temperatures throughout the year at Corvallis, Oregon (Oregon State University Climatic Research Institute).

Figure 1



CHAPTER 2

SEASONAL ADJUSTMENTS IN METABOLISM AND INSULATION IN THE DARK-EYED JUNCO

INTRODUCTION

Small, cold temperate-wintering passerine birds undergo seasonal acclimatization which facilitates maintenance of thermoregulatory homeostasis under winter conditions. Insulatory adjustments assist in winter improvement of cold tolerance in some passerines, but it appears that acclimatization is primarily metabolic in those birds inhabiting regions with relatively harsh winters (Pohl and West, 1973; Dawson and Carey, 1976; Dawson et al. 1983a). The primary feature of metabolic acclimatization in these birds is enhanced thermogenic capacity, which allows prolonged maintenance of greatly elevated metabolism to support shivering thermogenesis (Dawson and Carey, 1976; Dawson and Smith, 1986).

Heretofore, seasonal patterns of cold tolerance in passerines have been investigated primarily in species subject to extreme winter cold [e.g. evening grosbeaks, european starlings, and house sparrows from Ottowa, Canada (Hart, 1962), gray jays from Alaska (Veghte, 1964), house sparrows from Illinois (Barnett, 1970), bramblings from Scandinavia (Pohl, 1971), common redpolls from Alaska (West, 1972a; Pohl and West, 1973), American goldfinches from Michigan (Dawson and Carey, 1976; Dawson and Smith, 1986), house finches from Colorado (Dawson et al. 1983a)].

However, the small size and high energy requirements of many passerines might require seasonal cold adjustments in species inhabiting less stressful temperate regions (Dawson et al. 1983a). The present study was undertaken to determine patterns of metabolic and insulatory cold-acclimatization in dark-eyed juncos (Junco hyemalis) which inhabit western Oregon, a region of relatively mild winter climate. Parameters investigated here include body mass and composition, body temperature (T_b), standard metabolic rate (SMR), and the relation of metabolic rate ($\dot{V}O_2$) to ambient temperature (T_a).

Body mass generally increases in passerines in winter. Increased fat stores account for the majority of mass gained, although winter increases in nonfat body components may also contribute (Helms et al. 1967, Carey et al. 1978, Dawson et al. 1983b). Increased fat stores act as fuel reserves and presumably enhance survival under cold stress.

Reducing the difference between T_b and T_a would diminish heat loss. Thus, a reduced set point for thermoregulation in winter might affect energy savings. In fact, thermoregulatory set point has been shown to vary seasonally in some birds: mute swan (Bech, 1980) and black grouse (Rintimaki et al. 1983), but not in others: willow ptarmigan (West, 1972a) and American goldfinch (Dawson and Carey, 1976). In addition, T_b in passerines usually varies diurnally by only a few degrees centigrade at most, but nocturnal hypothermia under cold stress, or cold stress

coupled with food deprivation, has been documented for several small passerines (Chaplin, 1976; Reinertsen, 1986; Reinertsen and Haftorn, 1986). Furthermore, circadian fluctuation in T_b has been shown to be greater in winter in non-torpid white-crowned sparrows (Southwick, 1980).

Elevation of SMR in winter might be indicative of alterations in metabolic physiology associated with augmented aerobic thermogenic powers. SMR in temperate-wintering passerines is usually seasonally stable with acclimatization state, although it appears more labile with acclimation (Dawson et al. 1985).

Metabolic response to T_a is described by the width and lower boundary (lower critical temperature = LCT) of the thermoneutral zone and the rate of increase in $\dot{V}O_2$ with declining T_a below thermoneutrality. Improved insulation in winter might result in reduced LCT and slopes of temperature on $\dot{V}O_2$, thus decreasing energy expenditure for thermoregulation. Seasonal patterns in LCT and metabolism/T_a slopes vary among passerines. LCT is seasonally variable in some species (Dawson, 1958; Veghte, 1964; Pohl and West, 1973), but not in others (Pohl, 1971; Dawson and Carey, 1976). Metabolism/T_a slopes also vary seasonally in some species (Hissa and Palokangas, 1970; Pohl, 1971; Dawson and Carey, 1976), but not in others (Pohl and West, 1973). Clearly, adequate assessment of the metabolic response to temperature requires measurement of SMR, LCT, and metabolism/temperature slopes.

METHODS AND MATERIALS

BIRDS

Dark-eyed juncos were captured before 1000 hr, near Corvallis, Benton Co., Oregon by mist-nets or live-traps. Birds captured from 15 May-Sept. were designated "summer birds"; those captured Dec.-Feb. were designated "winter birds." Body mass to the nearest 0.1g was determined immediately upon capture with a Pesola spring balance (0-50g). Visible fat depots were also scored immediately upon capture using a scale of 0-5 (Helms and Drury, 1960).

BODY CONSTITUENTS

For determination of body constituents, juncos were sacrificed by cervical dislocation immediately upon return from the field and stored frozen at -20^{o}C for later measurement. Plumage mass was measured by plucking and drying contour feathers to constant mass in an open-ended vial at 80^{o}C. Carcass, remiges, and retrices were homogenized, lyophilized, and weighed. Neutral lipid was extracted from the lyophilized carcass by Soxhlet extraction for four hours in diethyl ether (OSU Agricultural Chemistry). Extractable neutral lipid was considered as fat content. Lean dry mass was calculated as lyophilized carcass weight minus fat content.

BODY TEMPERATURE

Determination of daily and seasonal T_b was accomplished by housing juncos individually, or in a few cases in pairs, in 25cmX30cmX38cm cages, at varying

temperatures in a constant temperature room capable of maintaining temperature within $\pm 2^{o}C$ of the desired value. These temperatures were $30^{o}C$, $15^{o}C$, $5^{o}C$, and $-10^{o}C$ (the last value during winter only, as summer birds became hypothermic at this temperature). Food and water were provided ad libitum. Every four hours, from 1200 to 2400 hr, birds were quickly removed from the cages and T_b was measured cloacally with a copper-constantan thermocouple and Cole-Parmer thermocouple thermometer (Model 8500-40), previously calibrated to $\pm 0.1^{o}C$ with a thermometer traceable to the U.S. Bureau of Standards. Data from birds that showed of signs of undue stress (e.g. panting) or that were difficult to capture quickly were omitted.

METABOLIC RATE AND AMBIENT TEMPERATURE

Juncos were weighed, placed in a metabolic chamber (3.8 L) at $30^{o}C$, and fasted for at least five hours prior to metabolic tests to ensure postabsorptive conditions. Tests were conducted from 2000 to 0300 hr in winter and from 2100 to 0300 hr in summer. Oxygen consumption ($\dot{V}O_2$) was measured by open-circuit respirometry with a Beckman Model E2 oxygen analyzer according to Swanson (1990). $\dot{V}O_2$ was calculated as mean steady state $\dot{V}O_2$ for the test period (DePocas and Hart, 1957) from values recorded every 60 seconds. Flow rates of dry, CO_2-free air through the metabolic chamber were maintained at 230-290 ml/min with a Cole-Parmer precision rotameter (Model FM082-03ST) calibrated to $\pm 1\%$ accuracy (Swanson, 1990). These rates

yielded changes in O_2 content between influx and efflux air of 0.3 to 0.5%, and maintained O_2 content of efflux air above 20.3%. Temperature within the metabolic chamber was controlled by immersion of the chamber into an anti-freeze bath capable of regulating temperature within $\pm 0.5^{o}C$. Chamber temperature was monitored continuously with a thermocouple thermometer.

Individual birds were exposed to a series of decreasing temperatures beginning with $30 \pm 1.2^{o}C$. After equilibration, $\dot{V}O_2$ at $30^{o}C$ was measured for one hour. Chamber temperature was then decreased until the desired test temperature was attained. Approximately 15 minutes equilibration were allowed at each new test temperature before $\dot{V}O_2$ measurements were initiated. $\dot{V}O_2$ at each test temperature below $30^{o}C$ was measured for 45 minutes. This procedure was continued until $\dot{V}O_2$ measurement at the lowest T_a desired was completed, at which time the bird was removed from the chamber and weighed. $\dot{V}O_2$ of individual birds was measured at a maximum of four test temperatures. Total time for metabolic tests was approximately 5.5 hrs.

STATISTICS

Data are reported as means $\pm$ SE, unless otherwise indicated. Means were compared by student's T test or by Mann-Whitney U test depending on the homogeneity of sample variance. Regression lines were fit by the method of least squares. Comparison of slopes and intercepts of regression lines was by analysis of covariance.

RESULTS

BODY MASS AND COMPOSITION

Dark-eyed juncos were significantly heavier in winter than in summer (Table 1). Mean winter body weight exceeded the summer value by 9.0%. This increase was largely due to increased fat stores, as total body lipid was increased in winter birds, although not significantly (P=.07) due to large sample variance (Table 1). Visible fat depots in furcular and abdominal regions were significantly increased in winter (Table 1). Total body lipid and visible fat scores were measured on birds caught and sacrificed in the morning, so fat stores were presumably at or near a daily minimum. Fat stores prior to nightly roosting probably show even greater seasonal differences. In contrast to stored fat, lean dry mass does not appear to vary seasonally (Table 1).

Plumage mass increased significantly in winter (Table 1), exceeding summer values by 31.7%. Some birds in late summer were undergoing varying degrees of molt, so summer plumage mass values may be correspondingly low. Nevertheless, birds must still thermoregulate when insulation is diminished by molt, so the seasonal difference in insulative capacity is noteworthy.

BODY TEMPERATURE

Cloacal temperature (T_b) showed a weak dependence on T_a at both seasons during the day and during winter nights. T_b was independent of T_a, over the range tested, for summer

nights (Figure 2). The only significant seasonal differences in T_b occurred at 5°C and 15°C during winter days, where mean values were significantly elevated over summer values, and at 30°C during winter nights, mean values were again significantly increased over summer values (Table 2). The mean fluctuation in T_b between resting and active daily periods was 2.3°C in summer and 2.5°C in winter. These values were not significantly different.

METABOLIC RATE AND AMBIENT TEMPERATURE

Standard metabolic rates (SMR) were $3.16 \pm .07$ $ml O_2 (gXhr)^{-1}$ ($0.87 \pm .02$ $ml O_2/min$, n=11) during summer and $3.45 \pm .13$ $ml O_2 (gXhr)^{-1}$ ($1.02 \pm .04$ $ml O_2/min$, n=8) during winter. These values were significantly different on both a total metabolism basis ($P<.01$) and a mass-specific basis ($P=.05$) [Note: SMR data from Swanson (1990) were reanalyzed to reduce rounding errors. Winter SMR was slightly reduced from previously reported values and P value for mass-specific SMR decreased from .08 to .05]. Winter SMR represents a 9.2% increase over summer SMR on a mass-specific basis, and an 17.2% increase over summer SMR on a total metabolism basis. Mass-specific SMRs were 5.0% and 17.3% greater than predicted values for passerines of equal mass, in summer and winter, respectively (Calder and King, 1974). On a total metabolism basis, summer SMR was lower than predicted by 2.3%, while winter SMR exceeded predicted by 7.4%.

The lower critical temperature (lower boundary of the thermoneutral zone) was 21.8°C in summer and 25.8°C in winter. The relations of mass-specific $\dot{V}O_2$ to temperature below thermoneutrality were best described by the equations given below (Figure 3).

Summer: $\dot{V}O_2(gXhr)^{-1}=7.32 - 0.19\ T_a$ ($n=23$, $R^2=0.93$) (1)

Winter: $\dot{V}O_2(gXhr)^{-1}=6.80 - 0.13\ T_a$ ($n=21$, $R^2=0.80$) (2)

The intercepts of the two regression lines were not significantly different. However, the slope of the summer line was significantly steeper than the winter slope ($P<.001$). Only the summer equation conformed closely to the "Newtonian" cooling model, as the temperature at zero metabolism extrapolated to 38.3°C. The winter equation extrapolated to zero metabolism at 52.3°C, which is more than 10°C higher than the nocturnal T_b.

Thermal conductance [$mlO_2(gXhrX^oC)^{-1}$] is equivalent to the slope of the line relating $\dot{V}O_2$ to ambient temperature only if the curve extrapolates to body temperature at zero metabolism. Consequently, only conductance for summer juncos was relatively constant at 0.19 $mlO_2(gXhrX^oC)^{-1}$ below thermoneutrality. This value exceeds that allometrically predicted for passerines (Aschoff, 1981) by 20.2%. If conductance is similarly calculated for winter birds, it decreases from 0.194 $mlO_2(gXhrX^oC)^{-1}$ at 15°C to 0.162 $mlO_2(gXhrX^oC)^{-1}$ at -10°C.

DISCUSSION

Dark-eyed juncos studied here showed a winter increment in body mass, due primarily to increased stored fat, rather than lean dry mass which did not vary seasonally. This pattern of seasonal fat storage is similar to that for most other temperate-wintering passerines (see King, 1972; Dawson et al. 1983b) although winter increments in non-fat body components usually accompany increased fat stores (Helms et al. 1967; Barnett, 1970; Carey et al. 1978; Dawson et al. 1983a). The seasonal stability of lean dry mass in this study may be an artifact of small sample size, as wet masses of pectoralis muscle and liver are greater in winter than in summer for these juncos (Swanson, unpublished data).

Winter fattening in American goldfinches varied little in response to daily temperature fluctuations (Dawson and Marsh, 1986). Instead, fat levels were maintained at a constant high level throughout the winter season, indicating temperature operates as an ultimate but not proximate factor regulating winter fattening. If this pattern also occurs in the junco, fat levels obtained during winter were presumably maximized prior to roosting the night before capture.

Winter juncos, captured in the morning soon after leaving their nightly roost, maintained a mean of 1.16g neutral lipid. If this were completely available for oxidation, at 9.4 kcal/g (Schmidt-Nielsen, 1983), it would

yield 10.9 kcal. This would support resting metabolism at 4°C (the mean daily temperature in January for Corvallis, OSU Climatic Research Institute) for 19.1 hr. However, not all neutral lipid is available for oxidation. It appears that minimal fat content in birds amounts to 2-4% of body mass (Barnett, 1970; Griminger, 1986). Given 2% initial body mass as non-metabolizable fat, this decreases fat-supported metabolism to 12.7 hr. These calculations suggest that juncos could endure fasting through the winter night and the following day, but not through a second night. Consequently, daily foraging may be required for winter survival in these birds. Coincidentally, foraging is seldom prevented by ice or snow storms in the relatively mild winter climate of western Oregon.

In contrast, winter-acclimatized Dark-eyed (slate-colored) juncos from Indiana have an estimated fasting capacity of one day and two nights (Stuebe and Ketterson, 1982), and winter-acclimatized juncos from Ohio exhibited a fasting endurance of 63.5 hr at 4°C (Ketterson and Nolan, 1978). This suggests that fasting endurance in winter-acclimatized juncos may be adjusted to the relative severity of their resident winter climate.

Little seasonal variation in body temperature was apparent, and what variation was present indicated a higher T_b for winter birds. Apparently, winter-acclimatization in juncos does not involve either a reduced set point for thermoregulation or an increased circadian variation in T_b.

No evidence of torpor or regulated hypothermia was noted under the conditions of this study. However, these results do not rule out hypothermia as a result of food deprivation coupled with cold, as demonstrated for some passerines (Reinertsen and Haftorn, 1986). At both seasons, cloacal temperature, T_b variation with T_a, and circadian variation in T_b were similar to those reported for other passerines (Dawson and Hudson, 1970; Dawson and Carey, 1976; Dawson et al. 1985).

Standard metabolic rate was elevated in winter-acclimatized birds. SMR for summer birds is very close to allometrically predicted values, whereas winter SMR exceeded predicted values by 7.4% on a per bird basis and 17.3% on a mass-specific basis. This is contrary to the generalization that SMR is seasonally invariant in passerines (Dawson et al. 1985). The adaptive significance of seasonal variation in SMR in juncos is unclear. Winter increment of SMR is coincident with elevation of thermogenic capacity and cold endurance (Swanson, 1990). Increased SMR may be indicative of metabolic alterations providing augmented thermogenic capacity, although some other passerines exhibiting seasonal variation in thermogenic capacity maintain seasonally static SMR (Dawson et al. 1985; Dawson and Smith, 1986). However, similar results have been reported for captive common redpolls (Pohl and West, 1973) and winter increases in SMR have been reported for some nonpasserines (Bech, 1980; Rintimaki et

al. 1983).

The elevated ratio of whole animal SMR to mass-specific SMR in winter probably resulted from the higher content of metabolically inert fat. Augmented fat stores contribute to increased mass but proportionately less so to metabolism. Thus, total metabolism may be more appropriate for seasonal comparisons.

Contour plumage dry mass was used as an index of insulation and was 31.7% greater in winter juncos. This increase was lower than that reported for several other small, cold temperate-wintering passerines, which range from 32.7%-72.7% (Barnett, 1970; Dawson and Carey, 1976; Dawson et al. 1983a). Perhaps the relatively small increase in plumage mass in winter juncos is related to wintering in the relatively mild climate of western Oregon.

Lower critical temperature in juncos varied little with acclimatization state and was similar to that recorded for other small passerines (Hart, 1962; King, 1964; Dawson and Carey, 1976; Weathers et al. 1980; Dawson et al. 1985). However, lower critical temperature in winter may be artificially high due nonconformity with Newtonian cooling. Forcing the winter data through a point at zero metabolism corresponding to T_b decreases lower critical temperature from 25.8^{o}C to 20.1^{o}C (Figure 3). This value, however, is little reduced from the summer value of 21.8^{o}C. The slope of the line relating metabolic rate to ambient temperature below thermoneutrality was significantly steeper in summer.

These suggest slightly better insulation in winter. Furthermore, the winter slope does not conform to Newtonian cooling, indicating modulation of thermal conductance at temperatures below thermoneutrality, resulting in a decrement of conductance with T_a. This pattern of nonconformity to Newtonian cooling has been noted in other passerines (West, 1972a; Dawson and Carey, 1976). Summer slope does conform to Newtonian cooling, suggesting an inability to modify conductance below thermoneutrality in summer birds. This implies that the ability to modify conductance below thermoneutrality, presumably through vasomotor changes, although plumage or postural adjustments might also be involved, is a component of winter-acclimatization in juncos.

Thermal conductance in summer-acclimatized juncos exceeded allometrically predicted values (Aschoff, 1981) by 20%, suggesting relatively poor insulation in summer birds. Calculation of conductance in winter birds is impaired by nonconformity to Newtonian cooling. According to standard methods of calculation from metabolism-temperature data, conductance at thermoneutrality exceeded allometrically predicted values by 23%, while conductance at $-10^{o}C$ exceeded predicted values by only 3%. If these calculations are accurate, the ability to decrease conductance below thermoneutrality allows relatively close approximation to allometrically predicted values at environmentally relevant temperatures.

Seasonal variation in insulation in passerines is not necessarily coincident with seasonal changes in cold tolerance (Hart, 1962; Dawson and Carey, 1976). Furthermore, increased insulation in winter juncos studied here did not obviate the requirement for elevation of metabolic rate to offset heat loss at lower winter temperatures. For example, the mean daily temperature for Corvallis is 4°C in January and 19°C in July/August (OSU Climatic Research Institute). Assuming a caloric equivalent of 4.8 kcal/L O_2 (Lasiewski and Dawson, 1967), incorporation of these temperatures into equations for VO_2 below thermoneutrality yield energy expenditures of 14.0 kcal/day in January and 7.6 kcal/day in July/August to maintain resting metabolic rates. Thus, on average, 1.8X as much energy is required for thermoregulation in winter. Below average winter temperatures would increase relative energy requirements. This demonstrates the primary importance of metabolic adjustments to seasonal acclimatization in the dark-eyed junco.

Overall, acclimatization in juncos from western Oregon appears primarily physiological, consisting of a capacity for maintenance of elevated metabolic rates in response to prolonged ambient cold. Insulation is increased in winter, but not to an extent preventing a requirement for increased metabolic rates even at moderate temperatures. This condition is similar to winter-acclimatization in passerines from more severe winter environments (Pohl and

West, 1973; Dawson and Carey, 1976; Schwan and Williams, 1978; Dawson et al. 1983a). However, winter juncos studied here show elevated SMR and a capacity to decrease conductance below thermoneutrality; these appear to be unusual physiological responses among passerines.

Table 1: Seasonal values for body mass and constituents and plumage. Sample size is indicated in parentheses. * and ** indicate significantly different means at P<.01 and P<.001, respectively (Student's t-test or Mann-Whitney U-test.

MEASUREMENT	SUMMER	WINTER
Total body mass	17.8±0.1g (59)	19.4±0.2g (56)**
Total body lipid	0.49±.03g (5)	1.16±.27g (6)
Visible fat-furcular	1.0±.1 (57)	3.1±.1 (57)**
-abdominal	1.2±.1 (57)	3.3±.1 (57)**
Lean dry mass	4.28±.13g (4)	4.50±.19g (6)
Plumage mass	0.60±.03g (14)	0.79±.02g (14)*

Table 2: Body temperatures of juncos housed at different temperatures in winter and summer. Sample sizes are indicated in parentheses. * and ** indicate significantly different means at P<.05 and P<.001, respectively (Student's t-test or Mann-Whitney U-test).

T_a (^oC)	W_{day}	S_{day}	W_{night}	S_{night}
30	42.5±0.3 (12)	42.3±0.1 (18)	40.6±0.2* (8)	39.6±0.4 (6)
15	42.6±0.2 (12)	41.7±0.1** (18)	39.8±0.3 (12)	39.2±0.5 (6)
5	42.4±0.3 (12)	41.5±0.3** (14)	39.9±0.2 (12)	39.6±0.8 (4)
-10	41.8±0.3 (12)	------	39.1±0.3 (12)	------

Figure 2: The relationship between T_b and T_a for winter- and summer-acclimatized juncos during day (o) and night (●). The slopes of all regressions except that for summer nights were significantly different from zero (winter day, $P<.05$, winter night, $P<.01$, summer day, $P<.01$). Equations were, Winter day : $T_b=42.1+.18(T_a)$, $R^2=.09$, $n=48$, Winter night: $T_b=39.5+.04(T_a)$, $R^2=.23$, $n=44$, and Summer day: $41.3+.03(T_a)$, $R^2=.19$, $n=50$.

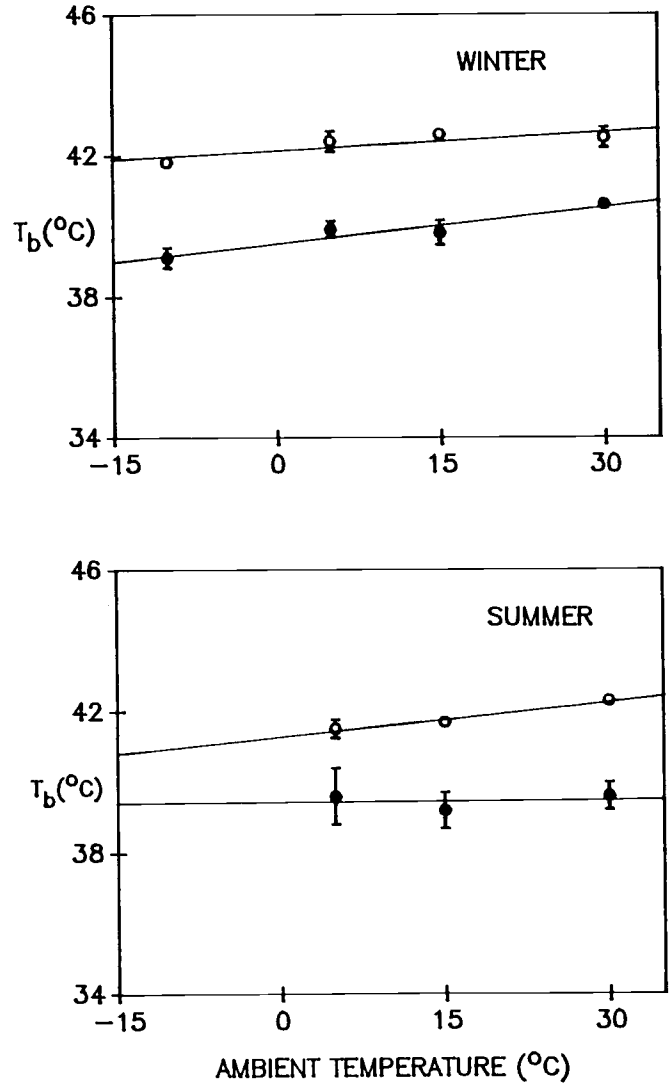
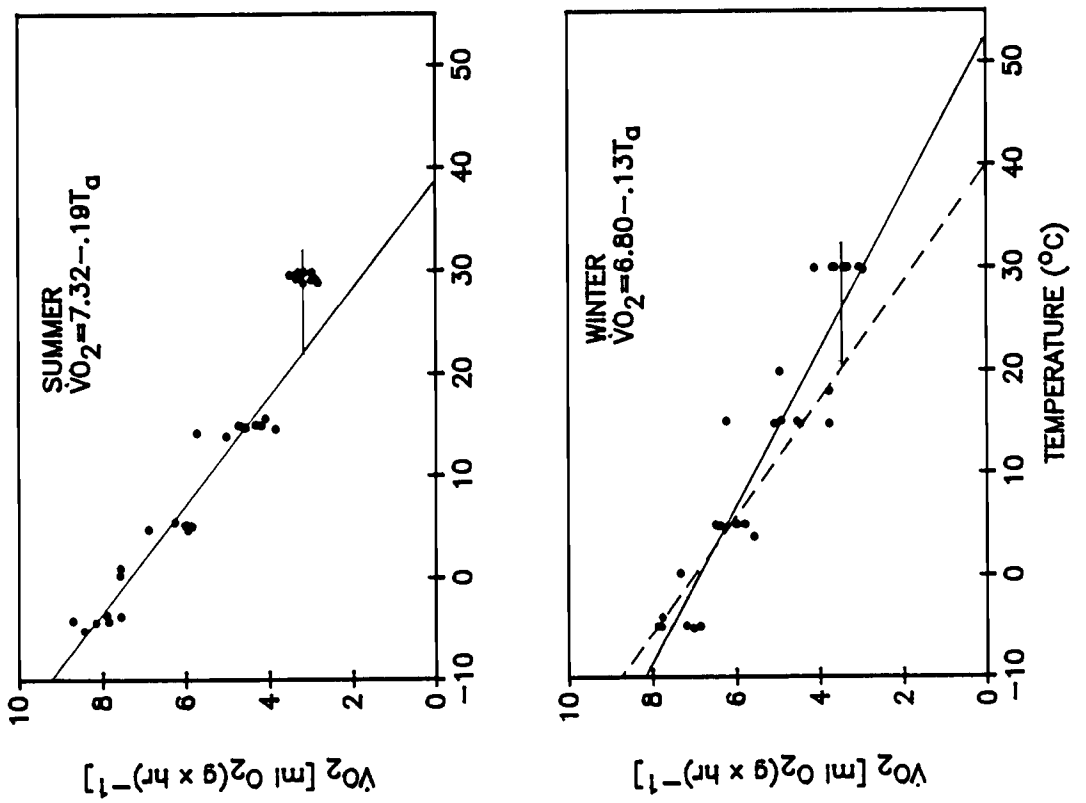


Figure 2

Figure 3: Relationship of mass-specific metabolism to ambient temperature in seasonally-acclimatized dark-eyed juncos. Equations given describe the regression line below thermoneutrality. The horizontal lines represent SMR. The dashed line in winter was obtained by forcing the mass-specific regression through T_b at zero metabolism, which yields $\dot{V}O_2=6.95-.17(T_a)$. On a total metabolism basis (ml O_2/min), equations below thermoneutrality were, Summer: $\dot{V}O_2=1.99-.054(T_a)$ and Winter: $\dot{V}O_2=1.95-.036(T_a)$.

Figure 3


SUMMER
$\dot{V}O_2$=7.32−.19T_a
WINTER
$\dot{V}O_2$=6.80−.13T_a
$\dot{V}O_2$ [ml O_2(g x hr)$^{-1}$]
TEMPERATURE (°C)
0
2
4
6
8
10
−10
0
10
20
30
40
50

CHAPTER 3

SEASONAL VARIATION IN COLD HARDINESS AND PEAK RATES OF COLD-INDUCED THERMOGENESIS IN THE DARK-EYED JUNCO, Junco hyemalis.

INTRODUCTION

Small birds that overwinter in temperate climates show marked seasonal changes in cold tolerance (Hart, 1962; Barnett, 1970; Pohl and West, 1973; Dawson and Carey, 1976; Dawson et al. 1983). Seasonal variation in peak rates of cold-induced thermogenesis ($\dot{V}O_{2max}$, defined here as maximal $\dot{V}O_2$ maintained over ten minutes) in these birds has received little attention, due mostly to problems associated with generating experimental temperatures low enough to elicit maximum metabolism. High specific heat helium-oxygen gas mixtures (heliox) facilitate heat loss, and induce $\dot{V}O_{2max}$ at less extreme temperatures (Rosenmann and Morrison, 1974).

Consequently, heliox gas mixtures have been used to document winter increases in maximal thermogenic capacity in several mammals, including Snowshoe Hares (Feist and Rosenmann, 1975), Red-backed Voles (Rosenmann, Morrison and Feist, 1975), and White-footed Mice (Wickler, 1980). In birds, Dawson and Smith (1986) documented increased $\dot{V}O_{2max}$ in winter-acclimatized American Goldfinches from Michigan compared with those acclimatized to spring conditions. Higher $\dot{V}O_{2max}$ in winter may indicate changes in the metabolic machinery involved in thermogenesis that increase thermogenic capacity and enable small birds to prolong cold

resistance. However, it remains unclear whether seasonal variation in maximal thermogenic capacity is widespread among small birds wintering in temperate climates.

I examined seasonal variation in cold resistance and VO_{2max} in dark-eyed juncos (Junco hyemalis) from western Oregon. These juncos appear to be local migrants wintering at low elevations throughout the breeding range (AOU Checklist, 1957; Bent, 1968). I measured $\dot{V}O_{2max}$ and cold resistance during heliox cold stress to determine if thermogenic capacity varies seasonally in this species.

METHODS

Dark-eyed Juncos were captured by mist-net near Corvallis, Benton County, Oregon, during June-Aug. 1987 and Dec. 1987-Feb. 1988. Birds were trapped in the morning, transported to the laboratory, and caged at room temperature (20°C - 25°C). Food and water were provided ad libitum. Birds were allowed to feed for at least 2 hr. prior to testing on the day of capture. Cold stress tests were conducted between 1100 and 1930 hr. Juncos tested from June through August were designated "summer birds". Those tested from December through February were designated "winter birds". Both males and females were used and male:female (sexed by plumage) ratios were 20:15 in winter and 15:13 in summer. All birds tested were adults.

The rate of oxygen consumption ($\dot{V}O_2$) during heliox cold stress was measured in an open-circuit metabolism system. Metabolic chambers were designed from one gallon (3.8 liter) paint cans with a black inner surface. Birds were weighed to the nearest 0.1g with a Pesola spring balance (50g) and placed into the metabolism chamber which was then lowered into a water/ethylene glycol bath capable of regulating chamber temperature to $\pm 0.5^{\circ}C$. Chamber temperature was monitored by a Cole-Parmer thermocouple thermometer, previously calibrated to $\pm 0.1^{\circ}C$ with a thermometer traceable to the U.S. Bureau of Standards. Gas flow (premixed gas mixtures of approximately 20.5%He:79.5%O_2) into the chamber was initiated upon

submersion into the water/ethylene glycol bath. Flow rates through the chamber were controlled by Cole-Parmer Precision rotameters (Model FM082-03ST) which were calibrated by timing water displacement by air and heliox from a six liter spirometer. Flow rates were corrected for water vapor pressure within the spirometer which was measured with an Extech (Model 5070) humidity meter. This procedure allowed calibration of flow rates to ±1% accuracy. Flow rates varied from 900-1025 ml/min, which provided approximately 0.5% difference between influx and efflux oxygen concentrations and kept efflux oxygen concentrations above 19.75%. A Beckman Model E2 paramagnetic oxygen analyzer was used for measurement of the fractional concentration of oxygen in the respiratory gas. Measurements of dry, CO_2-free efflux gas were recorded every 60 seconds. Oxygen consumption values were calculated as instantaneous rates (Bartholomew et al. 1981). The initial ten minutes of $\dot{V}O_2$ measurements were deleted from calculations.

Procedures utilized to measure standard metabolic rate (SMR) were similar to those for $\dot{V}O_2$ under cold stress. SMR measurements were conducted at night after at least 5 hr fasting within the metabolic chamber. Chamber temperature was maintained within the thermal neutral zone for the junco (Swanson, unpublished data). Flow rates of dry, CO_2-free air were maintained at 225-290 ml/min for SMR measurement. SMR was determined as the mean $\dot{V}O_2$ over a 60

min period. $\dot{V}O_2$ was calculated both by steady state (Depocas and Hart, 1957) and instantaneous (Bartholomew et al. 1981) methods. Mean $\dot{V}O_2$ calculated by the two methods differed by 4% or less, so gas mixing within the chamber was considered satisfactory. Steady state values for SMR were used for metabolic expansibility determinations.

Juncos were exposed to a series of decreasing temperatures in heliox until $\dot{V}O_{2max}$ was attained and hypothermia was induced in a majority of the birds. These temperatures were 8°C, 4°C, 2°C, and 0°C in summer and 0°C, -3°C, -6°C, and -9°C in winter. Individual birds were exposed to a single temperature within the series for 90 minutes, or until they experienced hypothermia, indicated by a steady decline in $\dot{V}O_2$ over several minutes. At the end of the test, birds were quickly removed from the chamber. Cloacal temperature was recorded with a Cole-Parmer thermocouple thermometer and 20 gauge copper-constantan wire thermocouple probe inserted to about 1 cm depth. This allowed measurement of cloacal temperature to within ± 0.1°C. Birds with a cloacal temperature greater than 36°C were considered normothermic. Following cloacal temperature measurement, birds were reweighed.

$\dot{V}O_{2max}$ data were analyzed by averaging instantaneous $\dot{V}O_2$ measurements over successive ten minute intervals. The highest average $\dot{V}O_2$ of these intervals was designated as $\dot{V}O_{2max}$ at the test temperature. The highest one-minute $\dot{V}O_2$ over the test period was considered maximal instantaneous

metabolic rate ($\dot{V}O_{2i}$). All values for $\dot{V}O_2$ were corrected to STP.

All values are presented as means $\pm$SD. Mean $\dot{V}O_2$ at each temperature, body mass, and mass loss were compared by Student's T-test, or by Mann-Whitney U test, when variances of mean values were unequal. Birds that became hypothermic in <30 min in summer, or <60 min in winter had substantially lower $\dot{V}O_{2max}$ than birds that remained normothermic for longer periods and were omitted from calculation of mean $\dot{V}O_{2max}$. The effect of heliox T_a on T_b was analyzed by one-way ANOVA. Statistical significance was accepted at $P<0.05$.

RESULTS

Cold tolerance in winter-acclimatized juncos increased markedly compared to summer-acclimatized juncos (Figure 4). At $0^{o}C$ in heliox in summer, 11 of 12 juncos tested became hypothermic before 90 minutes. In winter, all birds tested at $0^{o}C$ remained normothermic (T_b>$36^{o}C$). A temperature of $-9^{o}C$ in heliox was required in winter to induce hypothermia in >50% of the birds tested. To elicit hypothermia in >50% of summer birds required only $4^{o}C$ in heliox. At temperatures below $0^{o}C$ in summer and $-9^{o}C$ in winter, all birds tested rapidly became hypothermic.

Mean T_b of normothermic birds after cold stress was $39.5\pm0.9^{o}C$ in summer and $39.6\pm0.4^{o}C$ in winter. For normothermic birds, T_b was not dependent on T_a in heliox in either summer or winter.

Mean mass at the initiation of cold stress tests was significantly greater ($P<.01$) in winter ($18.2\pm1.5g$, $n=35$) than in summer ($16.9\pm1.1g$, $n=28$). Mass-specific $\dot{V}O_2$ is commonly assumed to account for variation in $\dot{V}O_2$ as a function of body mass. Hence, all $\dot{V}O_2$'s are reported on both a per bird and a mass-specific basis.

SMR was $0.87\pm.09$ mlO_2/min ($3.16\pm.30$ $mlO_2g^{-1}hr^{-1}$) in summer and $1.03\pm.14$ mlO_2/min ($3.49\pm.48$ $mlO_2g^{-1}hr^{-1}$) in winter. SMR in winter was significantly greater than in summer on a per bird basis ($P<0.01$), but not on a mass-specific basis.

$\dot{V}O_{2max}$ was significantly greater in winter than summer

on both a per bird and a mass-specific basis (Table 3). $\dot{V}O_{2max}$ in summer occurred at 2°C, but mean $\dot{V}O_2$ at 2°C was not significantly different from 0°C or 4°C on a per bird basis (combined $\dot{V}O_2$=5.78±0.39 mlO_2/min, n=20), or from 4°C on a mass-specific basis (combined $\dot{V}O_2$=20.75±1.64 $mlO_2g^{-1}hr^{-1}$, n=11). In winter, $\dot{V}O_{2max}$ occurred at -9°C. On a per bird basis, $\dot{V}O_2$ at -9°C was significantly greater than at other temperatures. However, on a mass-specific basis, $\dot{V}O_2$ at -9°C was not significantly different from -6°C (combined $\dot{V}O_2$=23.42±1.25 $mlO_2g^{-1}hr^{-1}$, n=14). $\dot{V}O_{2max}$ in summer represents an increment of 6.6X SMR in both total and mass-specific $\dot{V}O_2$. In winter, $\dot{V}O_{2max}$ increased to 7.2X SMR on a per bird basis and 6.7X on a mass-specific basis.

Maximal instantaneous oxygen consumption ($\dot{V}O_{2i}$) did not vary seasonally on either a per bird or mass-specific basis (Table 4). In summer, $\dot{V}O_{2i}$ at 2°C and at 4°C were not significantly different (combined $\dot{V}O_2$=9.30±2.01 mlO_2/min, 33.40±6.69 $mlO_2g^{-1}hr^{-1}$, n=11). Winter $\dot{V}O_{2i}$ at -9°C, -6°C, and -3°C were not significantly different (combined $\dot{V}O_2$=9.73±1.42 mlO_2/min, 32.35±4.97 $mlO_2g^{-1}hr^{-1}$, n=26). Summer $\dot{V}O_{2i}$ exceeded SMR by 10.9X and 10.8X on a per bird and mass-specific basis, respectively. In winter, $\dot{V}O_{2i}$ was 9.8X SMR on a per bird basis, and 9.6X SMR on a mass-specific basis.

Cold exposure-induced maximal rates of mass loss did not vary seasonally at any temperatures (Figure 5). Mean mass loss values were -0.6 ± 0.3 g/hr in summer (n=28) and

-0.6 ± 0.4 g/hr in winter (n=35). Absolute values for mass loss in terms of percent wet mass were -3.6%/hr in summer and -3.3%/hr in winter. Smaller individuals exhibited slightly decreased capacities for cold tolerance at both seasons (Table 5).

DISCUSSION

Increased thermogenic capacity in winter, indicated by augmented $\dot{V}O_{2max}$, appears to be a feature of metabolic acclimatization in the Dark-eyed Junco. In addition, winter-acclimatized juncos exhibited a marked increase in cold tolerance over summer-acclimatized juncos. These results are similar to others in which small passerines exposed to severe cold showed increased metabolic rates and improved cold tolerance at colder times of the year (Hart, 1962; Pohl and West, 1973; Southwick, 1979; Dawson and Smith, 1986). However, this pattern is not inviolate as House Finches in Colorado showed no seasonal differences in VO_{2max} (Dawson et al. 1983), and Gray Jays from Alaska had higher metabolic rates at -50°C in spring than in winter, although summer rates were lower (Veghte, 1964). A winter increment of $\dot{V}O_{2max}$ elicited by cold stress indicates augmented thermogenic capacity and seems to be associated with increased shivering endurance. Enhanced shivering endurance is primarily responsible for increased cold tolerance in other passerines (Dawson and Carey, 1976; Dawson et al. 1983).

Birds in this study exhibited a relatively wide seasonal fluctuation in heliox temperatures that elicited $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ occurred at 0°C to 4°C in summer and -6°C to -9°C in winter. Heliox cold stress in seasonally-acclimatized American Goldfinches from Michigan produced $\dot{V}O_{2max}$ from 0°C to 6°C in spring and 0°C to -6°C in winter

(Dawson and Smith, 1986).

Elevated winter $\dot{V}O_{2max}$ might be attributed to differential feeding prior to cold stress in winter-acclimatized and summer-acclimatized birds. Winter juncos might have eaten more prior to tests which allowed them to maintain elevated $\dot{V}O_2$ longer. However, fasted juncos also demonstrated increased cold tolerance in winter birds (Swanson, unpublished observations). I suggest that the seasonal variation in $\dot{V}O_{2max}$ and cold tolerance can be attributed to metabolic changes.

Cold tolerance of Dark-eyed Juncos appears to be influenced by body mass as larger birds tolerated cold more effectively. For both seasons, at higher test temperatures in heliox only the smaller birds became hypothermic while at lower test temperatures only the larger birds remained normothermic (Table 5). This improved cold tolerance could be due to increased substrate (fat or glycogen) reserves or enhanced mobilization of these reserves in larger birds, or by some size-dependent effect on thermogenic abilities.

I estimated air temperature equivalents to heliox test temperatures by extrapolation. Heliox $\dot{V}O_2$ values were inserted into equations that relate $\dot{V}O_2$ to T_a (Swanson, unpublished data) and solved for T_a. Estimated T_a's at $\dot{V}O_{2max}$ were $-69^{o}C$ in summer and $-125^{o}C$ in winter. Actual air temperatures were probably not this low as thermal conductance often changes with T_a. Nevertheless, juncos tolerated extreme cold stress (90 minutes maximum) far in

excess of any temperatures experienced under natural climatic conditions. Extreme minimum temperatures are 0°C in June-August and -26°C in December-February in Corvallis, Oregon (Oregon State University Climatic Research Institute). Effective extreme minimum T_a's are probably below these values due to environmental factors such as wind, humidity, or radiation. Cold tolerance data suggests that as long as food supplies are readily available, juncos probably face little danger from environmental cold stress.

However, these data may inaccurately predict environmental cold tolerance because experimental cold exposure was acute and severe. Chronic exposure to more moderate environmental cold stress (i.e. "normal" winter conditions) probably necessitates prolonged elevation of metabolic rates (although not to maximal levels). Wind, humidity, and radiation might further reduce effective T_a's below actual values, which necessitates further metabolic enhancement. Environmental conditions most challenging to cold tolerance capabilities are overnight fasting and fasting induced by severe weather limiting foraging. Stuebe and Ketterson (1982) predicted survival of fasting winter-acclimatized juncos at 4°C through the night, and the following day and night, if the birds were near the peak of the daily fat cycle at the onset of fasting. Colder temperatures would probably decrease survival time. Fasting capacities for summer-acclimatized juncos are unknown. Nevertheless, it seems juncos have a considerable

margin of safety when confronted with natural cold stress.

I calculated minimal thermal conductance from $\dot{V}O_2$ at heliox temperatures that elicited $\dot{V}O_{2max}$ (assuming T_b=39.5^oC and 4.85 cal/mlO_2). For winter-acclimatized juncos minimal thermal conductance was 2.81 mW/(gX^oC) and for summer-acclimatized juncos it was 3.21 mW/(gX^oC). This represents a 14.2% increase in conductance for summer birds and indicates that winter birds are somewhat better insulated. Values for minimal thermal conductance at equivalent air temperatures are 0.94 mW/(gX^oC) in winter and 1.05 mW/(gX^oC) in summer. Conductance in heliox exceeded that in air by 3.0X in winter and 3.1X in summer. These values slightly exceed increments in heliox reported in Common Redpolls (2.6X, Rosenmann and Morrison, 1974) and American Goldfinches (2.7X, Dawson and Smith, 1986).

$\dot{V}O_{2i}$ did not vary seasonally. $\dot{V}O_{2i}$ probably reflects activity but may represent intense short-term shivering bouts. Either would increase heat production over the short term. Apparently, changes in the metabolic machinery that produce increased $\dot{V}O_{2i}$ are not important to cold tolerance in the junco, while those that elevate $\dot{V}O_{2max}$ are.

Metabolic expansibility ($\dot{V}O_{2max}$/SMR; Dawson and Carey, 1976) for Dark-eyed Juncos, 6.6X in summer and 7.2X in winter on a per bird basis, is the highest yet reported for passerine birds (Rosenmann and Morrison, 1974; Dawson and Carey, 1976; Dawson and Smith, 1986; Koteja, 1986). Dawson (pers. comm.) found winter Dark-eyed Juncos from Michigan

capable of $\dot{V}O_{2max}$ equal to 6.3X SMR. The values for cold-induced metabolic expansibility in the Oregon juncos are surpassed by those from several winter-acclimatized small mammals, which equal or exceed 8X BMR (Feist and Rosenmann, 1975; Rosenmann et al. 1975; Wickler, 1980).

Consideration of $\dot{V}O_2$ on a per bird or mass-specific basis had some effect on metabolic expansibility determinations. In winter, $\dot{V}O_{2max}$ per bird was 7.2X SMR, while mass-specific $\dot{V}O_{2max}$ was only 6.7X SMR. In summer, this effect disappeared, as metabolic expansibility on both a per bird and mass-specific basis was 6.6X SMR. The increase in body mass in passerines in winter is due largely to an increase in fat which is relatively inert metabolically (Dawson and Smith, 1986). This accounts for the difference in metabolic expansibility between per bird and mass-specific $\dot{V}O_2$ in winter. Thus, per bird $\dot{V}O_2$ may be more appropriate than mass-specific $\dot{V}O_2$ for seasonal comparisons (Dawson and Smith, 1986).

Mass loss over the period of the cold stress tests was consistent and independent of test temperature at both seasons. Seasonal stability of mass loss rates in juncos during cold stress suggests that substrate mobilization is not a factor that limits $\dot{V}O_{2max}$ and cold tolerance. Decreased cold tolerance in smaller juncos in both seasons, assuming low body mass indicates low fuel reserves, may suggest that depletion of fuel reserves beyond a certain critical level diminishes capacity to further mobilize

metabolic substrates. However, the assumption that low body mass indicates low fuel reserves is tenuous as birds were allowed to feed prior to cold stress. Mass loss trends might also be explained by differences in feeding intensity prior to cold stress, differences in assimilation of digestive tract contents, or differences in respiratory water loss. In addition, hypothermia occurs in some passerines with substantial remaining fat stores (Carey et al. 1978), presumably above the putative critical level.

Table 3: Maximal $\dot{V}O_2$ sustained over a ten minute period at heliox test temperatures in summer (S) and winter (W) juncos. Body masses are means for the treatment group. Birds that became hypothermic in <60 minutes in winter and <30 minutes in summer had substantially lower $\dot{V}O_2$ and were excluded from calculations.

Temp./ Season	n	Mass (g)	$\dot{V}O_2$ (mlO_2/min)	XSMR
8°C/S	5	17.8	5.28±.23	6.1
4°C/S	5	17.3	5.67±.42	6.5
2°C/S	6	17.0	5.93±.22	6.8
0°C/S	9	17.1	5.75±.46	6.6
0°C/W	9	18.7	6.21±.68	6.0
-3°C/W	7	18.7	6.51±.46	6.3
-6°C/W	8	18.2	6.95±.25	6.7
-9°C/W	6	18.6	7.39±.35	7.2

Table 4: Maximal instantaneous oxygen consumption ($\dot{V}O_{2i}$) at heliox test temperatures in summer (S) and winter (W) juncos.

Temp./ Season	n	$\dot{V}O_2$ ($ml O_2$/min)	XSMR
8°C/S	5	6.81±.63	7.8
4°C/S	5	8.41±1.83	9.7
2°C/S	6	10.05±1.98	11.6
0°C/S	12	8.13±1.09	9.3
0°C/W	9	8.26±1.48	8.0
-3°C/W	9	9.03±1.50	8.8
-6°C/W	8	9.72±.79	9.4
-9°C/W	9	10.44±1.53	10.1

Table 5: Cold tolerance of juncos according to body mass at the initiation of cold stress. Cold stress tests were undertaken without regard to body mass so unequal numbers of birds in each mass class were exposed to each heliox test temperature. Data for 0°C in winter is excluded since all birds remained normothermic at that temperature. Hypo. and Normo. Temps. columns indicate heliox temperatures that resulted in hypothermia or normothermia for birds in the specified mass class. Numbers in parentheses indicate the number of birds that remained normothermic or became hypothermic at the given temperature in heliox. t_H represents the mean time to hypothermia for birds that became hypothermic.

SUMMER

Mass(g)	Normo./ Total	Hypo. Temps.(°C)	Normo. Temps.(°C)	t_H(min)
<16	0/4	8,2,0(2)	---	54.3
16-17	2/6	2(2),0(2)	4,8	52.3
17-18	1/7	4,2,0(4)	4	52.3
>18	4/7	2,0(2)	8(3),0	62.0

WINTER

Mass(g)	Normo./ Total	Hypo. Temps.(°C)	Normo. Temps.(°C)	t_H(min)
<17	0/1	-3	---	36.0
17-18	2/7	-3,-6,-9(3)	-6(2)	58.0
18-19	5/7	-6,-9	-3(2),-6(3)	61.5
>19	8/11	-9(3)	-3(5),-6,-9(2)	72.7

Figure 4: Cold tolerance in seasonally-acclimatized juncos over the 90 minute test period. The numbers over the bars indicate sample size. In winter at 0°C all birds tested (n=9) remained normothermic.

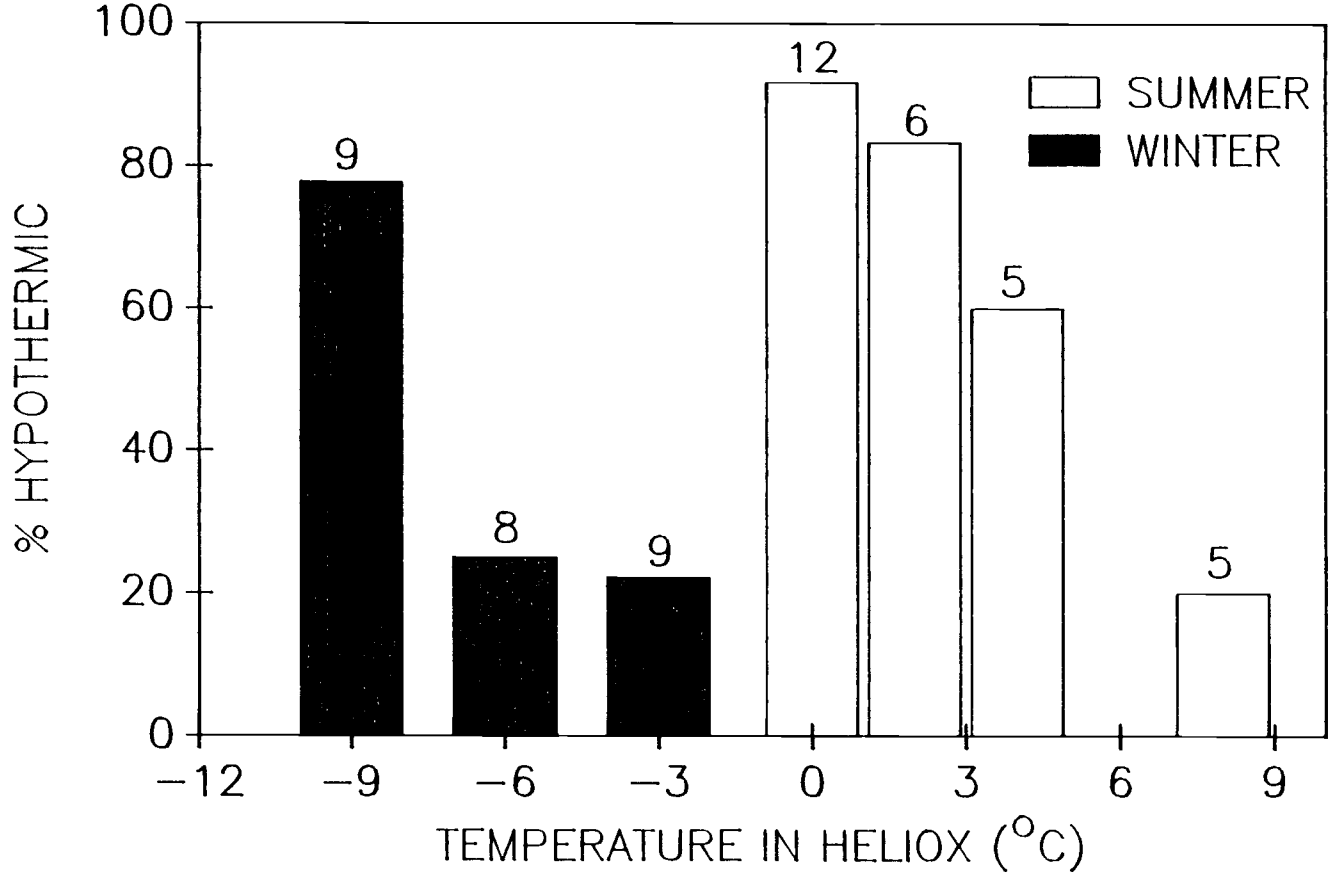
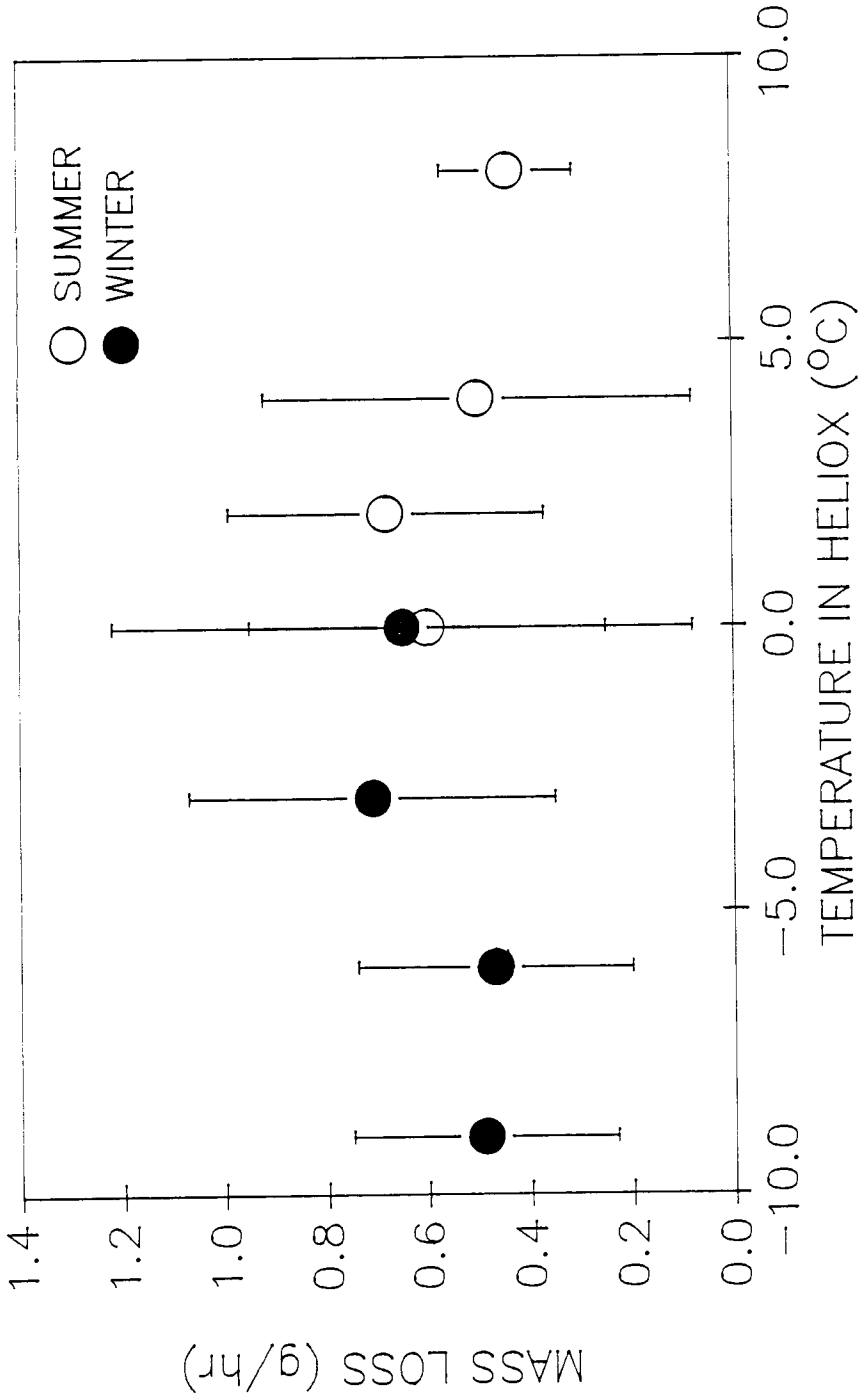

100
80
60
40
20
0
% HYPOTHERMIC
9
8
9
12
6
5
5
SUMMER
WINTER
−12
−9
−6
−3
0
3
6
9
TEMPERATURE IN HELIOX (°C)

Figure 4

Figure 5: Mass loss rates as a function of heliox test temperature in seasonally-acclimatized juncos. All values were statistically indistinguishable.

Figure 5


SUMMER
WINTER
MASS LOSS (g/hr)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
−10.0
−5.0
0.0
5.0
10.0
TEMPERATURE IN HELIOX (°C)

CHAPTER 4

SEASONAL VARIATION OF VASCULAR OXYGEN TRANSPORT IN THE DARK-EYED JUNCO.

INTRODUCTION

Small birds wintering in temperate climates show increased cold tolerance during the winter months (Hart, 1962; Barnett, 1970; Pohl and West, 1973; Dawson and Carey, 1976; Dawson et al. 1983). However, mechanisms underlying this response are incompletely understood. Apparently, increased cold tolerance in winter-acclimatized small birds depends primarily upon metabolic, rather than insulative, adjustments (Dawson and Carey, 1976). Consequently, metabolic rates in winter must be elevated well above basal levels, even at moderately cold temperatures (Dawson and Carey, 1976; Dawson et al. 1983). Augmented capacities for aerobic thermogenesis appear necessary to meet winter thermoregulatory demands.

Enhanced shivering thermogenesis appears primarily responsible for increased thermogenic capacity in winter-acclimatized birds (Dawson and Carey, 1976). Sustained shivering in response to cold stress results in an increased demand for oxygen in the muscles involved. Therefore, seasonal adjustments in vascular oxygen transport, allowing increased oxygen delivery in winter-acclimatized birds, might be expected. Oxygen unloading to shivering tissues might be increased by several mechanisms, including increased oxygen carrying capacity, decreased

hemoglobin oxygen affinity, increased cardiac output, or altered conditions at the tissues resulting in increased arteriovenous P_{O2} differences. Shivering efficiency would presumably be enhanced if oxygen delivery could be improved, at low metabolic cost, thereby facilitating increased shivering endurance.

Investigation of possible seasonal variation in avian capacities for vascular oxygen transport has received little attention. Consequently, an overview of avian vascular oxygen transport responses associated with seasonal acclimatization is lacking. Although a winter increment in hematocrit has been reported for some passerines (Carey and Morton, 1976; deGraw et al. 1979), seasonal trends in oxygen affinity remain largely uninvestigated. This study examines some aspects of vascular oxygen transport in a small passerine indigenous to western Oregon, the dark-eyed junco, Junco hyemalis, to determine if seasonal acclimatization is accompanied by variation in the vascular oxygen transport system.

METHODS AND MATERIALS

Dark-eyed juncos (Emberizidae: Junco hyemalis) were captured by mist-netting and live-trapping at several sites near Corvallis, Benton County, Oregon. Elevation at the capture sites ranged from about 200m to about 400m. After capture, birds were transported to the laboratory, or held overnight in outdoor cages with food and water provided ad libitum. Birds tested from 1 December - 28 February, 1985-86, were designated as winter birds, those tested from 20 May - 31 August, 1986, as summer birds. All birds were adults. Mean and extreme minimum daily temperatures in Corvallis were 0.4°C and -7.8°C for the winter period and 10.7°C and 3.9°C for the summer period (Oregon State University Climatic Research Institute).

Blood samples were withdrawn from the right jugular vein. Clotting was prevented by rinsing needle and syringe with 0.05 ml, or less, Na-heparin. No correction was made for this slight change in volume. Since individual samples were small (about 0.5 ml), blood from three to six individuals was pooled for each dissociation curve.

Oxygen dissociation curves (O_2DC's) were generated by a modification of the method of Tucker (1967). The pooled blood sample was placed into a 20 ml water-jacketed (41°C) vessel for equilibration. Equilibration was accomplished by continuously passing water vapor-saturated gas mixtures, containing 5%CO_2 and varying amounts of O_2 and N_2, over the blood, with gentle shaking. Oxygen carrying capacity

(O_2Cap) was determined by equilibration with 95% air/5% CO_2 and calculated according to Laver et al. (1965). Use of 95% air/5%CO_2 instead of higher O_2 concentrations probably results in a slight underestimation of O_2Cap and possibly a slight underestimation of P_{50}.

For each O_2DC entry, a 0.1 ml sample was anaerobically withdrawn from the equilibration vessel and placed into a Radiometer Copenhagen BMS 3 Mk 2 Blood Gas Analyzer at 41^oC for P_{O2} and pH determination. A second 0.05 ml sample was anaerobically withdrawn and placed into a 3.0 ml syringe filled with low P_{O2} potassium-ferricyanide reagent (Tucker, 1967). The solution was anaerobically mixed to dissociate all oxygen from hemoglobin and placed into the Blood Gas Analyzer for P_{O2} determination. The difference in P_{O2} between the ferricyanide reagent and the blood/ferricyanide mixture allowed calculation of blood O_2 content and percent saturation (Laver et al. 1965).

A winter O_2DC was generated from nine separate curves composed from the blood of 41 birds, the summer O_2DC from 11 separate curves from the blood of 45 birds.

The Bohr coefficient ($\phi = \Delta \log P_{50}/\Delta pH$) was determined by generation of O_2DC's at varying concentrations of CO_2 (%CO_2=0%,5%,and 12%) in spring and fall. Hematocrit was determined on residual blood from O_2DC's by centrifugation in a microhematocrit centrifuge (International Clinical Centrifuge) for six minutes.

Data on dissociation were fitted by non-linear

regression to a Weibull distribution describing non-symmetric sigmoid curves, according to the equation:

$$\%Sat=100(1-e^{B_0[PO2]^{B_1}})$$

Weibull parameters, B_0 (scale parameter) and B_1 (curve shape parameter), were compared by T-test. The data for hematocrit and oxygen carrying capacity are presented in terms of mean $\pm$ SD. Sample comparisons were made using student's T-test. Statistical significance was accepted at the 0.05 level.

RESULTS

Oxygen dissociation curves, generated at 41°C and 5%CO_2 (mean pH 7.43±.04 [n=47] and 7.46±.04 [n=49] in summer and winter, respectively), for summer- and winter-acclimatized juncos are given in Figure 6. Summer and winter O_2DC's did not vary significantly (P>.05) in either of the Weibull parameters (B_0:t=1.30, B_1:t=1.62; 42 d.f.). P_{50}'s for summer- and winter-acclimatized juncos were 54.1 torr and 54.0 torr, respectively, at 41°C and pH 7.5.

Hill plots derived from O_2DC's for summer- and winter-acclimatized juncos were not linear (Figure 7) as slope and the corresponding Hill coefficient ($n=\Delta\log[\%Sat/100-\%Sat]/\Delta\log P_{O2}$) increased with increasing saturation. At low saturations, below 20%, n-values for summer and winter were 2.0 and 2.3, respectively. N-values around P_{50} were 2.5 in summer and 2.9 in winter. At saturations exceeding 80%, n was greater than 4.0, and n increased to greater than 5.4 above 90% saturation.

The effect of pH on the O_2DC was determined in spring and fall as CO_2 Bohr effect. The combined mean Bohr coefficient ($\phi=\Delta\log P_{50}/\Delta pH$) from spring and fall for the dark-eyed junco was -0.46. This value is consistent with those recorded from other birds, which range from -0.39 to -0.67 (Baumann and Baumann, 1977; Palomeque et al. 1980).

Seasonal mean values for hematocrit varied significantly [47.2 ± 3.3% in summer; 52.6 ± 2.0% in winter; P<.001]. Oxygen carrying capacity means were 13.35

± 1.15 vol % and 14.48 ± 1.20 vol % in summer and winter, respectively. These values were also significantly different ($P<.05$).

DISCUSSION

Oxygen affinity and O_2DC shape of the blood of the dark-eyed junco did not vary seasonally. Thus, under standard conditions, oxygen unloading does not appear to be enhanced in winter as a result of a decrease in oxygen affinity. This is not completely surprising since inositol pentaphosphate, the principle organic phosphate controlling oxygen affinity in avian blood, is metabolically unreactive (Lutz, 1980). However, oxygen affinity has been shown to decrease in some birds upon altitude acclimation (Bouverot, 1976; Black et al. 1978). Apparently, mechanisms capable of altering oxygen affinity in response to environmental conditions are invariant during seasonal acclimatization in the junco.

Although no seasonal change in hemoglobin oxygen affinity was apparent under standard conditions, a functional change in oxygen affinity may occur as a result of seasonal differences in conditions at the tissues. Increased muscular activity associated with sustained shivering in winter, without a corresponding increase in blood flow, could result in conditions at the tissues which would tend to decrease oxygen affinity and enhance unloading, irrespective of seasonal stability in blood oxygen affinity under standard conditions. Indeed, arteriovenous differences in P_{O2} can vary profoundly with exercise at essentially the same oxygen carrying capacity (Nunn, 1987). However, arteriovenous P_{O2} differences in the

Pekin duck showed no variation upon cold exposure (-20^{o}C) even though oxygen consumption increased 2.4 times above thermoneutral rates (Bech et al. 1984).

Under standard conditions of 41^{o}C and pH 7.5, oxygen affinity of the blood of the dark-eyed junco (P_{50}=54 torr) was lower than that for most other birds, and was at the upper end of the range of P_{50}'s reported for other passerine birds (Palomeque et al. 1980). The Hill plot for junco blood showed increasing Hill coefficients with increasing saturation, n-values exceeding 4.0 at saturations above 80%. This pattern has been recorded for several other birds (Lutz, 1980; Lapennas and Reeves, 1983; Johansen et al. 1987). It is contrary to the situation in mammals, where Hill coefficients decrease at high and low saturations. Hill plots of this shape denote increased hemoglobin cooperativity as saturation increases, with very high cooperativity at high saturations. This allows high oxygen saturations to occur at relatively low partial pressures of oxygen. While an O_2DC with a high, constant, n-value would also result in efficient oxygen loading at relatively low P_{O2}'s, it would presumably result in higher oxygen saturation at P_{O2}'s characteristic of the tissues and thereby provide less efficient oxygen unloading to the tissues. The type of O_2DC found in the junco may be of great importance to animals with hemoglobins having low affinities for oxygen, allowing them to reach high saturation at physiologically relevant P_{O2}'s and

effectively unload oxygen to the tissues (Johansen et al. 1987).

For example, assuming an arterial pO_2 of 95 torr, the value claimed for the pigeon Columba livia (Bouverot et al. 1976), oxygen saturation of junco blood at 41°C and pH 7.5 would be 92.4% in winter and 88.7% in summer. If Hill coefficients around P_{50} are extended to 95 torr, oxygen saturation would become 87.4% in winter and 83.3% in summer. Thus, the pattern of increasing cooperativity with increasing saturation results in relative improvements in O_2 binding of 5.7% in winter, and 5.3% in summer. This allows for a corresponding increase in oxygen delivery which might be significant to small birds with high metabolic rates like the dark-eyed junco. The mechanisms responsible for this type of Hill plot are unknown, but may involve aggregation or association of hemoglobin molecules, thus modifying binding capabilities (Lapennas and Reeves, 1983).

Hematocrit and oxygen carrying capacity were increased in winter-acclimatized juncos relative to summer juncos, hematocrit by 11.1% and O_2Cap by 8.6%. This adjustment functionally provides the same effect as a decrease in oxygen affinity: an increase in oxygen available to metabolizing tissues. Hematocrit has also been shown to increase in winter in American goldfinches from Michigan (Carey and Morton, 1976) and in white-crowned sparrows from eastern Washington (deGraw et al. 1979). A winter

increment of hematocrit in the junco presumably reflects increased erythropoiesis. Hematocrit could also be increased by reduction of plasma volume, but this would entail redistribution of body water among body compartments, since total body water as a percentage of lean wet body mass does not appear to vary seasonally in the junco (Helms et al. 1967).

Winter increases in hematocrit and O_2Cap may be energetically advantageous in assisting support of elevated winter oxygen demands. An increase in O_2Cap allows more oxygen to be delivered to the tissues per unit blood. Consequently, reduced blood flow rates may be required for delivery of a given amount of oxygen to the tissues, thus decreasing metabolic costs associated with pumping of blood. However, elevated hematocrits result in increased blood viscosity which would increase cardiac work and partially offset energetic advantages associated with reduced blood flow requirements. Nevertheless, augmentation of oxygen delivery to tissues by increased hematocrit and O_2Cap is probably energetically more economical than simply increasing blood flow and would seem better suited to increase capacity for shivering and cold endurance.

Further study is needed to determine if seasonally fixed oxygen affinities are characteristic of other passerines, especially those inhabiting regions with harsher winters than western Oregon, and to determine the

extent to which seasonally changing tissue conditions influence oxygen affinity and oxygen unloading at the tissues. However, these data indicate that Junco hyemalis deals with increased winter demands for oxygen, at least in part, by increasing the blood oxygen carrying capacity while oxygen affinity, under standard conditions, remains constant. This pattern concurs with other evidence (Isaacks et al. 1982) suggesting that oxygen transport in birds may be less plastic than that of mammals, with reference to altering hemoglobin oxygen affinity in response to changing oxygen demands. If so, birds must employ other mechanisms for increasing oxygen availability under hypoxic conditions. Accordingly, the response observed in the junco may be characteristic of the general syndrome of seasonal acclimatization of vascular oxygen transport in small passerine birds.

Figure 6. Oxygen dissociation curves for summer-(triangle) and winter-acclimatized (square) juncos at 41°C and 5%CO_2 (P_{CO2} 38 torr). Mean values for pH were 7.43±.04 in summer and 7.46±.04 in winter. The curves are not significantly different (P>.05). The equations are: Winter, %Sat=100(1-$e^{-0.0001[PO2]^{2.208502}}$) and Summer %Sat=100(1-$e^{-0.0004[PO2]^{1.859872}}$).

Figure 6

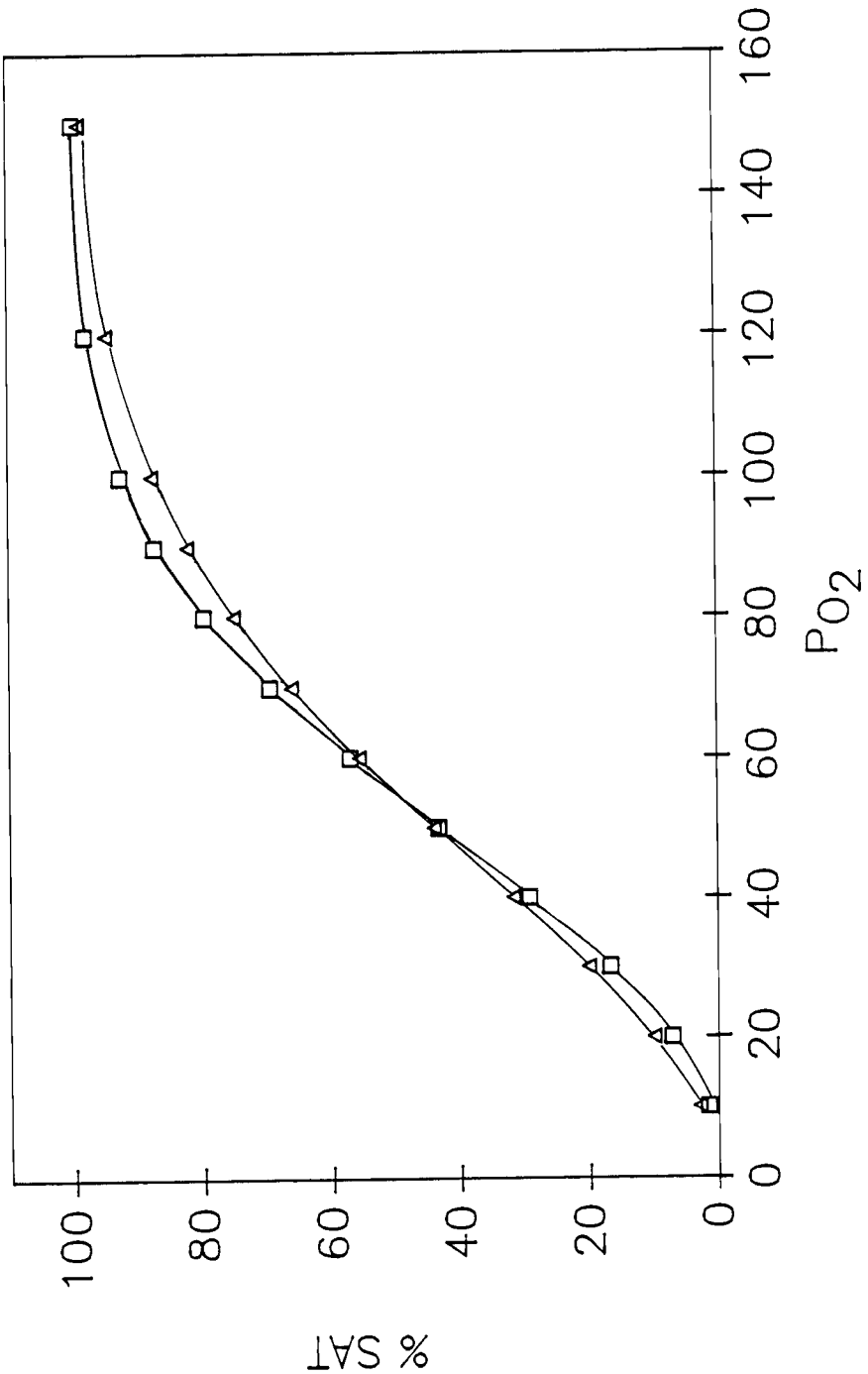
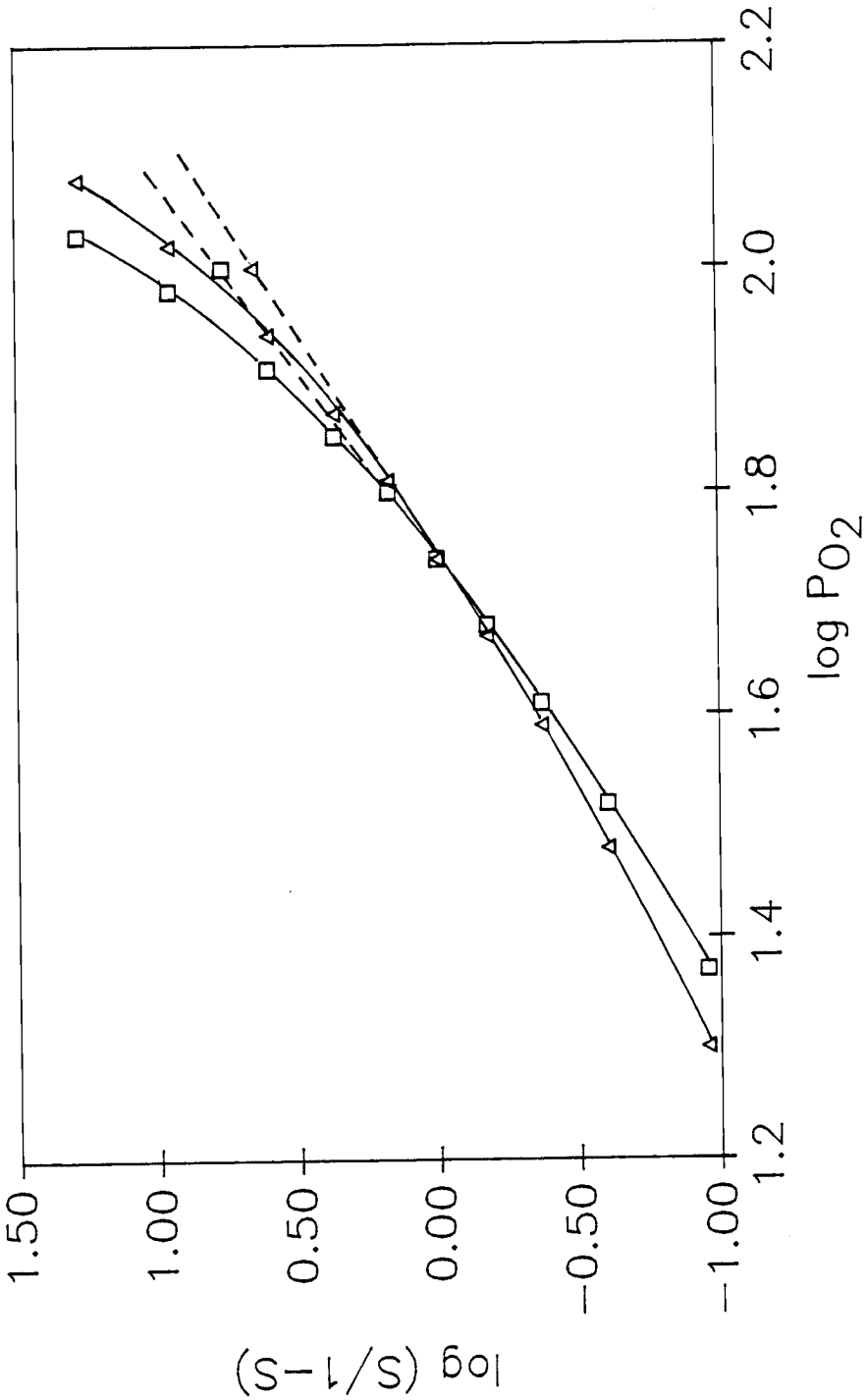


Figure 7. Hill plots derived from the oxygen dissociation curves in Figure 1 for summer-(triangle) and winter-acclimatized (square) juncos. Note the increasing n-values (slopes) at high saturations. The dashed lines correspond to a projection of the n-values around P_{50} to higher saturations for summer (triangle) and winter (square) birds.

Figure 7



CHAPTER 5

SUBSTRATE METABOLISM UNDER COLD STRESS IN SEASONALLY ACCLIMATIZED DARK-EYED JUNCOS

INTRODUCTION

Winter enhancement of cold tolerance is common among small, temperate-wintering passerine birds (Hart 1962, Barnett 1970, Dawson and Carey 1976, Dawson et al. 1983a, Dawson and Smith 1986, Swanson 1990). This is apparently associated with metabolic adjustments for prolonged maintenance of high rates of shivering thermogenesis (Dawson and Carey 1976, Swanson unpubl. data). However, the mechanistic basis for seasonal changes in shivering endurance is unclear.

Several factors have been proposed to account for seasonal changes in cold tolerance. For example, simple winter elevation of stored fuel reserves, as triacylglycerides, could allow for increased shivering endurance. However, exhaustion of energy reserves may not be a factor limiting cold tolerance, as cold-stressed passerines become hypothermic with significant triacylglycerol reserves still remaining (Dawson, Marsh, and Yacoe 1983b). Improved mobilization of stored fuels during winter might also contribute to increased cold resistance, but under cold stress passerine plasma metabolite levels differ little seasonally (Marsh and Dawson 1982, Marsh, Carey, and Dawson 1984), suggesting adequate mobilization at both seasons. Improved mass-

specific aerobic capacity in winter in the muscles involved in shivering might also assist in improving cold tolerance. However, there are no apparent changes in vascularity of the pectoralis muscles of seasonally-acclimatized American goldfinches (Carey et al. 1978) and no indications of seasonal variation in the ability of homogenates or isolated mitochondria from these muscles to oxidize pyruvate or free fatty acids (FFA) (Yacoe and Dawson 1983). Furthermore, mass-specific aerobic capacity of pectoralis muscles in migratory passerines does not appear to vary seasonally (Marsh 1981).

Marsh and Dawson (1982) suggest winter augmentation of FFA utilization, relative to carbohydrates, to support shivering may be integral to enhanced shivering endurance. This scenario allows for increased shivering endurance while possibly avoiding fatigue associated with depletion of muscle glycogen stores (Hermansen, Hultman, and Saltin 1967; Fitts et al. 1975; Gollnick, 1985). Indeed, American goldfinches exposed to -15^{o}C for 2 h exhibited elevated muscle glycogen levels and reduced glucose turnover in winter relative to summer (Marsh and Dawson, 1982). However, Yacoe and Dawson (1983) documented no seasonal variation in the ability of pectoralis muscle homogenates or isolated mitochondria from American goldfinches to oxidize fatty acids relative to carbohydrates. Furthermore, house finches, which show limited, but significant, winter expansion of cold tolerance, exhibited

little seasonal change in carbohydrate metabolism (Marsh et al. 1984). Thus, validation of the glycogen sparing hypothesis in winter-acclimatized passerine birds has also proven problematic.

The contribution of anaerobic metabolism to shivering and anaerobic effects on shivering endurance in passerine birds are unknown. Anaerobic pathways might contribute to energy provision as cold stress becomes more severe and muscular activity for thermogenesis increases. If this scenario is correct, then lactate accumulation from increasing anaerobic metabolism at colder temperatures might affect fatigue and provide limits to shivering endurance. Seasonal differences in anaerobic contributions to shivering thermogenesis at a given level of cold stress might affect differential lactate accumulation and thereby influence seasonal differences in shivering endurance.

The dark-eyed junco (Family Emberizidae, Junco hyemalis) is characteristic of boreal habitats and occupies some regions with relatively severe winter climates. The dark-eyed juncos studied here overwinter in western Oregon, a region of relatively mild winters. Nevertheless, these juncos exhibit marked seasonal changes in cold tolerance (Swanson 1990), and may provide a useful model for investigating correlation of seasonal variation in substrate metabolism and cold tolerance in small passerines. This study proposes to determine if the seasonal pattern of substrate metabolism under varied cold

stress in juncos is consistent with the glycogen sparing hypothesis (Marsh and Dawson 1982). In addition, to elucidate anaerobic effects on shivering endurance, this study attempts to discern anaerobic involvement in shivering thermogenesis at different levels of cold stress. Accordingly, plasma levels of glucose and FFA, and tissue levels of glycogen (pectoralis muscle and liver) and lactate (pectoralis muscle only) in juncos were measured after exposure to varied cold stress regimes.

MATERIAL AND METHODS

BIRDS

Dark-eyed juncos were trapped by mist-net near Corvallis, Benton Co., Oregon. Birds were captured in the morning before 1030 hour. Juncos captured from Dec.-Feb. were designated "winter birds," and those captured from June-Sept. were designated "summer birds." Juncos were tested without regard to sex and all individuals used in the experiments were adults. Mean total body mass was 19.8±.4g (n=25) in winter and 17.9±.2g in summer (n=24).

OXYGEN CONSUMPTION UNDER COLD STRESS

Juncos were fasted for five h prior to testing to assure post-absorptive conditions. Cold stress tests were conducted from 1830-2300 hours in winter and 1930-2400 hours in summer. Birds were exposed to one of three temperature regimes: 30°C in air (thermoneutrality), -12°C in air (moderate cold), and 2°C in a gas mixture containing approximately 20.9% oxygen and 79.1% helium (heliox, severe cold) for two h or until they became hypothermic, as indicated by a steady decline in metabolic rate over several min. Chamber temperature was controlled by immersion into an ethylene glycol-water bath capable of regulating temperature to ±0.5°C. Chamber temperature was continuously monitored with a thermocouple thermometer (Cole-Parmer Model 8500-40) and copper-constantan thermocouple previously calibrated to ±0.1°C with a thermometer traceable to the U.S. Bureau of Standards.

Oxygen consumption ($\dot{V}O_2$) was measured using an open circuit system with a 3.8 liter metabolism chamber and Beckman E2 oxygen analyzer as described previously (Swanson 1990). Flow rates of dry, CO_2-free gas through the chamber were regulated at 290 cc/min at thermoneutrality, 600 cc/min at moderate cold, and 1000 cc/min at severe cold by a Cole-Parmer precision rotameter (Model FM082-03ST) previously calibrated to $\pm$1% accuracy. These flow rates maintained oxygen content of excurrent air above 20.2% O_2 and yielded incurrent-excurrent O_2 content differences of 0.3-0.7%. Oxygen consumption was calculated as instantaneous $\dot{V}O_2$ from readings taken every min (Bartholomew, Vleck, and Vleck 1981). For 30°C and -12°C, $\dot{V}O_2$ was calculated as the mean value over the second h of measurement. $\dot{V}O_2$ at 2°C in heliox was regarded as the highest ten min mean $\dot{V}O_2$. All values for $\dot{V}O_2$ were corrected to STP.

PLASMA AND TISSUE METABOLITES

Upon completion of metabolism tests, birds were quickly removed from the metabolism chamber and decapitated. Blood was collected in heparinized beakers, then centrifuged for immediate determination of plasma glucose (Sigma glucose oxidase kit) and free fatty acids (Novak 1965). In a few cases, plasma was rapidly frozen in a dry ice-acetone bath and stored at -80°C for later metabolite determination. Plasma metabolite values from fresh and frozen samples did not vary significantly.

Pectoralis muscle and liver were quickly dissected and frozen in dry ice-acetone and stored at -80^{o}C for later measurement of glycogen (Keppler and Decker 1974) and lactate (by a modification of Sigma lactate dehydrogenase kit #826-UV). Muscle and liver were weighed while still frozen, minced, and homogenized in 0.6M perchloric acid. This homogenate was used for metabolite assays.

STATISTICS

Data are presented as means $\pm$ SE, unless otherwise indicated. Seasonal means were compared by Student's T-test or by Mann-Whitney U-test depending on the homogeneity of sample variances. Temperature effects within seasons were analyzed by oneway ANOVA or Kruskal-Wallis test if sample variances were not equal. Statistical significance was accepted at $P<.05$.

RESULTS

METABOLISM

Oxygen consumption was inversely related to ambient temperature (fig. 8). Summer and winter $\dot{V}O_2$ did not differ significantly at -12°C in air or at 2°C in heliox. However, $\dot{V}O_2$ at thermoneutrality was significantly higher ($\underline{P}$=.01) in winter (1.13±.08 $cc O_2$/min) than in summer (0.90±.02 $cc O_2$/min). Cold tolerance was improved in winter-acclimatized juncos relative to summer-acclimatized juncos (table 6).

PLASMA AND TISSUE METABOLITES

Winter pectoralis and liver wet masses were significantly greater than in summer at all temperatures ($\underline{P}$<.01), except for liver mass at severe cold (table 7). Wet mass of pectoralis muscle was independent of temperature at both seasons. Liver wet mass did not vary with temperature in winter, but in summer was significantly greater at severe cold than at thermoneutrality or moderate cold ($\underline{P}$<.05). Pooled temperature-independent pectoralis and liver wet masses were significantly elevated in winter relative to summer ($\underline{P}$<.001), by 28% for pectoralis muscle and 39% for liver (fig. 9).

Plasma glucose was significantly greater at thermoneutrality than at severe cold in winter. No significant differences in plasma glucose were detected at any temperature in summer (fig. 10a). The only seasonal difference in plasma glucose was at thermoneutrality, where

winter values were significantly greater than summer values (table 8). Winter plasma FFA were significantly elevated under severe cold. No difference in plasma FFA levels with temperature was documented in summer (fig. 10b). Summer plasma FFA were significantly elevated over winter levels at moderate cold, otherwise there was no significant seasonal variation in plasma FFA (table 8).

Pectoralis muscle glycogen decreased with increasing severity of cold stress at both seasons (fig. 11a). In winter, muscle glycogen stores at severe cold were significantly diminished compared to stores at thermoneutrality. Summer muscle glycogen levels under moderate and severe cold were both significantly lower than thermoneutral levels. The only significant seasonal difference in muscle glycogen occurred under severe cold, as winter values were greater than summer values (table 8), even though all winter birds in which muscle glycogen was measured remained normothermic for 2 h at this temperature, whereas all summer birds become hypothermic (mean time to hypothermia = 34.2 min). Mean winter muscle glycogen levels were 2.4X greater than summer levels under moderate cold , but this difference was not significant (P=.16). Liver glycogen stores showed no variation with temperature (fig. 11b). Pooled mean liver glycogen (winter = 2.78$\pm$.678 mg/g tissue, n=22; summer = 1.01$\pm$.156 mg/g tissue, n=21) was significantly greater in winter than in summer (P<.01).

Pectoralis muscle lactate was significantly lower

under severe cold than at thermoneutrality or moderate cold at both seasons (fig. 12). Winter pectoralis lactate levels were significantly elevated over summer levels at moderate cold, but not at thermoneutrality or severe cold (table 8). Seasonal comparisons at severe cold are hampered because all summer birds at this temperature became hypothermic. The mean pectoralis lactate level in birds becoming hypothermic in both summer (n=7) and winter (n=1) was 4.39$\pm$1.80 mg/g tissue. This was significantly lower than that for birds remaining normothermic at the same test temperatures (10.41$\pm$0.66 mg/g tissue, n=14, $\underline{P}$=.02).

DISCUSSION

Metabolic rates did not differ significantly between seasons, except at thermoneutrality, where $\dot{V}O_2$ was greater in winter than in summer (fig. 8). This is in agreement with previous results documenting increased SMR in winter-acclimatized juncos (Swanson 1990). $\dot{V}O_2$ at thermoneutrality, at night, in a post-absorptive state in this study were not significantly different from previously reported values (Swanson 1990).

Winter birds were capable of withstanding severe cold far longer than their summer counterparts (table 6). This seasonal increase in cold tolerance in fasted juncos is in accord with previous results documenting increased cold tolerance and thermogenic capacity in fed winter-acclimatized dark-eyed juncos (Swanson 1990).

Wet mass of both pectoralis muscle and liver increased in winter birds (fig. 9). This is consistent with previous studies documenting increases in mass of nonfat body components in association with winter fattening in passerines (Helms et al. 1967, Carey et al. 1978, Dawson et al. 1983b). However, lean dry mass apparently does not vary seasonally in these juncos, although body mass is significantly greater in winter (Swanson unpubl. data). Increased pectoralis muscle mass in winter may allow for elevation of total thermogenic capacity via shivering and thus contribute to increased cold tolerance capabilities. Alternatively, muscle mass in summer might be maintained at

reduced levels to allocate more energy for reproduction.

Plasma levels of glucose were significantly reduced and plasma FFA were significantly elevated in winter-acclimatized juncos under severe cold (fig. 10). No such changes in plasma metabolites with temperature were detected for summer juncos. Elevation of plasma levels of FFA under severe cold stress have also been reported for American goldfinches (Marsh and Dawson 1982) and house finches (Marsh et al. 1984), although in these species, increased FFA concentrations occurred regardless of season. Plasma glucose was also significantly decreased in winter-acclimatized American goldfinches exposed to severe cold (Marsh and Dawson 1982). The pattern of variation detected for plasma metabolites in winter juncos might be explained by increased lipolysis and decreased glycogenolysis under severe cold, although the data does not rule out variation in FFA and glucose uptake or gluconeogenesis as being causal factors.

Seasonal differences in plasma metabolites are difficult to explain. Perhaps the winter elevation of glucose at thermoneutrality is associated with an elevated SMR at that season. Increased plasma FFA in summer birds at $-12^{o}C$ might be indicative of moderate cold being a relatively greater thermoregulatory stress at that season, thereby inducing higher levels of FFA mobilization.

Pectoralis muscle glycogen declined with increased cold stress at both seasons (fig. 11a). Winter muscle

glycogen levels were significantly greater than summer levels under severe cold. This is noteworthy since all summer birds became hypothermic before completion of the two h exposure period (mean time to hypothermia = 34 min), while all winter birds examined for muscle glycogen remained normothermic for two h. Mean muscle glycogen levels were 2.4 times higher in winter than in summer at moderate cold, although this increase was not significant due to large sample variation. In absolute terms, the reduction in mean thermoneutral muscle glycogen stores due to moderate cold stress was 3.1 times greater in summer birds than in winter birds. These absolute reductions represent a 79% reduction in muscle glycogen stores for summer birds, but only a 34% reduction for winter birds. Thus, over ecologically relevant temperatures, muscle glycogen depletion appears reduced in winter-acclimatized juncos relative to summer-acclimatized juncos, even though mean levels of muscle glycogen under moderate cold stress did not differ significantly. These results suggest reduced depletion of muscle glycogen stores in winter-acclimatized juncos under cold stress and are consistent with results from other passerines exhibiting marked seasonal changes in cold tolerance (Marsh and Dawson 1982). A similar pattern of substrate metabolism, based on premigratory changes in β-oxidative and glycolytic enzyme activities, may also operate during long-distance migration in passerines (Marsh 1981).

At both seasons, severe cold stress resulted in significant depletion of pectoralis muscle lactate levels relative to thermoneutral levels (fig. 12). This could be accounted for by several mechanisms, not necessarily mutually exclusive, including reduced mobilization of muscle glycogen, reduced reliance on anaerobiosis, or increased lactate clearance. With regard to the latter, no evidence of increased glycogenesis in the liver (an important lactate clearing mechanism) under cold stress was detected in this study. Elevated glycogenesis in livers of other cold-stressed passerines has been reported (Marsh and Dawson 1982, Marsh et al. 1984). However, the data do not exclude augmented lactate clearance via other metabolic pathways (e.g. oxidation, conversion to alanine, etc.). High VO_2 recorded under severe cold (fig. 8) might allow decreased reliance on anaerobiosis, but generally greatly increased muscular activity results in increased lactate accumulation in vertebrates (Bennett 1978, Ruben and Battalia 1979). Reduction of muscle glycogen mobilization as glycogen stores become depleted under severe cold, thereby resulting in diminished lactate production, might be more plausible as muscle glycogen levels were low at these temperatures.

Birds becoming hypothermic at both seasons had significantly lower pectoralis lactate than normothermic birds at the same temperatures. Low lactate accumulation could be explained by either diminished production or

increased clearance of lactate. Increased clearance seems improbable, given that metabolic rates declined precipitously with the onset of hypothermia. Low lactate accumulation might then be explained by depression or failure of glycogen mobilization systems with the onset of hypothermia.

From a seasonal perspective, winter levels of pectoralis lactate significantly exceeded summer levels at moderate cold and were also greater, although not significantly so due to large variance in the summer sample, at severe cold where all summer birds became hypothermic (table 8). It appears that pectoralis lactate accumulation is correlated with absolute quantities of pectoralis glycogen. Therefore, lactate accumulation under cold stress may be related to glycogen mobilization. At severe cold in winter, FFA may play a proportionately more prominent role in energy provision, sparing muscle glycogen. Decreased muscle glycogen mobilization could result in lower lactate accumulation at severe cold relative to other temperatures. Since summer birds became hypothermic at severe cold, decreased lactate levels might be associated with decreased lactate production due to low glycogen mobilization and/or Q_{10} effects. Elevated lactate in winter birds relative to summer birds at moderate cold might be explained by lower absolute levels of pectoralis muscle glycogen in summer at this temperature, or by -12^{o}C being a relatively more severe cold stress for summer

birds, thus resulting in lower levels of glycogen mobilization and elevated mobilization of FFA, as suggested by increased plasma FFA levels at moderate cold in summer relative to winter.

Substrate metabolism under cold stress in the dark-eyed junco appears consistent with the glycogen sparing hypothesis (Marsh and Dawson 1982). Winter-acclimatized juncos exhibited reduced depletion of pectoralis muscle glycogen, elevated plasma concentrations of FFA, and reduced plasma concentrations of glucose under severe cold, all of which are consistent with the glycogen sparing hypothesis. Furthermore, winter juncos maintained elevated levels of pectoralis lactate under cold stress, possibly due to prolonged availability and/or improved accessibility of pectoralis muscle glycogen. In light of failure to document seasonal differences in the relative oxidation of carbohydrates and FFA in passerines in vitro (Yacoe and Dawson 1983) more study is needed to determine precisely how winter improvement of glycogen sparing is accomplished and what significance, if any, this has for improving cold tolerance.

Table 6. Cold tolerance in juncos fasted for 5 h prior to cold stress tests. Numbers in parentheses represent sample size.

% Hypothermic before 2 h

	$30^{o}C$	$-12^{o}C$	$2^{o}C/HeO_2$
Summer	0% (7)	29% (7)	100% (6)
Winter	0% (8)	0% (8)	11% (9)

Table 7. Pectoralis muscle and liver wet mass after various degrees of cold stress. Pectoralis mass is from one-half of the pectoralis muscle. Sample sizes are given in parentheses.

	30°C	-12°C	2°C/HeO_2
LIVER (g)			
Summer	0.435±.018 (8)	0.431±.018 (7)	0.534±.035 (7)
Winter	0.590±.042 (8)	0.636±.039 (7)	0.576±.099 (7)
PECTORALIS (g)			
Summer	1.196±.099 (8)	1.073±.080 (8)	1.130±.115 (8)
Winter	1.460±.176 (8)	1.466±.096 (8)	1.441±.081 (8)

Table 8. Plasma and tissue metabolites under varying cold stresses. Sample sizes are given in parentheses. * indicates significant differences between summer and winter values ($P<.05$, Student's t-test or Mann-Whitney U-test).

SUBSTRATE	30°C	-12°C	2°C/HeO_2
Plasma Glucose (mg/mL)			
Summer	3.23±.10 (6)	2.87±.25 (7)	2.96±.37 (6)
Winter	3.60±.10* (8)	3.42±.20 (8)	2.85±.25 (8)
Plasma FFA (meq/L)			
Summer	0.85±.27 (6)	1.04±.11* (7)	1.22±.23 (6)
Winter	0.59±.05 (8)	0.56±.10 (8)	1.48±.11 (8)
Pectoralis Glycogen (mg/g tissue)			
Summer	5.92±1.96 (8)	1.22±.54 (7)	0.26±.05 (6)
Winter	4.72±1.60 (8)	2.90±.93 (8)	0.65±.10* (8)
Liver Glycogen (mg/g tissue)			
Summer	0.80±.15 (8)	0.88±.22 (7)	1.46±.42 (6)
Winter	3.41±1.48 (7)	2.50±.81 (7)	2.49±1.27 (8)
Pectoralis Lactate (mg/g tissue)			
Summer	11.74±1.47 (7)	9.99±1.45 (6)	5.57±2.22 (6)
Winter	14.33±0.51 (8)	14.02±0.84 (8)	10.73±0.50 (8)

Fig. 8. Oxygen consumption under varying cold stress regimes for the last h of metabolic tests, except for summer birds at 2°C in heliox which all became hypothermic within 1 h. For 30°C and -12°C, values are means over the last h of tests. For 2°C in heliox, values represent the highest ten-min mean oxygen consumption. Sample sizes in order of decreasing temperature were 6, 8, and 4 in winter, and 8, 6, and 5 in summer.

Figure 8

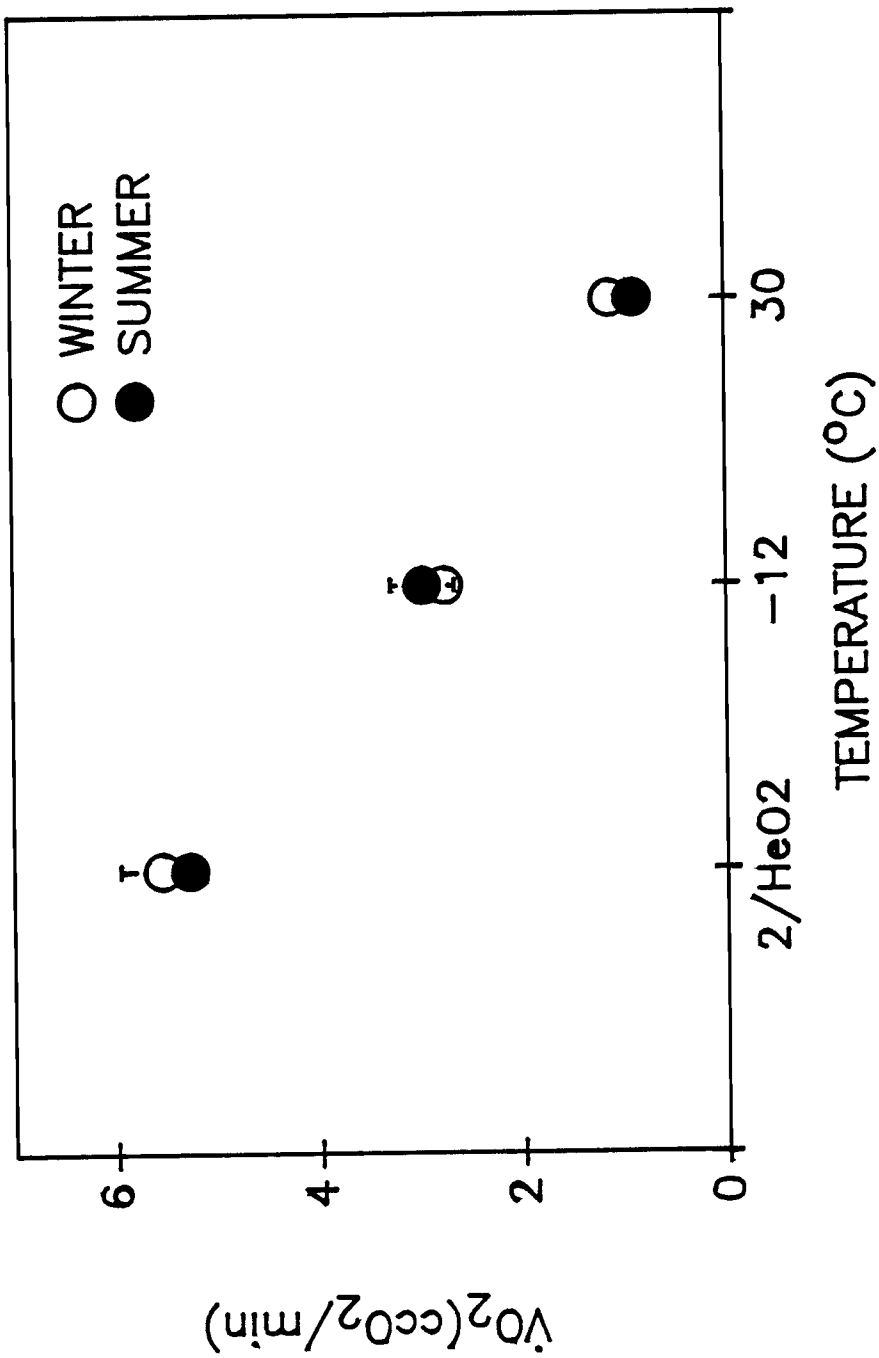


Fig. 9. Wet masses of frozen pectoralis muscle halves and liver from seasonally acclimatized dark-eyed juncos. Data was pooled when mass was independent of temperature. Both pectoralis muscle and liver were significantly heavier in winter. Numbers over bars represent sample sizes.

Figure 9

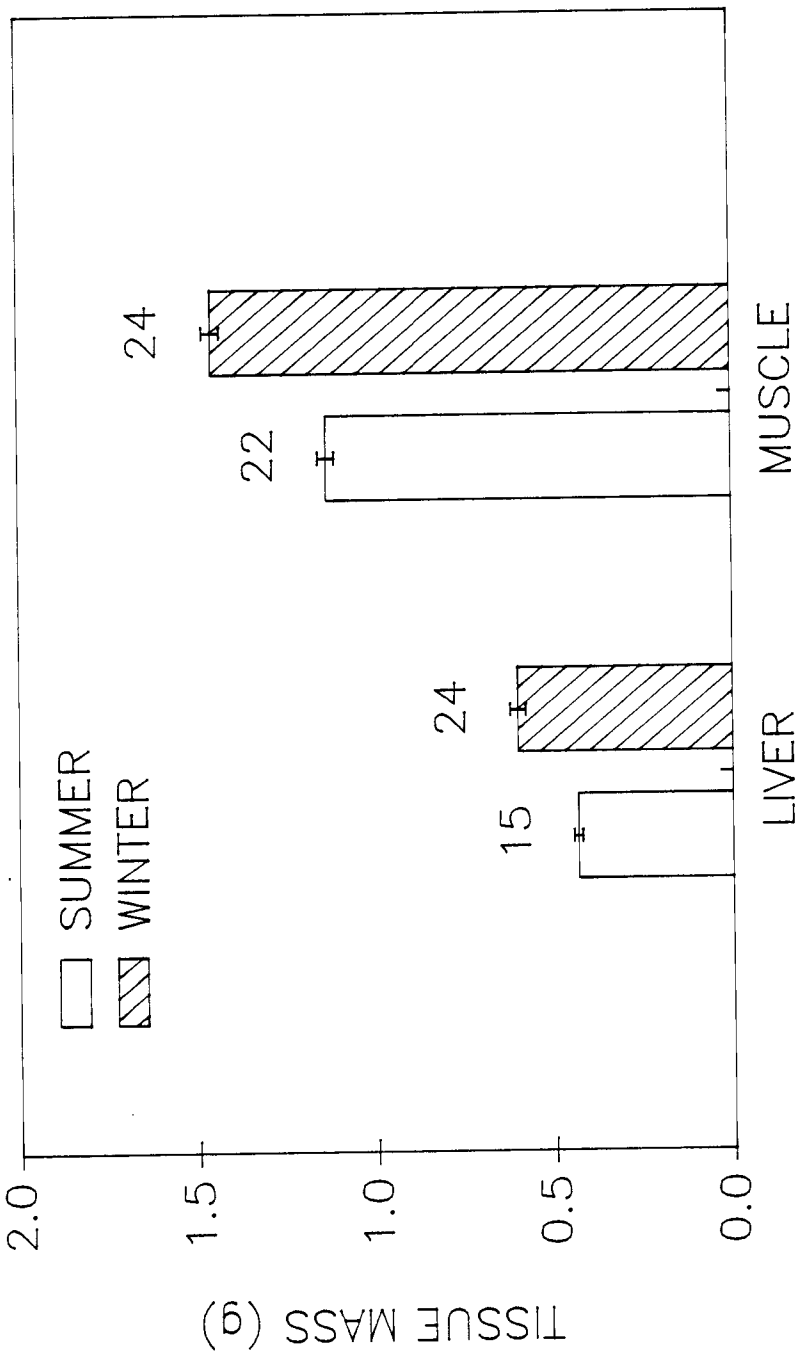

TISSUE MASS (g)
2.0
1.5
1.0
0.5
0.0
SUMMER
WINTER
24
22
24
15
MUSCLE
LIVER

Figure 10: Plasma metabolites in seasonally acclimatized juncos exposed to different cold stress regimes.

Figure 10

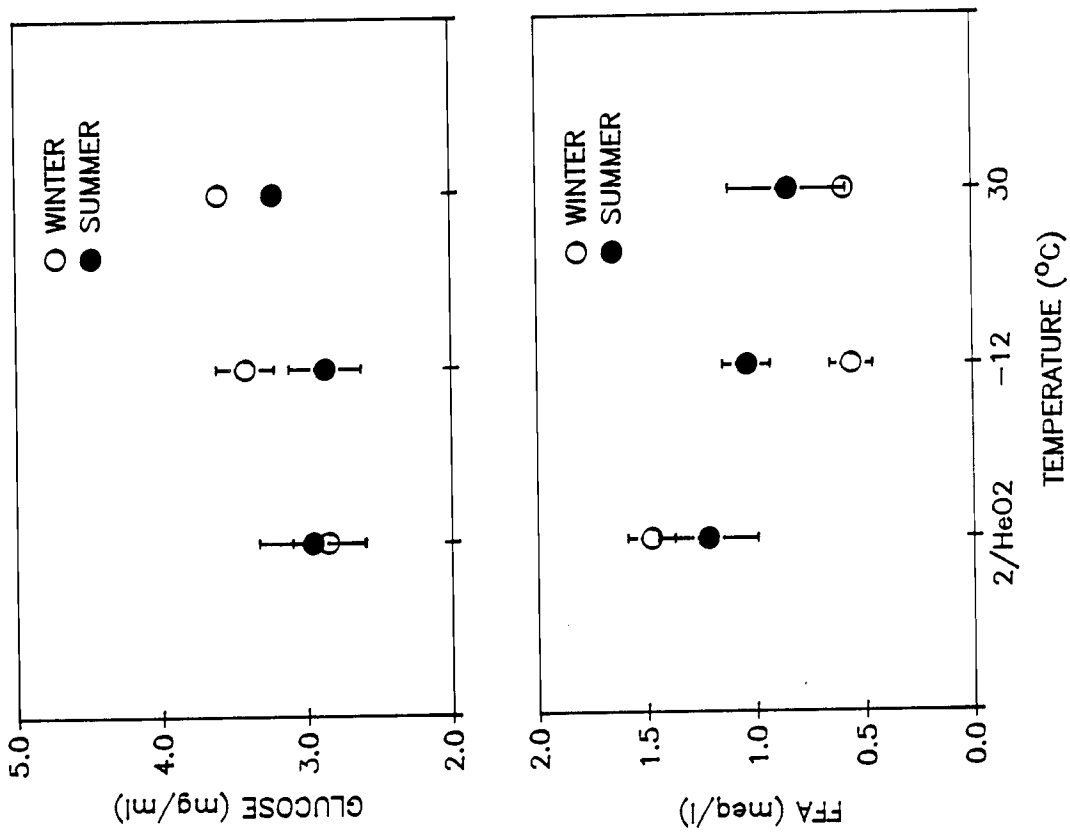

O WINTER
● SUMMER
GLUCOSE (mg/ml)
5.0
4.0
3.0
2.0
FFA (meq/l)
2.0
1.5
1.0
0.5
0.0
2/HeO2
-12
30
TEMPERATURE (°C)

Figure 11: Pectoralis muscle and liver glycogen at different levels of cold stress in seasonally acclimatized dark-eyed juncos.

Figure 11

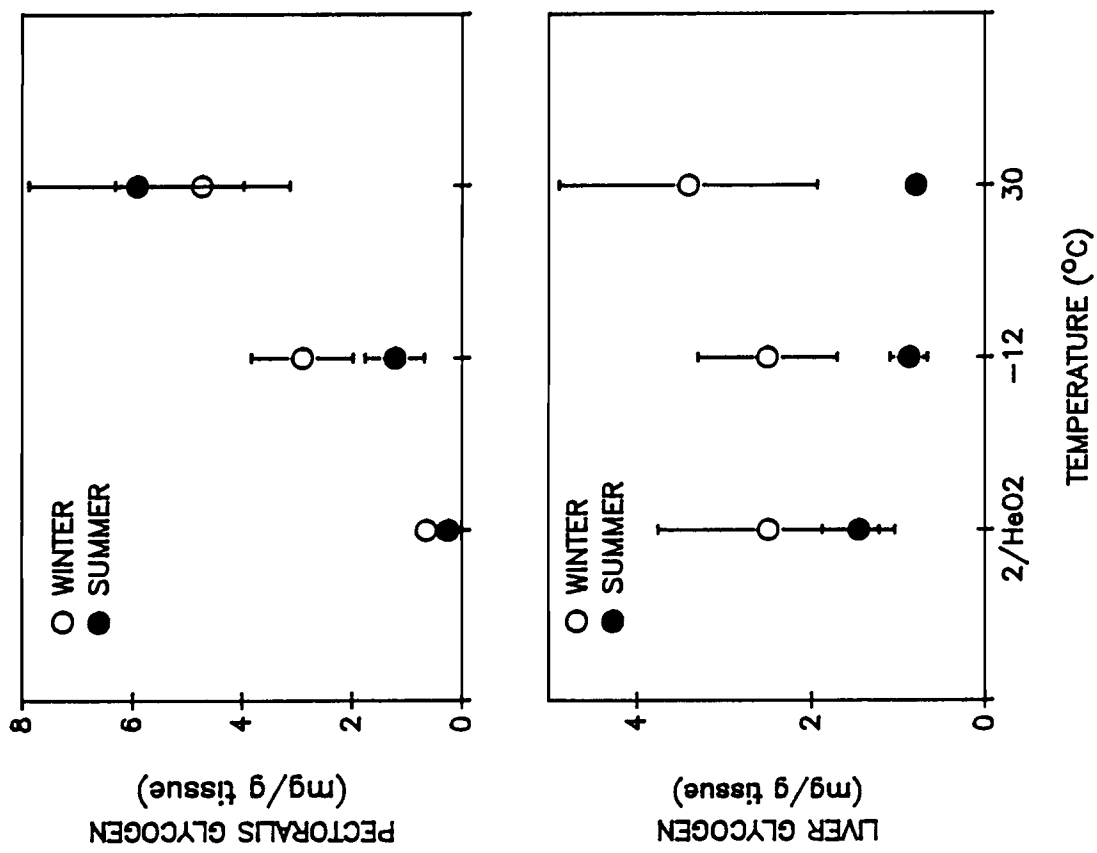
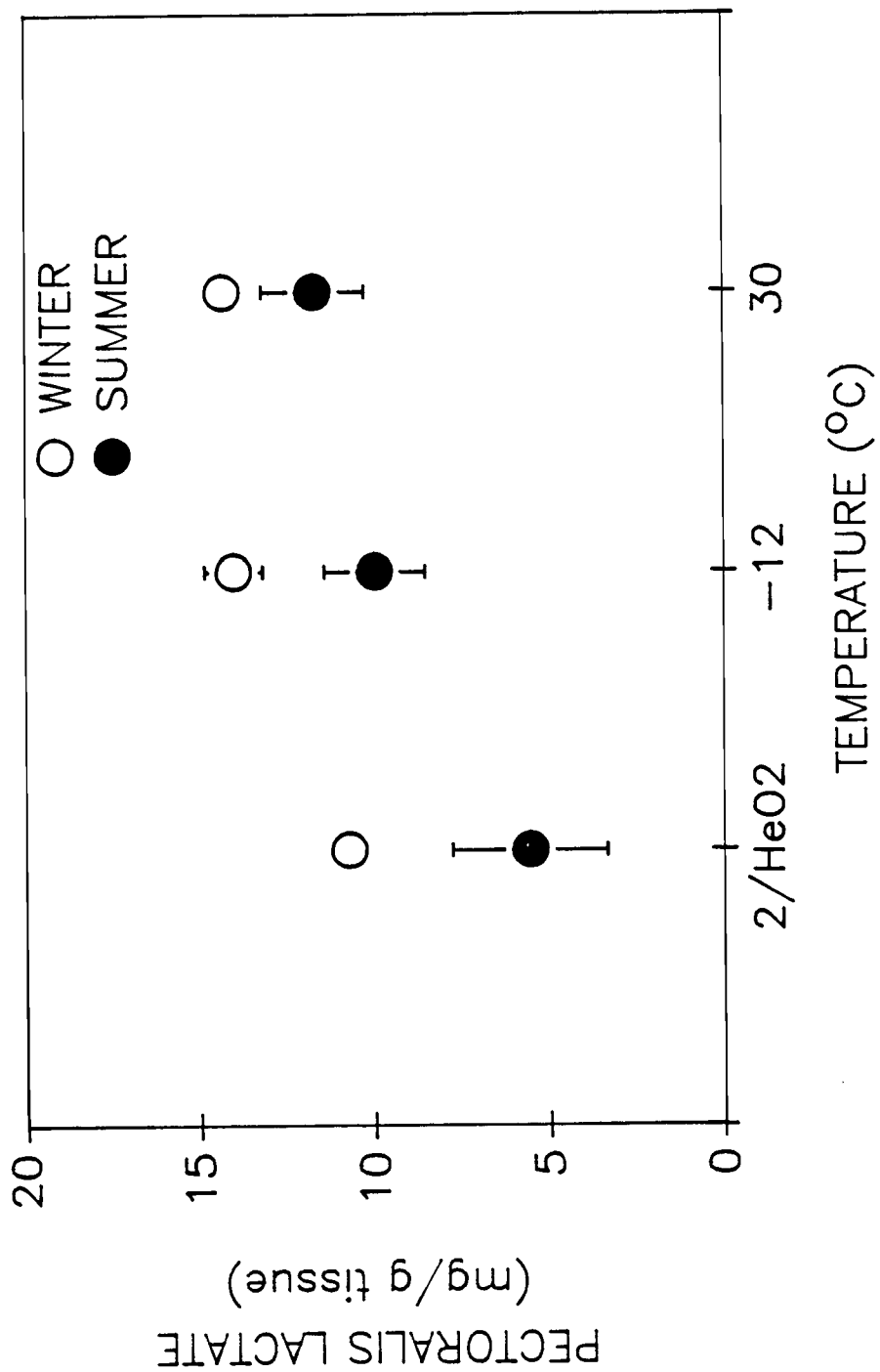

PECTORALIS GLYCOGEN
(mg/g tissue)
LIVER GLYCOGEN
(mg/g tissue)
WINTER
SUMMER
0
2
4
6
8
2/He02
-12
30
TEMPERATURE (°C)

Figure 12: The effect of varying cold stress on pectoralis lactate in seasonally acclimatized juncos.

Figure 12


PECTORALIS LACTATE
(mg/g tissue)
20
15
10
5
0
O WINTER
● SUMMER
2/He02
-12
30
TEMPERATURE (°C)

CHAPTER 6

CONCLUSIONS AND FUTURE RESEARCH

Seasonal acclimatization in the Dark-eyed Junco allows successful overwintering in western Oregon. Data presented here indicate that seasonal acclimatization in the junco is primarily metabolic rather than insulatory. This acclimatization provides increased thermogenic capacity and cold tolerance in winter birds. These results are consistent with previous studies on temperate-wintering passerines from harsh climates (Dawson et al. 1983b) and demonstrate the primary importance of metabolic adjustment to seasonal acclimatization in small passerines.

Winter increments in blood oxygen carrying capacity, exclusive of any change in oxygen affinity, contribute to meeting elevated elevated oxygen demands in an energetically-efficient manner. This was the first attempt to examine possible seasonal changes in oxygen affinity in bird blood and the demonstrated seasonal stability of oxygen affinity strengthens the conclusion that avian oxygen affinity has little capacity for modification.

Winter birds also exhibit enhanced shivering endurance correlated with seasonal changes in the metabolism of carbohydrates and lipids. This facilitates reduced winter depletion of pectoralis muscle glycogen. The mechanism and significance of winter glycogen sparing remains unclear. Hormones stimulating lipolysis and/or decreasing glycogenolysis and increasing calorigenesis may be involved

in facilitating seasonal changes in substrate metabolism and thermogenic capacity which lead to increased cold tolerance.

Future studies should examine hormonal regulation of acclimatization and an aspect of my future studies will include investigation of the possible role of GH in seasonal acclimatization. Preliminary results on summer-acclimatized Dark-eyed Juncos indicate no differences in carbohydrate or lipid metabolism between GH-injected or control birds. Perhaps metabolic response to GH is enhanced in winter-acclimatized birds, or alternatively, acute GH injection may not be effective in eliciting a metabolic response. In any case, further experiments on winter-acclimatized birds are necessary before any conclusions may be reached.

The propensity for enhanced glycogen sparing in winter juncos is similar to that documented for the American Goldfinch (Marsh and Dawson, 1982), but differs from the pattern in the House Finch (Marsh et al. 1984). Both the American Goldfinch (Dawson and Smith, 1986) and the juncos in this study show substantial winter increases in cold tolerance, while the House Finch demonstrates only limited increases (Dawson et al. 1983a). Dawson et al. (1983a) suggest that the limited winter enhancement of cold tolerance in House Finches may be associated with their mainly tropical and subtropical distribution and their relatively recent (post-glacial) invasion of regions with

harsh winters.

Based mainly on present-day distribution patterns, Miller (1941) proposed that the genus Junco arose in North or Middle America. Juncos are adapted to boreal conditions and it is likely that their immediate ancestors were similarly adapted. The present assemblage of juncos probably evolved from southern ancestors accompanying the retreat of Pleistocene glaciers. The southern highlands of Mexico have been suggested as possible sites of origin (Miller, 1941).

In light of the presumed similarity in evolutionary exposure of juncos and American Goldfinches to boreal climates, the similarity of acclimatization responses in these birds and the differences from the House Finch (Dawson et al. 1983a, Marsh et al. 1984) may be evolutionarily significant. The ability to increase thermogenic capacity and shivering endurance appear to be components of winter-acclimatization common among small temperate-wintering passerine birds (Dawson et al. 1983b). This capacity to maintain greatly elevated metabolism for prolonged periods allows successful overwintering in energetically-demanding temperate climates. Presumably, the evolution of elevated thermogenic capacity was a prerequisite to invasion of temperate or boreal environments.

The present diversity of birds decreases with increasing distance from the tropics, tropical regions

being much richer in families, genera, and species than north-temperate regions. It appears that oscine (advanced) passerines may have dispersed and radiated during the mid-Tertiary from tropical or subtropical regions of the Old World (Darlington, 1957), although this is not certain (Mayr, 1964; Vuilleumier, 1975). Nonetheless, a tropical or subtropical origin seems reasonable.

Penetration of temperate environments during the breeding season presumably reduces competition for food and nesting habitats and increases nesting success (Perrins and Birkhead, 1983). This was the presumed selective factor behind the origin of migratory behavior among low latitude residents. Present day migration routes probably evolved very recently during the last post-glacial period (Perrins and Birkhead, 1983). Early arrival on the breeding grounds appears to provide a selective advantage in establishment of territories and breeding success and this may have led to overwintering in close proximity to the breeding grounds in passerines (Ketterson and Nolan, 1976; Myers, 1981; Horvath and Sullivan, 1988). Development of high thermogenic capacity would appear necessary for successful overwintering on or near breeding grounds in temperate climates.

Temperate-wintering passerine birds exhibit high thermogenic capacities which are correlated with high levels of cold tolerance. Maximal metabolism under cold stress exceeds basal levels by 4.7 to 7.2 times in these

birds (Hart, 1962; Rosenmann and Morrison, 1974; Dawson and Carey, 1976, Dawson et al. 1983a; Dawson and Smith, 1986; Koteja, 1986; Swanson, 1990). Maximal thermogenic capacity and cold tolerance are elevated in winter in passerines wintering in temperate climates (Dawson and Smith, 1986; Swanson, 1990). Thus, it appears that high thermogenic capacities are required for temperate-wintering in passerines. Correlation of maximum thermogenic performance with geographic distribution has been demonstrated for rodents (Bozinovic and Morrison, 1989).

Very little is known of relative thermogenic mechanisms and capacities between temperate, tropical, and migrant birds. Standard metabolic rate is lower in many tropical birds than in temperate birds of equal mass (Vleck and Vleck, 1979; Weathers, 1979; Weathers and Van Riper, 1982; Bartholomew et al. 1983; Hails, 1983). Furthermore, SMR is broadly correlated with latitude in birds, showing approximately a 1% increase for every degree increase in latitude (Weathers, 1979). In addition, growth rates in tropical birds appear to be lower than in their temperate counterparts, suggesting reduced metabolic rates (Ricklefs, 1976). This reduced resting metabolic level may indicate a corresponding reduction in maximal thermogenic capacity. However, House Finches acclimatized to winter in Colorado and southern California showed significantly different maximal thermogenic capacity (15% greater in Colorado birds) but statistically indistinguishable SMR's (Dawson et

al. 1983a).

Maximal thermogenic capacity under cold stress has been determined only for temperate-wintering passerines so nothing is known of maximal thermogenic capacities in neotropical migrants or in tropical residents. Perhaps inability to sustain high metabolic rates necessary for overwintering in temperate environments is a physiological factor precluding expansion of ranges to temperate regions in these birds. Elucidation of thermogenic abilities in tropical and migratory birds might provide insight into the evolution of present-day passerine distribution.

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