INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

PHYSIOLOGICAL ADAPTATIONS FOR OVERWINTERING BY THE BLACK-CAPPED CHICKADEE (*Parus atricapillus*) IN INTERIOR ALASKA (64°N)

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Susan Marie Sharbaugh, B. A.

Fairbanks, Alaska

December 1997

PHYSIOLOGICAL ADAPTATIONS FOR OVERWINTERING BY THE BLACK-CAPPED CHICKADEE (*Parus atricapillus*) IN INTERIOR ALASKA (64°N)

By

Susan Marie Sharbaugh

RECOMMENDED:

Advisory Committee Chair

Department Head, Biology and Wildlife

APPROVED:

Dean, College of Science, Engineering, and Mathematics

Dean of the Graduate School

12-1-97

Date

ABSTRACT

Winter in interior Alaska (64° N) is characterized by short photoperiod (LD 5:19) and chronic subfreezing temperatures. These conditions present a physiological challenge to overwintering animals. This challenge increases as body size decreases. In small animals, higher surface to volume ratio and concomitant higher mass-specific metabolic rate (compared to larger animals) vastly increase energetic demand. This thesis focuses on the physiological adaptations of the smallest (12 g) overwintering bird in interior Alaska, the Black-capped Chickadee (*Parus atricapillus*).

To determine if seasonal acclimatization of Black-capped Chickadees at high latitude differs from that of conspecifics at lower latitudes, standard metabolic rates (SMR), metabolic response to low temperature (-30° C), use of nocturnal hypothermia, body mass, fat reserves, and conductance were measured over two winters and one summer in three groups of seasonally acclimatized captive birds. Body mass and conductance did not vary with season, but furcular fat levels were higher in winter. Birds used nocturnal hypothermia when exposed to -30° C in summer or winter. Although SMR did not vary seasonally, winter SMRs differed between the two winters studied. Nocturnal hypothermia in summer and decreased SMR during winter have not been reported in conspecifics at lower latitudes.

Lipid reserves play an important role in fueling the energy demands of overwintering birds. Black-capped Chickadees exhibit a daily and seasonal cycle of fattening. At high latitudes (64° N), fat to fuel an 18 hour nocturnal fast is deposited during 6 hours of daylight available for foraging. Daily fattening rates

are highest in December when ambient conditions are most limiting. Activity of the fat deposition-promoting enzyme, adipose lipoprotein lipase (ALPL), was measured in furcular fat samples from seasonally acclimatized captive birds to determine its role in daily and seasonal fattening cycles. ALPL activity levels were 20 - 30 times higher than those previously reported for passerines. Seasonal variation in enzyme activity positively correlated with changes in fattening rates from December to April. Alaskan birds displayed the highest level of activity when compared to conspecifics at lower latitudes. All subpopulations measured exhibited activity higher than previously reported for small birds.

Table of Contents

Abstract....iii

List of Figures and Tables....vii

List of Tables....viii

Acknowledgments....ix

Introduction....1

Chapter One - Seasonal Acclimatization to Extreme Climatic Conditions by Black-capped Chickadees (*Parus atricapillus*) in Interior Alaska (64° N)....15

- Abstract....16
- Introduction....17
- Material and Methods....19
- Results....23
- Discussion....25
- Acknowledgments....31
- Literature Cited....32

Chapter Two - Adipose Lipoprotein Lipase Activity and Winter Fattening in the Black-capped Chickadee (*Parus atricapillus*) in Interior Alaska....44

- Abstract....45
- Introduction....46
- Material and Methods....48
- Results....52
- Discussion....53

Acknowledgments....58
Literature Cited....59
Chapter Three - Latitudinal Variation in Adipose Lipoprotein Lipase Activity in Winter-Acclimatized Black-capped Chickadees (*Parus atricapillus*)....71
Abstract....72
Introduction....73
Methods and Materials....75
Results....77
Discussion....78
Acknowledgments....84
Literature Cited....85
Summary and Conclusions....92
Literature Cited....96

List of Figures

Chapter One

Figure 1 Monthly Record Low, Normal Low, Normal High and Record High temperatures (°C) in Fairbanks, AK from 1941 to present.....39

Figure 2 Oxygen consumption of seasonally acclimatized Black-capped Chickadees over two winters and one summer.....40

Figure 3 Overnight oxygen consumption by a seasonally acclimatized Black-capped Chickadee at three different ambient temperatures.....41

Figure 4 Body temperatures before and after metabolic trials at -30° C and 25° C in 1995.....42

Figure 5 Minimum daily temperatures during the time of metabolic experiments in 1994 and 1995.....43

Chapter Two

Figure 1 Monthly Record Low, Normal Low, Normal High and Record High temperatures (°C) in Fairbanks, AK from 1941 to present.....64

Figure 2 Changes in winter fat levels of individual wild Black-capped Chickadees in January 1991 and 1992.....65

Figure 3 Seasonal change in fattening rate and associated changes in photoperiod in wild Black-capped Chickadees in interior Alaska...66

Figure 4 Seasonal *in vitro* adipose lipoprotein lipase of winter-acclimatized Black-capped Chickadees.....67

Figure 5 *In vitro* adipose lipoprotein lipase activity in three species.....68

Figure 6 Apparent Michaelis-Menten constants for winter-acclimatized Black-capped Chickadee adipose lipoprotein lipase.....69

Figure 7 *In vitro* adipose lipoprotein lipase activity vs. substrate concentration at five temperatures.....70

Chapter Three

Figure 1 Distribution of the Black-capped Chickadee in North America.....89

Figure 2 Mass and wing length of winter-acclimatized Black-capped Chickadees captured in western Washington, northwestern New York and interior Alaska.....90

Figure 3 In vitro adipose lipoprotein lipase activity in three populations of winter-acclimatized Black-capped Chickadees. Samples incubated at five temperatures.....91

List of Tables

Chapter One

Table One Summer and winter standard metabolic rate of Black-capped Chickadees in North America and Willow Tits in Norway.....38

Chapter Two

Table One Black-capped Chickadee adipose lipoprotein lipase activity (μMol free fatty acids (FFA)·unit time^{-1}·mg protein^{-1}) as a function of duration of incubation, NaCl concentration and incubation temperature.....63

Acknowledgments

This project would not have been possible without the financial and moral support of many people. Monetary support has come from the Department of Biology and Wildlife in the form of Teaching Assistantships and travel money, the American Heart Association provided a yearlong fellowship and supply money to collect samples in the Lower 48, a National Institutes of Health startup grant provided funding for the initial adipose lipoprotein lipase (ALPL) work, the North American Bluebird Association granted money to begin the metabolic work, Dr. Robert White provided additional funding from the Institute of Arctic Biology to continue that work. Sigma Xi and the Arctic Institute of North America provided travel and supply money to develop the ALPL assay in Fairbanks and the Graduate School provided travel money and tuition scholarships for my final two semesters.

I would like to thank my advisory committee, Dr. Pierre Deviche, chair, and Drs. Brian Barnes, Dale Feist, and Jim Sedinger. I appreciate the role they have played in my professional development and I know I am a better scientist because of their teaching. I am also grateful to the faculty of the Institute of Arctic Biology and the Department of Biology and Wildlife. I have benefited greatly from interacting with them on various topics; they were instrumental in broadening my appreciation of all fields in biology.

Personally, I am indebted to my friends and colleagues for their unfailing support over the last six years. It's a long row to hoe, but it is made tolerable by commiseration. Thanks to fellow grad students, Maria Berger, Amy Larsen, Maggie MacCluskie, Kate Doran, Jim Lawler, Paul Matheus, Christa Mulder, Tina Moran, Renee Crain, and Cindy and Jay Gulledge. My buddies at the University

of Washington, Drs. Tom Hahn, Julie Coombs, Marilyn Ramenofsky, John Wingfield, Ken Morgan and Jim Kenagy, encouraged me to start this adventure. Special thanks to Paul Cotter and Loren Buck, from them I learned much about science, life, and the internal combustion engine.

Chapter Two would not have been possible without the collaboration of my two co-authors, Drs. Pierre Deviche and Marilyn Ramenofsky. A fruitful discussion in a sunny Oregon courtyard resulted in the pursuit of ALPL in northern chickadees. Thanks to Dr. Deviche for the use of his fattening rate data and assisting in late night sampling. Dr. Ramenofsky provided the inspiration and expertise, instruction in assay protocol, and undying encouragement.

At last, I must acknowledge my family as the core of my support system. Steve, Tressa, Gretchen, Sally, Mom and Dad (Juanita and Burley) provided just the right words of encouragement at just the right times. In an evolutionary light, it makes little sense in terms of parental investment to devote so many emotional (and financial) resources in an offspring with rapidly diminishing reproductive potential pursuing a non-traditional career choice. I am eternally grateful for their love and support.

INTRODUCTION

Winter Acclimatization in Birds

The central tenet of ecological physiology is the determination of the physiological mechanisms that allow animals to live where they do (Bennett 1987). This research generally focuses on animals in extreme environments or at times of high energy expenditure in their annual cycle. It is during these challenging times that the subtle details of basic physiological processes come to light. The physiological adaptations that allow an organism to cope with a clearly definable challenge (e.g., extremes in temperature, high pressures or extremes of pH) illustrate mechanisms that underlie the usually inconspicuous aspects of fundamental physiology (Bartholomew 1987).

An extreme environment that has long fascinated physiologists is the Arctic. How animals live through the long periods of extremely low temperatures and short daylength of the arctic winter has been a question that has intrigued scientists for generations. The work of Per Scholander, Laurence Irving, Peter Morrison, and Mario Rosenmann has laid a solid foundation in the study of arctic adaptations. The classic papers of these investigators introduced the concept of lower critical temperature (Scholander et al. 1950a), defined the role of insulation in cold adaptation (Scholander et al. 1950b), and pioneered the use of a helium/oxygen (helox) mixture to facilitate maximum conductance (which in turn allows measurement of maximal energy expenditure) (Rosenmann and Morrison 1974). Modern techniques can be combined with this established framework to help understand the physiology of numerous arctic residents yet to be studied.

One group of animals that has puzzled biologists is the small passerines that overwinter at high latitudes. The physiological challenge to meet the energetic demands of maintaining basic physiological processes increases as body size decreases (Schmidt-Nielsen 1990). In these birds, higher surface to volume ratio, coupled with higher mass specific metabolic rates compared to larger animals, increases rates of mass-specific energy expenditure. This is not a liability when food is abundant, but when access to these resources is restricted by short daylength or inclement weather, maintaining a positive energy balance presents a considerable challenge to these small birds (King 1974).

The smallest (12 grams) bird overwintering in Interior Alaska is the Black-capped Chickadee (*Parus atricapillus*). With a geographic distribution extending to the Carolinas and Arizona, this bird is not adapted exclusively to the extremes of the Far North. The ability of chickadees to persist across a wide range of environmental conditions suggests great flexibility in their basic physiological makeup. The Black-capped Chickadee is divided into 9 subspecies (Smith 1991) across its range. One subspecies, the Yukon Chickadee (*P. a. turneri*), resides in Interior Alaska. In terms of temperature and photoperiod, this subspecies lives in the most severe climatic conditions encountered by the species.

The study of avian adjustments to cold began with the work of Miller and Dontcheff and Kayser in the 1930's on sparrows and domestic pigeons. Almost seventy years of experiments have established that overwintering birds can meet the metabolic challenge of low temperature by modifying heat production or heat retention (increased insulation through ptiloerection and local vasoconstriction), substrate utilization, and through behavioral adjustments (favorable roost site selection, caching) (Marsh and Dawson 1989). My thesis has

focused on the first two mechanisms. Specifically, I used Black-capped Chickadees to investigate metabolic adjustments (seasonal variation of standard metabolic rate and the use of nocturnal hypothermia) and the use of fat as a fuel to meet the energetic demands of overwintering in Interior Alaska (64°N).

In an environment with large seasonal fluctuations in ambient temperature and daylength, maintaining a stable body temperature necessitates modifications in the mechanisms of thermoregulation. King and Farner (1961) define these climate-induced changes as acclimatization. This homeostatic process allows the organism to maintain body temperature within normal limits at a minimal energetic cost. In contrast, acclimation consists of metabolic changes that are induced by exposure to varying temperatures in a laboratory setting (Davenport 1992). The study of seasonal acclimatization has focused on the seasonal change in standard metabolic rate (SMR) as a measure of the metabolic intensity (Farner and King 1961) and the metabolic cost of thermoregulation at low temperatures. Measuring SMR (the metabolic rate of a resting, post-absoptive animal in a dark, thermoneutral environment) at different seasons will show if the basic metabolic expenditure increases during the winter in response to increased thermoregulatory demands. During the winter, small birds at high latitudes sometimes experience a temperature gradient of 91° C, internal body temperature (41°C) to ambient conditions (-50°C) across 1 cm of feather insulation. Determining the metabolic rate of the bird exposed to low temperatures provides a measure of the thermogenic capacity of the bird (Dawson and O'Connor 1996), and provides information on the extent to which metabolism can increase above SMR in response to a thermal challenge.

Variation in Seasonal Acclimatization and Nocturnal Hypothermia

My interest in the seasonal acclimatization of Black-capped Chickadees is founded on previous work on the family Paridae. Odum (1943) was the first to report that Black-capped Chickadees exhibit a winter increase in cold resistance. Work by Chaplin (1974, 1976), Cooper and Swanson (1994) and Dutenhoffer and Swanson (1996) has added to this knowledge. Examining birds obtained from two latitudinally similar (42°N), but geographical distinct (upstate New York and South Dakota) areas, these authors found that both populations display a greater tolerance for cold during the winter than during other seasons. In South Dakota, SMR varied with season (higher in the winter) but the New York birds exhibited no variation in SMR with season. When exposed to subfreezing temperatures at night, winter-acclimatized birds in both areas reduced their core body temperature (nocturnal hypothermia). This drop in body temperature is a controlled process used to reduce energy expenditure, not the loss of thermoregulatory capabilities (Chaplin 1974). Nocturnal hypothermia was not used by summer-acclimatized birds in either region. Reinertsen and Haftorn (1983, 1986) studied a related parid, the Willow Tit (*Parus montanus*), in Norway at 63°N. These birds did not increase their SMR during the winter (no seasonal variation), and, as in the above studies, only winter-acclimatized birds used nocturnal hypothermia. All of these studies consistently showed use of an energy-saving mechanism, nocturnal hypothermia, by winter-acclimatized birds. In contrast, an increase in SMR in response to more thermally challenging conditions was recorded in only one population (South Dakota).

Seasonal variation in SMR has been measured in other species as well. An annual cycle in SMR was first described in pigeons by Dontcheff and Kayser in

1934. An increase in winter SMR also has been reported for the European Goldfinch (*Carduelis carduelis*), and the Mute Swan (*Cygnus olor*) (Dawson and Marsh 1989). In contrast, a few species, such as two high latitude residents, King Penguin chicks, *Aptenodytes patagonicus* (Barre 1986) and Willow Ptarmigan, *Lagopus lagopus* (West 1972), reduce their SMR in winter. Finally, some species show no seasonal variation in SMR, including Yellow Bunting, *Emberiza citrinella*, (Wallgren 1954), Northwest Crow, *Corvus caurinus*, Black Brant, *Branta nigricans*, (Irving et al. 1955), Great Tit, *Parus major*, (Hissa and Palokangas 1976), Greenfinch, *Carduelis chloris*, and Siskin, *C. spinus* (Saarela 1988).

Although the role of a seasonally variable SMR is uncertain, use of nocturnal hypothermia by Black-capped Chickadees and Willow Tits increases the ability of these birds to survive long winter nights (Reinertsen 1989). By reducing core body temperature at night, the thermal gradient between ambient temperature and core body temperature is reduced. This lower gradient decreases heat loss. Thus, the overnight cost of thermoregulation is lowered. Hiebert (1990) examined the net energy savings of nocturnal hypothermia in Rufous Hummingbirds (*Selasphorus rufus*), including the cost of rewarming. She calculated that when the ambient temperature is lower than 18 ° C, any nocturnal hypothermia reduces total energy expenditure, even if the bird rewarms immediately. Above 18 ° C, hypothermic and normothermic birds incur the same costs. Steen (1958) calculated that the use of nocturnal hypothermia by Willow Tits resulted in a 32 - 45% savings in metabolic costs.

Being diurnal feeders, parids undergo a daily nocturnal fast. During this fast, metabolic expenditures are fueled by energy stored in their body (Blem 1990) in the form of fat and glycogen (Chaplin 1974). On a per gram basis, fat is a

more energy-dense substrate (4.0 kcal g^{-1} for glycogen vs. 9.0 kcal g^{-1} for fat) (Blem 1990). In small birds, glycogen depletion may require only a few hours (Whittow 1986). So, even the maximal contribution of glycogen stores are minor when compared to fat (Dawson and O'Connor 1996). Regardless of the substrate used, lowering metabolic costs allows a more efficient use of all energy reserves.

In the light of these findings, it is surprising that Black-capped Chickadees residing in interior Alaska have been reported not to use nocturnal hypothermia (Grossman and West 1977). In this study, birds exposed to temperatures as low as -50°C retained high body temperature (39° - 41°C) throughout the night, and their SMRs were much higher than those recorded for conspecifics residing at lower latitudes. If this population does not use hypothermia to reduce nocturnal energy expenditure, does it use other physiological adaptations to survive severe environmental conditions? Is there a constraint on the use of nocturnal hypothermia? I address these questions in Chapter One.

Winter Fattening

Chapters Two and Three focus on another component of overwintering at high latitudes. One aspect of cold acclimatization is the series of metabolic adjustments made to maintain normothermy. A second mechanism used to regulate positive energy balance is modulation of energy stores. Fat is an important energy source when foraging is not possible (nightly fasts). How do birds constrained by short daily periods for foraging and severe climatic conditions meet the demands of fueling their daily energy requirements and build fat stores large enough to last from dusk until the next morning?

Winter fattening is a common phenomenon among many species of birds in the northern hemisphere (Blem 1976). King and Farner (1966) postulated that this process evolved in response to the energetic demands of the long, cool (or cold) winter nights coupled with limited access to food due to inclement weather or snow cover and short duration of foraging time. The magnitude of winter fattening varies with latitude and regional environmental conditions, with the highest levels of fattening occuring at the highest latitudes (Biebach 1996). In some small passerines, a cycle of daily fattening is superimposed upon this seasonal pattern. These birds, due to their small size, are unable to store enough fat to fuel more than overnight costs and a few hours the following morning (Blem 1976). They must replace these lipid stores daily to survive another night.

This daily and seasonal fattening cycle reaches its peak amplitude in winter (Lehikoinen 1987). At this time, available foraging time is at its shortest and thermal demands may be highest. Wintering chickadees in temperate and subarctic regions lose ≈ 8% of their body mass overnight (Chaplin 1974, Haftorn 1989). This weight loss is due to a decrease in fat stores, not lean body mass (Chaplin 1974). Each winter day chickadees must replace the energy stores they used the previous night (Blem 1990). The predicted maximum fat deposition rate for a 10 - 20 gram passerine is 4.3 - 5.4% of total body mass day $^{-1}$ (Lindstrom 1991). Thus, winter-acclimatized chickadees are doubling this rate. How do these birds manage to nearly double this rate under harsh environmental conditions?

The limiting factors that determine the amount of fat that birds can deposit (and the rate of deposition) during winter or before migration have traditionally been thought to be either the amount of food available or the ability of the gut to process ingested food (Lindstrom 1991). One other limiting factor

may be the ability of adipose tissue to sequester free fatty acids. Chapter Two of my thesis examines the mechanism of actual fat deposition.

What controls the breakdown of triacylglycerols circulating in the bloodstream into the free fatty acids necessary for lipid accumulation? Fat deposition and utilization depend on the presence of the enzyme lipoprotein lipase (LPL), which catalyzes the hydrolysis of circulating triacylglycerols into free fatty acids (FFA) and glycerol (Eckel 1987). Free fatty acids can then diffuse across cell membranes to be used for energy production (in muscle) or fat storage (in adipose tissue). Thus, increased LPL activity induces a higher rate of lipid deposition in adipose tissue and of lipid utilization in muscle (Ramenofsky 1990). Differential activity of lipoprotein lipase also has been proposed in another instance of high energy demand in small birds. During migration, small passerines use increased levels of very low density lipoproteins (VLDL) in the blood to deliver fatty acids to flight muscles (Jenni-Eiermann and Jenni 1992). Increased activity of muscle lipoprotein lipase would enhance the uptake of FFAs during endurance locomotion (Nilson et al. 1980). Could a seasonal increase in adipose lipoprotein lipase (ALPL) activity be responsible for the high fattening rate observed in free-living Black-capped Chickadees? Chapter Two tests this hypothesis and describes the role of ALPL in fat deposition on a daily and seasonal basis.

Winter fattening levels are modulated by environmental conditions (Blem 1990). Chapter Three compares ALPL activity in three geographically different populations of Black-capped Chickadees. Fat samples were collected from birds overwintering in the Pacific Northwest (mild winter conditions), upstate New York (temperate winter conditions), and interior Alaska (subarctic winter

conditions). This chapter examines the influence of local climate on the activity of ALPL.

The goal of this project was to elucidate the physiological mechanisms used by a small passerine to survive in an extremely challenging environment. Modifications of two aspects of basic physiology, thermoregulation and fat deposition, are fundamental to homeostasis at high latitude. Comparing these alterations with those of conspecifics residing in less challenging climates helps us understand patterns of physiological adaptation.

Literature Cited

Barre H. 1986. Metabolic and insulative changes in winter- and summer-acclimatized king penguin chicks. Journal of Comparative Physiology B 154: 317 - 323.

Bartholomew G. A. 1987. Interspecific comparison as a tool for ecological physiologists. In: G. A. Bartholomew (ed), New Directions in Physiological Ecology. Cambridge University Press, New York, pp. 5 - 15.

Bennett A. T. 1987. New Directions in Physiological Ecology. In: G. A. Bartholomew (ed), New Directions in Physiological Ecology. Cambridge University Press, New York, pp. 1 - 8.

Biebach H. 1996. Energetics of winter and migratory fattening. In: C. Carey (ed), Avian Energetics and Nutritional Ecology. Chapman and Hall, New York, pp. 280 - 323.

Blem C. R. 1976. Patterns of lipid storage and utilization in birds. American Zoologist 16:671 - 684.

Blem C.R. 1990. Avian energy storage. In: D. M. Power (ed), Current Ornithology. Vol. 7. Plenum Press, New York, pp. 59 - 113.

Chaplin S. B. 1974. Daily energetics of the Black-capped Chickadee, *Parus atricapillus*, in winter. Journal of Comparative Physiology 89:321 - 330.

Chaplin S. B. 1976. The physiology of hypothermia in the Black-capped Chickadee, *Parus atricapillus*. Journal of Comparative Physiology 112:335 - 344.

Cooper S. J. and D. L. Swanson. 1994. Seasonal acclimatization of thermoregulation in the Black-capped Chickadee. Condor 96:638 - 646.

Dawson W. R. and R. L. Marsh. 1989. Metabolic acclimatization to cold and season in birds. In: C. Blech and R. E. Reinertsen (eds), Physiology of Cold Adaptation in Birds. Plenum Press, New York, pp. 83 - 94.

Dawson W. R. and T. P. O'Connor. 1996. Energetic features of avian thermoregulatory responses. In: C. Carey (ed), Avian Energetics and Nutritional Ecology. Chapman and Hall, New York, pp. 85 - 124.

Davenport J. 1992. Animal Life at Low Temperature. Chapman and Hall. London.

Dutenhoffer M. S. and D. L. Swanson. 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiological Zoology 69:1232 - 1254.

Eckel R. H. 1987. Adipose tissue lipoprotein lipase. In: J Borensztajn (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago, pp 277 - 329.

Grossman A. F. and G. W. West. 1977. Metabolic rate and temperature regulation of winter acclimatized Black-capped Chickadees, *Parus atricapillus*, of interior Alaska. Ornis Scandinavica 3:127 - 138.

Haftorn S. 1989. Seasonal and diurnal body weight variations in titmice, based on analyses of individual birds. Wilson Bulletin 101:217 - 235.

Hiebert S. M. 1990. Energy costs and temporal organization of torpor in the Rufous Hummingbird (*Selasphorus rufus*). Physiological Zoology 63:1082 - 1097.

Hissa R. and R. Palokangas. 1970. Thermoregulation in the titmouse (*Parus major* L.). Comparative Biochemistry and Physiology 33:941 - 953.

Hochachka P. and G. Somero. 1973. Strategies of Biochemical Adaptation. W. B. Saunders Company, Philadelphia, PA.

Irving L., J. Krog, and M. Monson. 1955. The metabolism of some Alaskan animals in winter and summer. Physiological Zoology 28:173 - 185.

Jenni-Eiermann S. and L. Jenni. 1992. High plasma triglyceride levels in small birds during migratory flight: a new pathway for fuel supply during endurance locomotion at very high mass-specific metabolic rates. Physiological Zoology 65:112 - 123.

King J. R. 1974. Seasonal allocation of time and energy resources in birds. In: R. A. Paynter, Jr. (ed), Avian Energetics. Nuttall Ornithological Club. no. 15, Cambridge, MA, pp. 4 - 70.

King J. R. and D. S. Farner. 1961. Energy metabolism, thermoregulation and body temperature. In: A. J. Marshall (ed), Biology and Comparative Physiology of Birds, vol 2. Academic Press, London, pp. 215 - 288.

Lehikoinen E. 1987. Seasonality of the daily weight cycle in wintering passerines and its consequences. Ornis Scandinavica 18:216 - 226.

Lindstrom A. 1991. Maximum fat deposition rates in migrating birds. Ornis Scandinavica 22:12 - 19.

Marsh R. L. and W. R. Dawson. 1989. Avian adjustments to cold. In: L. C. H. Wang (ed), Advances in Comparative and Environmental Physiology. Springer-Verlag, Berlin, pp. 203 - 253.

Miller D. S. 1939. A study of the physiology of the sparrow thyroid. Journal of Experimental Zoology 80:259 - 269.

Odum E. P. 1943. Some physiological variations in the Black-capped Chickadee. Wilson Bulletin 55:178 - 191.

Ramenofsky M. 1990. Fat storage and fat metabolism in relation to migration. In: E. Gwinner (ed), Bird Migration. Physiology and Ecophysiology. Springer-Verlag, Berlin, pp 214 - 231.

Reinertsen R. E. 1989. The regular use of nocturnal hypothermia and torpor during energetically-demanding periods in the annual cycles of birds. In: A. Malan and B. Canguilhem (eds). Living in the Cold. John Libbey Euortext Ltd., London, pp. 107 - 116.

Reinertsen R. E. and S. Haftorn. 1983. Nocturnal hypothermia and metabolism in the Willow Tit *Parus montanus* at 63°N. Journal of Comparative Physiology 151:109 -118.

Reinertsen R. E. and S. Haftorn. 1986. Different metabolic strategies of northern birds for nocturnal survival. Journal of Comparative Physiology B 156:655 - 663.

Rosenmann M. and P. Morrison. 1974. Maximum oxygen consumption and heat loss facilitation in small homeotherms by HeO_2. American Journal of Physiology 226: 303 - 310.

Saarela S., B. Klapper and G. Heldmaier. 1989. Thermogenic capacity of greenfinches and siskins in winter and summer. In: C. Blech and R. E. Reinertsen (eds), Physiology of Cold Adaptation in Birds. Plenum Press, New York, pp. 115 - 122.

Schmidt-Nielsen K. 1990. Animal Physiology. Adaptation and Environment. Cambridge University Press, New York, New York.

Scholander P. F., R. Hock, V. Walters, F. Johnson and L. Irving. 1950a. Heat regulation in some arctic and tropical mammals and birds. Biological Bulletin 99:237 - 258.

Scholander P. F., R. Hock, V. Walters, and L. Irving. 1950b. Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. Biological Bulletin 99:259 - 271.

Smith S. M. 1991. The Black-capped Chickadee. Behavioral Ecology and Natural History. Cornell University Press, Ithaca, New York.

Steen J. 1958. Climatic adaptation in some small northern birds. Ecology 39:625 - 629.

Wallgren H. 1954. Energy metabolism of two species of the genus Emberiza as correlated with distribution and migration. Act Zoologica Fennica 84:1 -110.

West G. C. 1972. Seasonal differences in resting metabolic rate of Alaskan ptarmigan. Comparative Biochemistry and Physiology A 42:867 - 876.

Chapter One

Seasonal Acclimatization to Extreme Climatic Conditions by Black-capped Chickadees (*Parus atricapillus*) in Interior Alaska (64° N)†

---

† Prepared for submission in Physiological Zoology

Abstract

Winters in interior Alaska (64° N) are characterized by short photoperiod (LD 5:19) and chronic subfreezing temperatures. To determine if seasonal acclimatization of Black-capped Chickadees (*Parus atricapillus*) at high latitude differs from that of conspecifics at lower latitudes, standard metabolic rates (SMR), metabolic response to low temperature (-30° C), nocturnal hypothermia, body mass, fat reserves, and conductance were measured over two winters and one summer in three groups of seasonally acclimatized birds. Body mass and conductance did not vary with season, although furcular fat levels were higher in the winter. Birds used nocturnal hypothermia when exposed to -30° C in summer or winter. Although SMR did not vary seasonally, winter SMRs differed between the two winters of the study. Nocturnal hypothermia in summer and decreased SMR in response to extreme conditions may either reflect plasticity inherent to all populations of Black-capped Chickadees or result from individual variation within this northern population.

Introduction

How endotherms survive in extreme environments has intrigued physiologists for decades and has been the object of numerous investigations (e.g., Bartholomew 1987). Short winter daylength and chronic subfreezing temperatures present a physiological challenge for organisms that remain active throughout the winter at high latitudes. The challenge to meet the energetic demands of maintaining basic physiological processes increases as body size decreases (Schmidt-Nielsen 1990). In small animals, higher surface to volume ratio and higher metabolic rates compared to larger animals increases mass-specific energy expenditure rates (Kleiber 1961).

Small passerines that overwinter at high latitudes maintain a high internal body temperature (41° C) in spite of ambient temperatures as low as -50° C, approximately a 90° C temperature gradient across 1 cm (between body core and ambient conditions). The rate of cooling of an object heated above ambient temperature can be expressed by Newton's equation $Q = k (T_s - T_a)$, where Q = rate of heat loss by conduction, convection, and radiation, k = a proportionality constant, T_s = surface temperature and T_a = ambient temperature. This equation was modified by King and Farner (1961) to apply to the thermal relationship of a bird to its environment yielding: $Q_p = Q_l = k' C \Delta T + Q_e$, ($Q_p$ = rate of heat production, Q_l = rate of heat loss by radiation, conduction, and convection, Q_e = rate of heat loss by evaporation, k' = constant, C = conductance of the insulating shell, and ΔT = the temperature gradient). We can use this equation to identify behavioral and physiological factors that may be modified to meet the energetic demands of overwintering at high latitude.

Studies of winter acclimatization in birds have been conducted mainly at middle latitudes (Pohl 1971; Weathers 1979; Dawson and Smith 1986; Marsh and Dawson 1989; Swanson 1991; O'Connor 1996). Chaplin (Budd 1973; Chaplin 1974; 1976) focused on a resident passerine, the Black-capped Chickadee (*Parus atricapillus*) (hereafter chickadee), in upstate New York (42° N). Its small size (≈ 12 g) and wide latitudinal distribution (67° N to 37° N; Smith 1991) make this chickadee a good candidate for examining variation in overwintering strategies. Use of nocturnal hypothermia (a mechanism used to lower overnight energy expenditure) and fat as a fuel in winter for New York chickadees were also important in North Dakota (42° N) (Cooper and Swanson 1994) and in some Scandinavian parids. Reinertsen and Haftorn (1983) described similar responses in winter-acclimatized Willow Tits (*Parus montanus*) residing in a maritime, subarctic climate (63°N). Black-capped Chickadees living in interior Alaska (64° N) experience the most thermally challenging winter days for the longest duration (Figure 1) for the species. However, they apparently do not exhibit nocturnal hypothermia and have mass-specific metabolic rates that are 50% higher than those of similar-sized parids (Grossman and West 1977).

This study investigates the metabolic responses to low temperature by Black-capped Chickadees in interior Alaska, providing a basis for comparing these responses with those reported by Chaplin (1974) and Cooper and Swanson (1996) for conspecifics residing at lower latitudes. I hypothesized that if this northern population is not using nocturnal hypothermia, then other metabolic adjustments must be used. I also examined the extent of seasonal acclimatization in this population. A short summer season (only one month with no recorded

sub-freezing temperatures) may result in little or no seasonal variation in metabolic response to low temperatures.

Material and Methods

Birds

I captured birds at feeders using mist-nets and Potter traps in upland birch forests around Fairbanks, Alaska (64° 54' N) in September 1993 , March 1994 and October 1994. I measured body mass (± 0.1 g) with a Pesola spring balance. Wing chord and furcular fat score were determined as described by Helms and Drury (1960). Age class and stage of molt were based on plumage characteristics (Pyle 1987). I placed a unique color combination of plastic leg bands on each bird.

I transported birds to the Animal Quarters Facility of the Institute of Arctic Biology (IAB), University of Alaska Fairbanks. Ten birds lived in each of two large (4 x 3 x 2.5 m) wooden outdoor aviaries. I constructed the aviaries on a cement pad (7 x 6 m) that was covered by a 5 to 3.5 m high slanted metal roof. This roof protected the area from snow but was tall enough to allow natural light into the aviaries, exposing the birds to ambient light and temperature. Wooden roost boxes (10 x 10 x 10 cm) lined with 1.4 cm blue foam insulation, spruce boughs and birch branches provided roosting and perching sites. I fed the birds an *ad libitum* diet of sunflower seeds, suet, peanuts, and peanut butter mixed with a commercial avian vitamin supplement (Avi-Con, Vet-A-Mix, Inc.). Mealworms ($2 \cdot bird^{-1}$) were placed in aviaries daily. I provided fresh water in the summer, and snow was available at other times.

I used a different group of birds for each series of tests. Individuals used during February 1994 were captured in September 1993; those used in July 1994

were captured in March 1994, and birds captured in October 1994 were used in February 1995. Previous work on Apapane (*Hemignathis parvus*) found no effect of long-term captivity on metabolic rate (Weathers et al. 1983).

I recorded daily maximum and minimum temperatures (± 1 °C) in the aviaries using a Taylor Max-Min® Thermometer. The local office of the National Weather Service supplied long term weather information.

Metabolic Measurements

I captured birds using Potter traps in the aviary one half hour before roosting time (≈ 1630 h, Alaska Standard Time (AST)) and measured their body mass, furcular fat scores (in 1994 and 1995), and body temperature (in 1995) (pre-trial values). I placed a randomly selected bird in a 2 liter cylindrical Plexiglas metabolic chamber containing a wooden perch, and then positioned the metabolic chamber in a controlled temperature box (So-low Environmental Equipment, 2 x 1 x 1.5 m). Lights remained on for 15 min and then were turned off until the end of the trial. I measured oxygen consumption (VO_2) by open-circuit respirometry. The fractional concentration of oxygen in respiratory gas was measured using an Ametek Model S-3A Oxygen Analyzer. I measured carbon dioxide production using a Beckman Model 864 Infrared CO_2 Analyzer. All metabolic trials were performed between 1700 - 0900 h (1994), or 1700 - 0100 h (1995). At the end of the trial, I measured body mass, furcular fat score, and body temperature (post-trial values).

A small pump pulled outside air through the chamber at a flow rate of 300-400 ml/min. A Hastings Mass Flowmeter (AFSC-10K (1994), 229H (1995)) measured the flow. Within the controlled temperature box, air flowed through a

3 m long section of copper tubing to equilibrate the outside air to the box temperature upstream of the metabolic chamber. The amount of CO_2 and O_2 in dry (air passed through a column of Drierite to remove all water) efflux gas was measured in series and recorded by computer every 60 sec. Custom software calculated instantaneous VO_2 and respiratory quotients (RQ) (Luick, unpub.). All measurements were corrected to standard pressure and temperature (STP). I estimated standard metabolic rate (SMR) from the lowest 10 min average rate of oxygen consumption after the first 4 hours of the trial (Swanson and Weinacht 1997). This delay ensured that birds were in a post-absorptive condition. Trial duration in 1994 averaged 16 h. I shortened the trial duration to 6 h in 1995 after determining that evening SMR did not change substantially between the fourth hour of roosting and morning arousal. I calibrated analyzers with outside air and span gases prior to, during (approximately every 2 hours), and after each trial. In 1994, I tested birds at three temperatures (+25°C, -10°C, -30°C) in February and July. In 1995, birds were tested at two temperatures (+25°C, -30°C) in February and July. A HOBO Temp data logger (Onset Computer Corp.) in the bottom of the chamber continuously recorded ambient temperature within the metabolic chamber. All birds except one regained normothermy without assistance. Age and sex have been shown not to influence metabolic rate in Black-capped Chickadees (Rising and Hudson 1974).

Body Temperature Measurement

At the conclusion of the trial in 1995, I quickly removed the bird from the metabolic chamber. Body temperature was measured within the next 60 -90 sec by inserting a 20 gauge copper-constantan thermocouple into the cloaca to a

depth of ≈ 1 cm. A Bailey Model Bat 12 digital reader displayed the temperatures. I recorded body temperature (± 0.1 °C) before and immediately after each metabolic trial. At low body temperature, parids respond to disturbance by rapidly raising their body temperature (Reinertsen and Haftorn 1983). Thus, post-trial body temperatures are an upper estimate of evening body temperature.

I calculated conductance using the equation $M = C(T_b - T_a)$, where M = oxygen consumption (ml/g/h), C = thermal conductance, T_b = body temperature and T_a = ambient temperature (Lasiewski et al. 1967).

Data Analysis

With the exception of fat scores, all data sets complied with assumptions of normality and equal variance and are reported as means ± standard deviations (s.d.). Multiple comparisons of means were made using one-way analysis of variance, followed with multiple pairwise comparisons using Tukey's test. Two-tailed Student's t-tests were used to determine the differences between two means. Fat scores were ranked and analyzed using Kruskal-Wallis analysis of variance of ranks followed by non-parametric equivalent of Tukey's multiple comparisons tests. Statistical significance was accepted at $P < 0.05$. Lower limit of sample sizes for metabolic trials were determined by an analysis of power using variances from similar data available in the literature (Zar 1984).

Results

Seasonal Change in Body Mass and Fat

Pre-trial mean mass did not vary across seasons ($F = 1.922$, $P = 0.189$), but this result may be due to insufficient power ($\beta = 0.70$). In summer 1994, birds weighed 11.4 ± 0.7 g (n=4). Mean winter mass was 12.4 ±1.0 g in 1994 and 12.4 ± 0.9 g in 1995 (n = 5, 6, respectively). Pre-trial fat scores varied seasonally ($P < 0.05$). Winter fat scores did not differ between 1994 and 1995 (median = 5), but both differed from the summer values (median = 3). Adipose tissue is metabolically relatively inactive; reporting metabolic rates on a per gram basis could artificially underestimate mass specific oxygen consumption (VO_2) values in fat individuals. As a result, metabolic rates are reported on a whole animal rather than a mass-specific basis. Additionally, whole animal values may be more instructive when doing seasonal comparisons (McNab 1978, Dawson and Smith 1986, Swanson 1991, Cooper and Swanson 1994).

Metabolic Rates

Adipose tissue is metabolically relatively inactive; reporting metabolic rates on a per gram basis could artificially underestimate mass specific oxygen consumption (VO_2) values in fat individuals. As a result, metabolic rates are reported on a whole animal rather than a mass-specific basis. Additionally, whole animal values may be more instructive when doing seasonal comparisons (McNab 1978, Dawson and Smith 1986, Swanson 1991, Cooper and Swanson 1994).

Standard metabolic rates did not differ between seasons. Metabolic rate estimates from either winter 1995 (1.01 ± 0.10 ml·min^{-1}, n = 5) or winter 1994

(0.79 ± 0.06 ml·min^{-1}, n = 6) did not differ significantly from those obtained during summer 1994 (0.90 ± 0.09 ml·min^{-1}, n = 4). Winter SMRs differed from each other ($P < 0.01$). SMR was higher than allometrically predicted values (Aschoff and Pohl 1970) in winter 1994 (17%), summer 1994 (41%) and winter 1995 (47%).

At ambient temperatures of -30 ° C, metabolic rates (1.62 ± 0.12 ml·min^{-1}, n = 6) of birds tested during the winter of 1994 were significantly lower than those of birds in summer (1.99 ± 0.09 ml·min^{-1}, n = 4) and birds in winter 1995 (2.16 ± 0.23 ml·min^{-1}, n = 5). Metabolic rates of summer birds and 1995 winter birds exposed to -30° C were not significantly different from each other (Figure 2).

In 1994, metabolic trials lasted throughout the night. Birds were tested at three different temperatures (25° C, -10° C and -30° C). Metabolic rate decreased at night, and the magnitude of this decrease varied with temperature. All birds exhibited a similar pattern of response regardless of temperature but nocturnal metabolic rate varied with temperature (Figure 3). After lights out, metabolic rate dropped immediately, stabilized, and remained near the lowered level until arousal the following morning. Some birds began to increase metabolic rate before lights were turned back on, 16 hours after the start of the trial. Others increased their metabolism 15 - 20 min after lights on.

Body Temperature and Conductance

All birds lowered their body temperature (T_b) at night. T_b at the beginning of all trials did not differ between treatments (Pre-SMR (25° C), 40.8 ± 1.0° C; Pre-

-30° C, 41.1 ±1.0° C). Body temperature decreased from beginning to end of trials at both 25° C and -30° C (Student's paired t-test, n = 5, P = 0.009 and P = 0.002, respectively). Post-trial body temperatures were significantly lower in birds exposed to -30° C (33.9 ± 1.3° C) than 25° C (38.7 ± 0.7° C) (Student's paired t-test, n =5, P = 0.002) (Figure 4).

During trials at -30° C, conductance did not differ with season (winter 1995 = 0.16 ± 0.019 $ml^{-1} \cdot g^{-1} \cdot h^{-1}$ °C, n = 5; summer 1994 = 0.16 ± 0.005 $ml^{-1} \cdot g^{-1} \cdot h^{-1}$ °C, n = 4). These values were 33% lower than the allometrically predicted value of 0.24 $ml^{-1} \cdot g^{-1} \cdot h^{-1}$ °C (Lasiewski et al. 1967).

Discussion

This study demonstrates that Alaskan chickadees use nocturnal hypothermia during winter and retain the capacity to use hypothermia in summer. In contrast, Willow Tits and Black-capped Chickadees from lower latitudes in North America, do not use nocturnal hypothermia during the summer months (Chaplin 1974; Reinertsen and Haftorn 1983; Cooper and Swanson 1994). This difference may reflect the great environmental variability of subarctic continental climates (Figure 1). In Fairbanks the mean annual daily maximum is 2° C, with freezing temperatures recorded in all months except July. Although summer temperatures can reach 36° C, summer is very short. The short summer combined with fluctuations in the severity of the climate may favor birds that do not lose their cold acclimatization during summer.

Over the course of a winter night, this reduction of 8° C in body temperature translated into 7% decrease in energy expenditure. Although a hypothermic bird gains a savings of only 4 min of fuel each hour, the cumulative

savings during an 18 hr night is impressive. Fasting endurance is increased by 72 minutes. This may be important for survival through the long subarctic night or to fuel early morning foraging bouts.

SMR reflects the energy necessary to perform basic physiological and biochemical processes (Bennett 1988). Based on the assumption that higher latitudes experience more severe climatic conditions, Weathers (1979) postulated that SMR should increase with increasing latitude. Comparison of Alaskan Black-capped Chickadees with other parids is consistent with this hypothesis (Table 1). Summer SMR of Alaskan chickadees is 15% higher than that of New York Black-capped Chickadees, somewhat less than the 20% increase predicted by the Weathers' model (1% degree latitude^{-1}). In Scandinavia, Willow Tit SMR was 10% above the SMR values of parids from lower latitudes.

Although winter and summer SMRs did not differ, SMR did differ from one winter to the next. Dutenhoffer and Swanson (1996) found SMR values for Black-capped Chickadees in one winter to be 24% higher than those of other Black-capped Chickadees sampled in the same region in a different winter (South Dakota, Cooper and Swanson 1994). This difference was explained by a difference in the severity of the winters, with higher SMR being correlated with colder temperatures. Increased thermogenic demand increased SMR in other studies (Kendeigh and Blem 1974, Dawson et al 1983, Marsh and Dawson 1985, and Heldmaier et al. 1986). While investigating seasonal acclimatization in geographically distinct American Goldfinch populations, Dawson and Smith (1986) found a 22% increase in winter SMR when compared with previous studies of winter acclimatization on the same population. In both studies there

was no seasonal difference in SMR. They offer no explanation for the difference in winter SMRs and report no climatic data for either winter.

In contrast, Alaskan chickadees had a lower SMR in the winter when ambient temperatures were lower. This relationship is opposite to that reported in other investigations (Marsh and Dawson 1989; Hammond et al. 1994; Dutenhoffer and Swanson 1996). It is, however, similar to studies of other high latitude residents. King Penguin chicks, *Aptenodytes patagonicus,* (Barre 1986) and Alaskan Willow Ptarmigan, *Lagopus lagopus,* (West 1972) both reduce their SMR as part of their winter acclimatization. King and Farner (1961) suggested that there is no adaptive advantage to increasing SMR in response to low temperature because any potential benefit would be lost due to the increased costs of supporting a higher metabolism. This would be especially true when the bird is both meeting daily energy requirements and depositing fat to fuel a nocturnal fast. If daily energy intake is limited by short daylength, which may be the case in interior Alaska, then energy conservation would become extremely important.

Mean minimum temperature in February 1994 was -29° C. During the -30°C metabolic trials, birds were placed in a chamber warmer than ambient evening temperatures. In addition, trials were performed during some of the coldest weather of the winter (Figure 5). The following winter the mean minimum temperature in February was -19° C. Winter 1995 was milder overall, and the SMR trials were performed during some of the milder periods (Figure 5). The increase in SMR was correlated with this 10° C increase in mean aviary temperatures during winter trials.

Nocturnal hypothermia reduces energy expenditure, but there is a lower limit to body temperature. Birds exposed to 0° C (Chaplin 1974), -20° C (Reinertsen and Haftorn 1983) and -30° C (present study) all lower their body temperatures to ≈ 32° C. Birds in New York study reached internal temperatures of 28° C, but needed assistance to regain normothermy (Chaplin 1974). Passerines that use nocturnal hypothermia successfully do not let their body temperature drop below 30° C (Reinertsen 1996). Therefore, the energy that chickadees can conserve by a reduction in body temperature is finite regardless of ambient temperature.

Pohl (1971) reported similar summer and winter SMR in Bramblings (*Fringilla montifringilla*). There was an autumnal decrease in SMR, concurrent with migratory hyperphagia. By reducing existence metabolism, even a small increase in food intake will result in fattening. If chickadees in winter 1994 were unable to maintain an elevated metabolism and store the necessary fat for a nightly 18 hour fast, one alternative would be to lower maintenance requirements. Although captive birds were fed *ad libitum*, processing time may limit the amount of food ingested. During the short winter days of the subarctic, the amount of time to forage and process food may be a limiting factor. Kessel (1976) reported that the general activity level of Alaskan Black-capped Chickadee is reduced on very cold days (< -40° C), but when feeding, intensity is increased. Reducing flight costs increases energy available for fat deposition.

The unpredictability of winter low temperature may also affect SMR. Brooks (1968) found that redpolls (*Carduelis hornemanni* and *C. flammea*) exposed to fluctuating low temperatures had relatively higher SMR than birds exposed to constant low temperature. Temperature variability does not influence SMR when

ambient temperatures are warmer than -10° C. In interior Alaska, minimum daily temperatures in winter rarely rise above -10° C and can fluctuate greatly. Thus, below a certain threshold of chronic low temperature, chickadees may lower SMR to reduce energy requirements. Fluctuating temperatures may not elicit this response, which could yield differences in SMR within and across winters.

In Alaska, Black-capped Chickadees fuel energy demands like some conspecifics at lower latitudes.Fat reserves are larger in winter than in summer. This pattern is similar to birds in upstate New York (Chaplin 1974), but differs from that of birds in South Dakota (Cooper and Swanson 1994). This result may reflect a difference in methodology. Cooper and Swanson (1994) captured birds in South Dakota before 1100h local time, when birds may not have reached their maximal daily winter fat levels. Chaplin reported that birds in New York come out of roosts with low fat reserves and replenish them during the day reaching a peak before dusk. At lower latitudes, lean body mass remains stable throughout the year.

Plasticity in regulating SMR may be essential for Black-capped Chickadees to survive severe environments. Lowering SMR in winter has been demonstrated in another avian subarctic resident, the Willow Ptarmigan (*Lagopus lagopus*) (West 1972), and arctic ungulates: muskoxen, caribou and reindeer (Blaxter 1985). A mammal more comparable in size and activity level to the chickadee, the shrew, (*Sorex sinosus*) also has lower SMR in winter than in summer (Newman and Rudd 1978) at temperate latitudes. This seasonal metabolic variation may also reflect individual variation within the population. The difference in mean SMR between winters was 28%, and SMR range across

seasons was 30%. The ability to modulate energetic demand to fit with available resources (e.g., food, foraging time, digestive efficiency) increases the odds of maintaining positive energy balance.

Root's (1986) model of northern limits to avian distribution correctly predicts the range in SMR of this northern population of chickadees. Root's model predicts that birds cannot inhabit a region where energy expenditure exceeds 2.5X basal metabolic rate in winter. In both winters, the metabolic rate of birds exposed to -30° C was 2.1X SMR. Although SMR differed between winters, the response to low temperature was similar, suggesting a link between SMR and daily energy expenditure. Even though peak metabolic rate in chickadees can reach 6X SMR (Cooper and Swanson 1994), birds may not be able to sustain this rate, and may instead adjust to chronic low temperatures by decreasing their existence metabolism, so that doubling SMR can be supported by available forage and foraging time.

Black-capped Chickadees in interior Alaska respond to the physiological challenge of overwintering much like their conspecifics at lower latitudes. Lowering overnight energy expenditure by using nocturnal hypothermia allows survival through an eighteen hour night using only body reserves. The ability to reduce body temperature in response to low temperatures in the summer is unique to this population. Given the short summer, these birds may experience sub-freezing temperatures 11 months out of the year. The option of reducing overnight energy expenditure may be advantageous during a late spring or early winter.

Acknowledgments

This study was supported by a North American Bluebird Association Research Grant to the author. The Institute of Arctic Biology at the University of Alaska Fairbanks through its director, Dr. Robert White, provided financial and scientific support for the metabolic equipment. Many thanks to Dr. Bret Luick for the numerous hours of instruction and troubleshooting. Dr. Luick also designed the computer software necessary for running the metabolic system. Capture, maintenance and laboratory study of Black-capped Chickadees were authorized by permits to the author from Alaska Department of Fish and Game (AK 93- 27 and 94-53) and the U. S. Fish and Wildlife Service (PRT-692453). All experimental procedures and animal care protocols were approved by the Institutional Animal Care and Use Committee of the Institute of Arctic Biology (protocol 92-31). Thanks are due to Drs. Pierre Deviche, Jim Sedinger, Dan Roby, Dale Feist and Brian Barnes for helpful comments on earlier drafts of the manuscript.

Literature Cited

Alexander R. M. 1996. Biophysical problems of small size in vertebrates. Pp. 3 -14 in J. P. Smith, ed. Miniature Vertebrates. The Zoological Society of London, London.

Aschoff J. and H. Pohl. 1970. Der Ruheumsatz von Vogeln als Funktion der Tageszeit und der Korpergrosse. Journal of Ornithology 111: 38 - 47.

Barre H. 1986. Metabolic and insulative changes in winter- and summer-acclimatized king penguin chicks. Journal of Comparative Physiology B 154: 317 - 323.

Bartholomew G. 1987. Interspecific comparison as a tool for ecological physiologists. Pp. 11-37 in M. E. Feder, A. F. Bennett, W. W. Burggren and R. B. Huey, eds. New Directions in Physiological Ecology. Cambridge University Press, New York, New York.

Bennett A. F. 1988. Structural and functional determinants of metabolic rate. American Zoologist 28: 699 -708.

Blaxter K. 1989. Energy Metabolism in Animals and Man. Cambridge University Press, New York, New York.

Brooks W. S. 1968. Comparative adaptations of the Alaskan Redpolls to the arctic environment. The Wilson Bulletin 80: 253 - 280.

Budd S. M. 1973. Thermoregulation, bioenergetics, and endocrinology of cold acclimatization of Black-capped Chickadees, *Parus atricapillus*. PhD diss., Cornell University, Ithaca, New York.

Chaplin S. B. 1974. Daily energetics of the Black-capped Chickadee, *Parus atricapillus*, in winter. Journal of Comparative Physiology 89: 321 - 330.

Chaplin S. B. 1976. The physiology of hypothermia in the Black-capped Chickadee, *Parus atricapillus*. Journal of Comparative Physiology. 112: 335 - 344.

Cooper S. J. and D. L. Swanson. 1994. Seasonal acclimatization of thermoregulation in the Black-capped Chickadee. Condor 96: 638 - 646.

Dawson W. R. and R. L. Marsh. 1986. Winter fattening in the American goldfinch and the possible role of temperature in its regulation. Physiological Zoology 59: 357 - 368.

Dawson W. R. and B. K. Smith. 1986. Metabolic acclimatization in the American Goldfinch (*Carduelis tristis*). Pp. 427 - 434 in H. C. Heller, X. J. Musacchia and L. C. H. Wang, eds. Living in the cold: Physiological and biochemical adaptations. Elsevier Science Publishing, New York.

Dawson W. R., R. L. Marsh, W. A. Buttemer, and C. Carey. 1983. Seasonal and geographic variation of cold resistance in House Finches *Carpodacus mexicanus*. Physiological Zoology 56: 353 - 369.

Dutenhoffer M. S. and D. L. Swanson. 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiological. Zoology 69: 1232 - 1254.

Grossman A. F. and G. W. West. 1977. Metabolic rate and temperature regulation of winter acclimatized Black-capped Chickadees, *Parus atricapillus*, of interior Alaska. Ornis Scandinavica 3: 127 - 138.

Hammond K. A., M. Konarzewski, R. M. Torres, and J. Diamond. 1994. Metabolic ceilings under a combination of peak energy demands. Physiological Zoology 67: 1479 - 1506.

Heldmaier G., H. Boeckler, A. Buchberger, S. Klaus, W. Puchalski, S. Steinlechner, and H. Wiesinger. 1986. Seasonal variation of thermogenesis. Pp. 361 - 372 in H. C. Heller, X. J. Musacchia, and L. C. H. Wang, eds. Living in the Cold: Physiological and Biochemical Adaptations. Elsevier Science Publishing Co., Inc., New York, New York.

Helms C. W. and W. H. Drury. 1960. Winter and migratory weight and fat field studies on some North American buntings. Bird Banding 31: 1 - 40.

Hiebert S. 1993. Seasonal changes in body mass and use of torpor in a migratory hummingbird. The Auk 110: 787 - 797.

Irving L. 1960. Birds of Anaktuvuk Pass, Kobuk, and Old Crow. A study in arctic adaptation. U.S. National Museum, Washington, D.C.

Kessel B. 1976. Winter activity patterns of Black-capped Chickadees in interior Alaska. Wilson Bulletin 88: 36 - 61.

Kendeigh S. C. and C. R. Blem. 1974. Metabolic adaptation to local climate in birds. Comparative Biochemistry and Physiology A 48: 175 - 187.

King J. R. 1974. Seasonal allocation of time and energy resources in birds. Pp. 4 - 70 in R. A. Paynter, Jr., ed. Avian Energetics. Nuttall Ornithological Club. no. 15, Cambridge, Mass.

King J.R. and D. S. Farner. 1961. Energy metabolism, thermoregulation and body temperature. Pp. 215 - 288 in A. J. Marshall, ed. Biology and Comparative Physiology of Birds. Academic Press, New York.

Kleiber M. 1961. The Fire of Life. Wiley, New York New York.

Lasiewski R. C., W. W. Weathers, and M. H. Bernstein. 1967. Physiological responses of the Giant Hummingbird, *Patagona gigas*. Comparative Biochemistry and Physiology 23: 797 - 813.

Marsh R. L. and W. R. Dawson. 1989. Avian adjustments to cold. Pp. 203 - 253 in L. C. H. Wang, ed. Advances in Comparative and Environmental Physiology. Springer-Verlag, Berlin.

McNab B. K. 1978. The evolution of endothermy in the phylogeny of mammals. American Naturalist 112: 1 - 21.

Newman J. R. and R. L. Rudd. 1978. Minimum and maximum metabolic rates of *Sorex sinuosus*. Acta Theriologica 23: 371 - 380.

O'Connor T. P. 1996. Geographic variation in metabolic seasonal acclimatization in House Finches. The Condor 98: 371 - 381.

Pennycuick C. J. 1992. Newton Rules Biology: A Physical Approach to Biological Problems. Oxford University Press, Oxford.

Pohl H. 1971. Seasonal variation in metabolic functions of Bramblings. Ibis 113: 185 - 193.

Reinertsen R. E. 1996. Physiological and ecological aspects of hypothermia. Pp. 125 - 157 in C. Carey, ed. Avian Energetics and Nutritional Ecology. Chapman and Hall, New York.

Reinertsen R. E. 1989. The regular use of nocturnal hypothermia and torpor during energetically-demanding periods in the annual cycles of birds. Pp. 107 - 116 in A. Malan and B. Canguilhem, eds. Living in the Cold. John Libbey Euortext Ltd., London.

Reinertsen R. E. and S. Haftorn. 1983. Nocturnal hypothermia and metabolism in the Willow Tit *Parus montanus* at 63°N. Journal of Comparative Physiology 151: 109 - 118.

Rising J. D. and J. W. Hudson. 1974. Seasonal variation in the metabolism and thyroid activity of the Black-capped Chickadee (*Parus atricapillus*). The Condor 76: 198 - 203.

Root T. 1988. Energy constraints on avian distributions and abundance. Ecology 69: 330 - 339.

Schmidt-Nielsen K. 1990. Animal Physiology. Adaptation and Environment. Cambridge University Press, New York, New York.

Smith S. M. 1991. The Black-capped Chickadee. Behavioral Ecology and Natural History. Cornell University Press, Ithaca, New York.

Steen J. 1958. Climatic adaptation in some small northern birds. Ecology 39: 625 - 629.

Swanson D. L. 1991. Substrate metabolism under cold stress in seasonally acclimatized Dark-eyed Juncos. Physiological Zoology 64: 1578 - 1592.

Swanson D. L. and D. P. Weinacht. 1997. Seasonal effects on metabolism and thermoregulation in Northern Bobwhite. Condor 99: 478 - 489.

Weathers W. W. 1979. Climatic adaptation in avian standard metabolic rate. Oecologia 42: 81 - 89.

Weathers W. W. and K. A. Sullivan. 1993. Seasonal patterns of time and energy allocation by birds. Physiological Zoology 66: 511 - 536.

Weathers W. W., D. L. Weathers and C. Van Riper III. 1983. Basal metabolism of the Apapane: Comparison of freshly caught birds with long-term captives. The Auk 100: 977 - 978.

West G. C. 1972a. The effect of acclimation and acclimatization on the resting metabolic rate of the Common Redpoll. Comparative Biochemistry and Physiology A 43: 293 - 310.

West G. C. 1972b. Seasonal differences in resting metabolic rate of Alaskan ptarmigan. Comparative Biochemistry and Physiology A 42: 867 - 876.

Zar J. H. 1984. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Table 1. Summer and winter standard metabolic rate (SMR) of Black-capped Chickadees belonging to different North American populations and of Willow Tits in Norway. Standard deviation is presented in parentheses if available. Temperature data correspond to winter in which SMR was collected.

Location/ latitude	Standard Metabolic Rate ($ml \cdot min^{-1}$) Summer	Winter	$\bar{x}$ min Feb temp (°C)	Reference
New York/ 42°N	0.76	0.77	-5.1	Chaplin 1976
South Dakota/ 42°N	0.73 (±0.09)	1.09 (±0.12)/ 0.86 (±0.08)	-15.8/ -3.5	Cooper and Swanson 1994; Dutenhoffer and Swanson 1996
Norway/ 63°N	0.84	0.84	-10	Reinertsen and Haftorn 1977
Alaska/ 64°N	0.89 (±0.09)	1.01 (±0.10)/ 0.79(±0.06)	-18/ -35	Present study

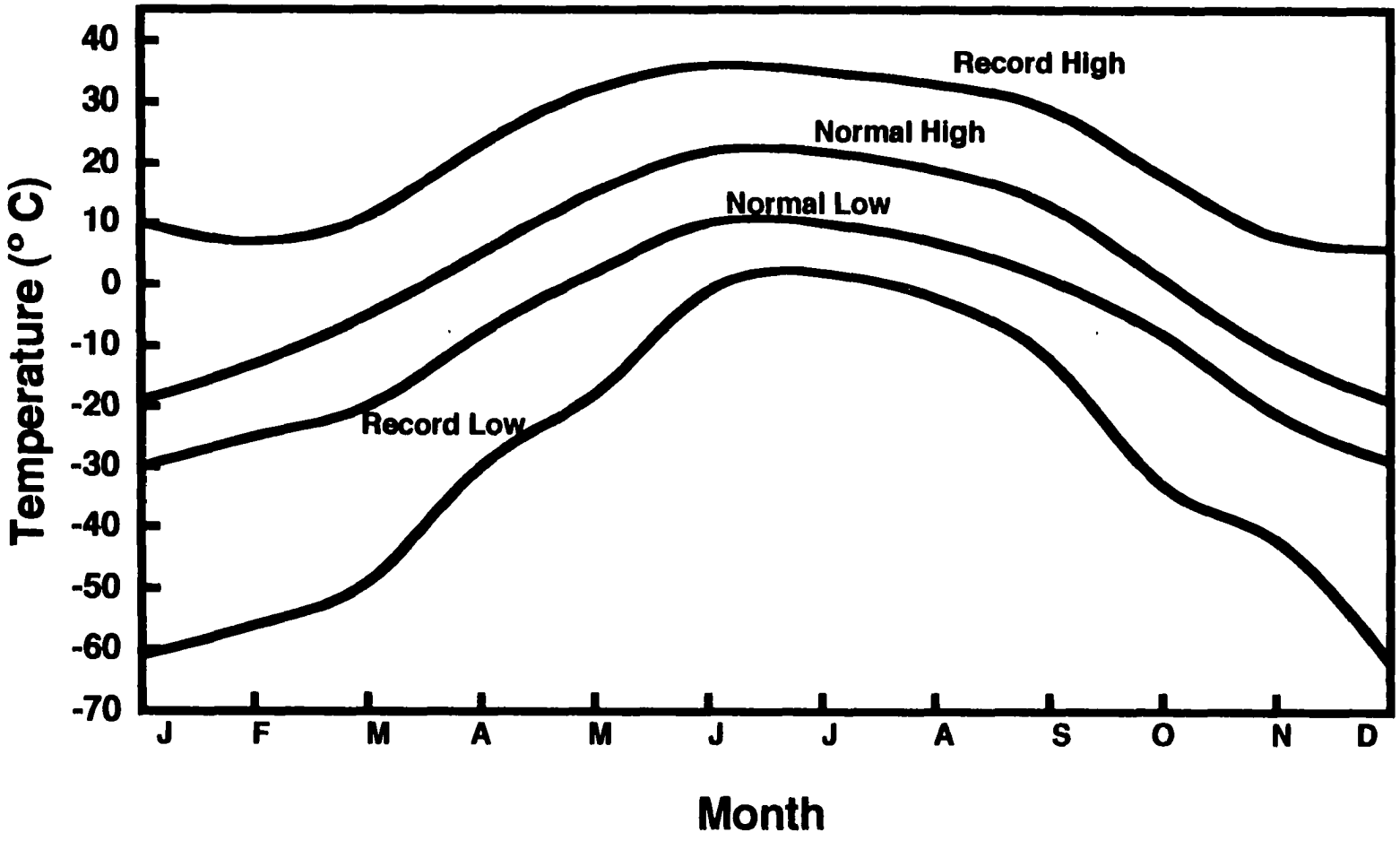


Figure 1. Monthly Record High, Normal Low, Normal High and Record Low temperatures (°C) in Fairbanks, AK (64° N) from 1941 to present. Data were obtained from the National Weather Service, Fairbanks, AK. Letters on the x-axis correspond to the months of the year.

Figure 2. Oxygen consumption (mean $\pm$ standard deviation) of seasonally acclimatized Black-capped Chickadees over two winters and one summer. Standard metabolic rate (SMR) (white bars) and metabolic rate at -30° C (black bars) were measured. Asterisks show significant differences in pairwise comparisons: *, winter of 94 is significantly lower than summer of 94 and winter of 94 ($P < 0.05$), and **, winter 94 and winter 95 are significantly different from each other ($P < 0.01$).

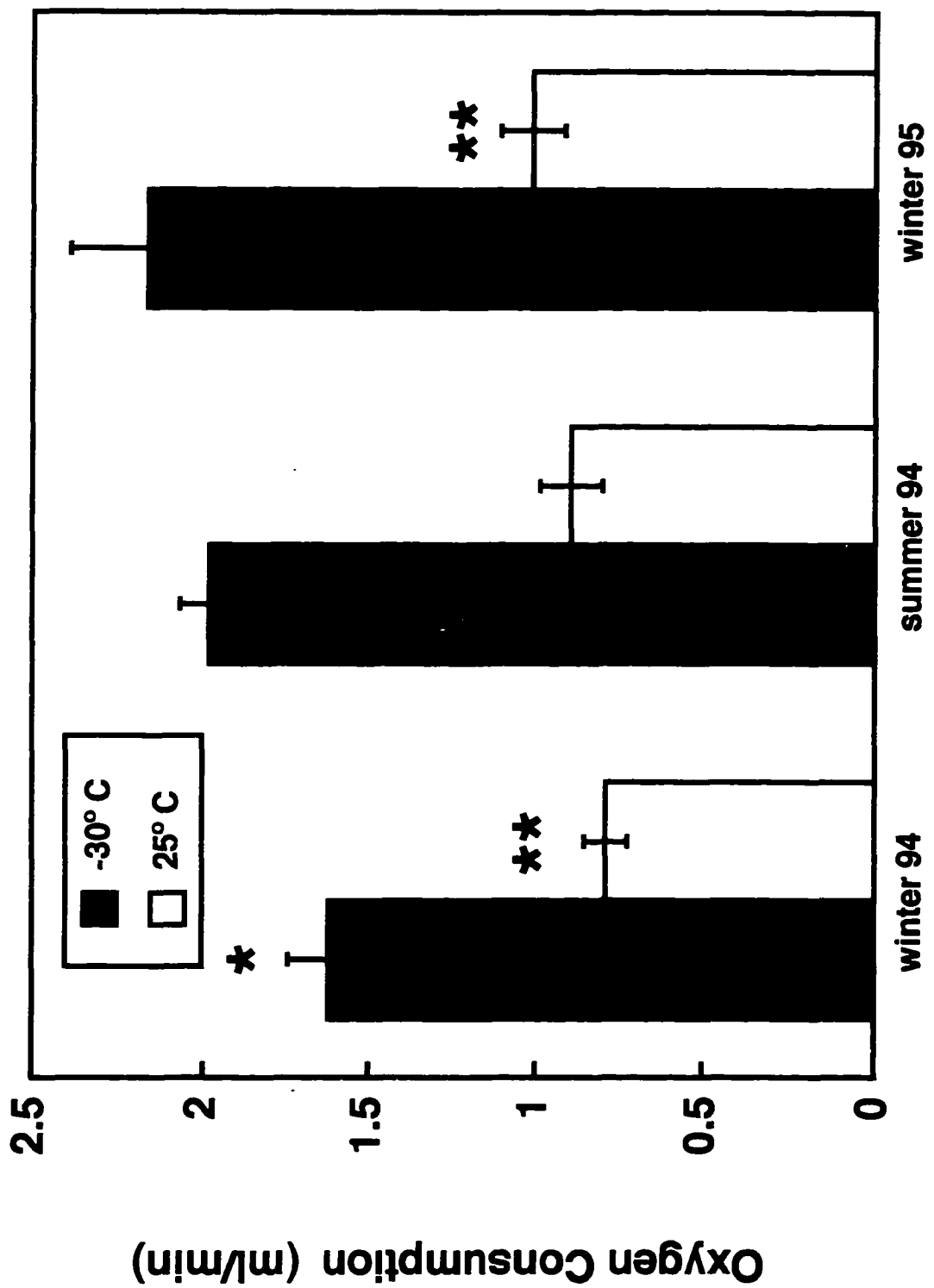


Figure 3. Overnight oxygen consumption ($ml \cdot min^{-1}$) by a seasonally acclimatized Black-capped Chickadee at three different ambient temperatures (upper panel: -30° C; middle panel: -10° C; bottom panel: 25° C). All trials were run in February 1994. Lights off and lights on are represented by dashed lines.

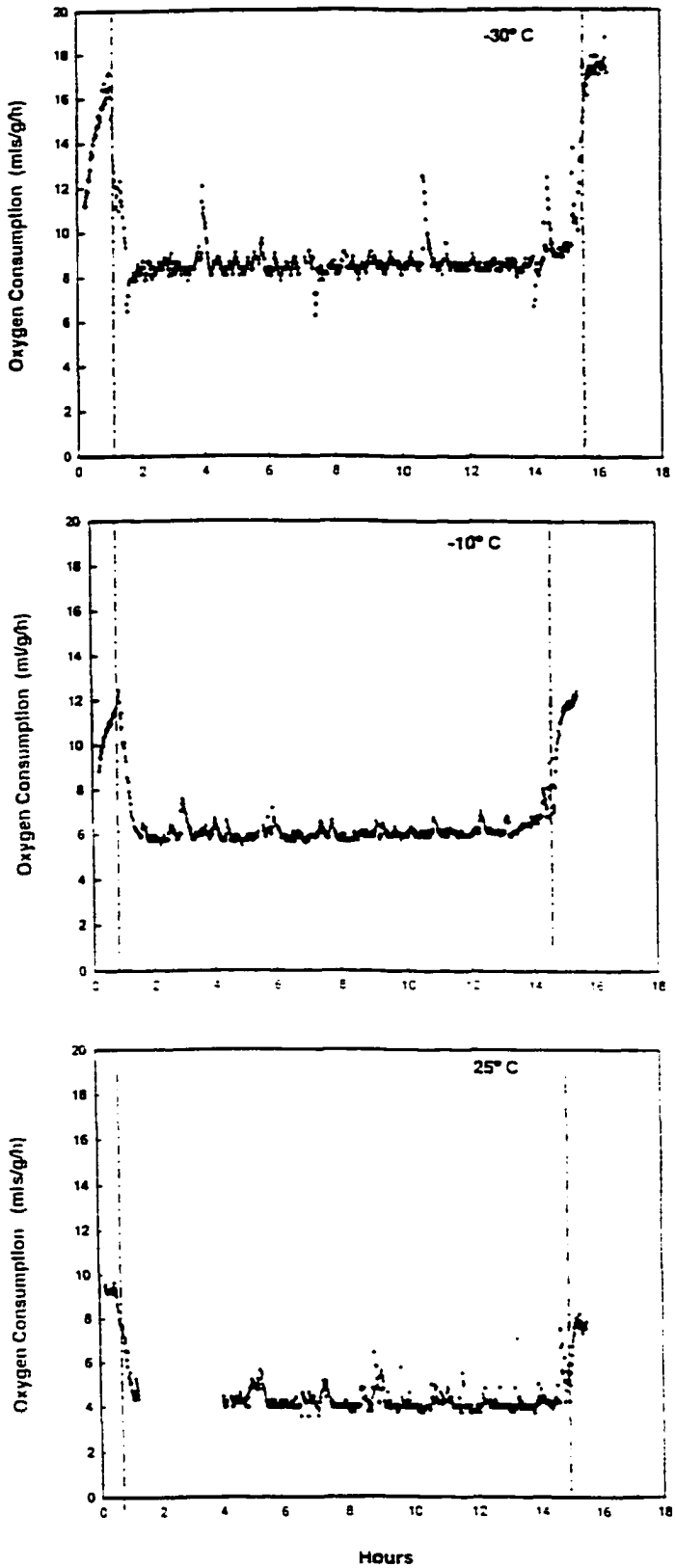


Figure 4. Body temperatures (mean $\pm$ s.d.) before (Pre-trial; black bars) and after (Post-trial; white bars) metabolic trials at -30° C and 25° C in 1995. Horizontal lines and asterisks indicate significant differences (Student's t-tests): **, $P < 0.01$.

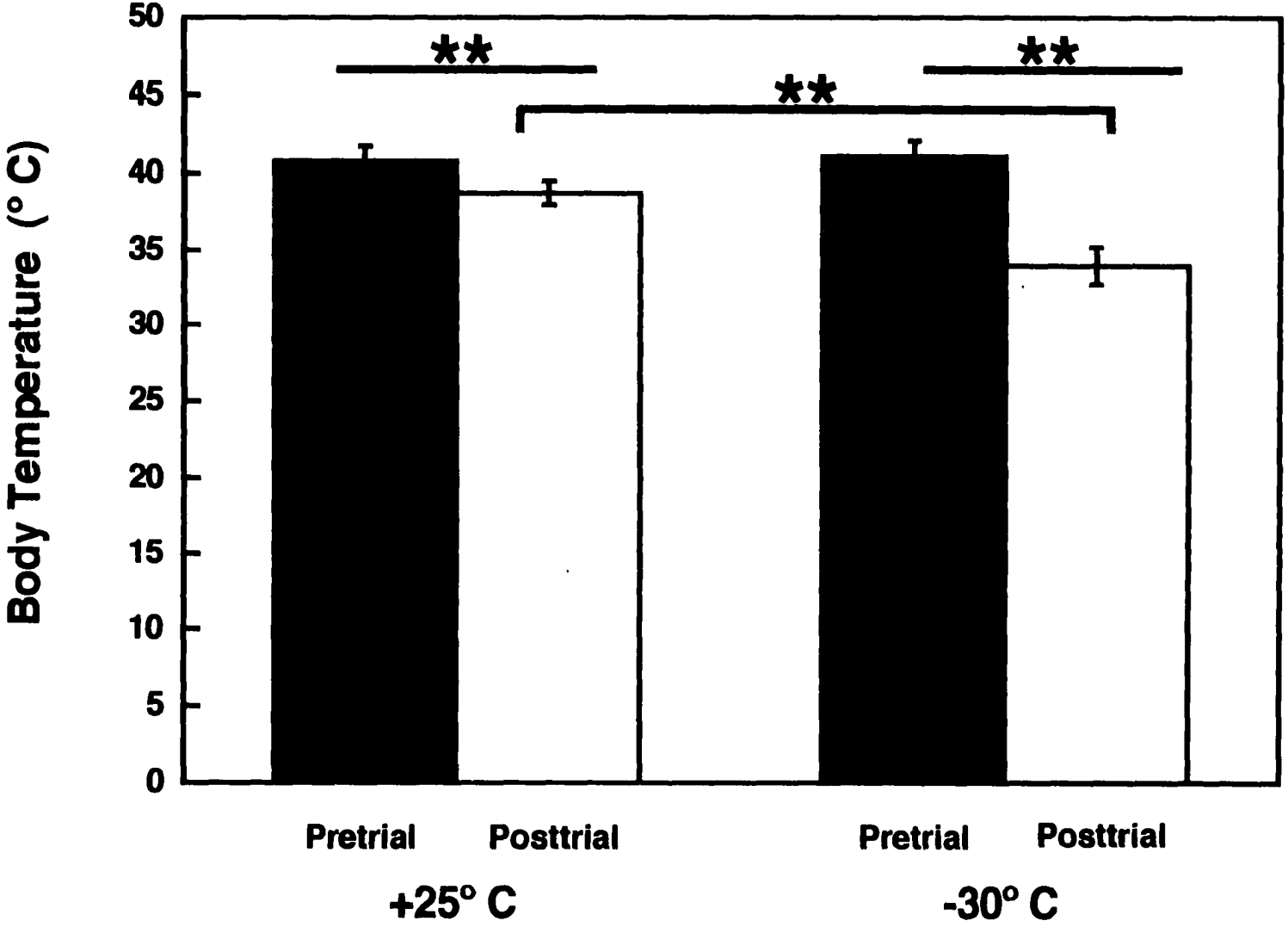
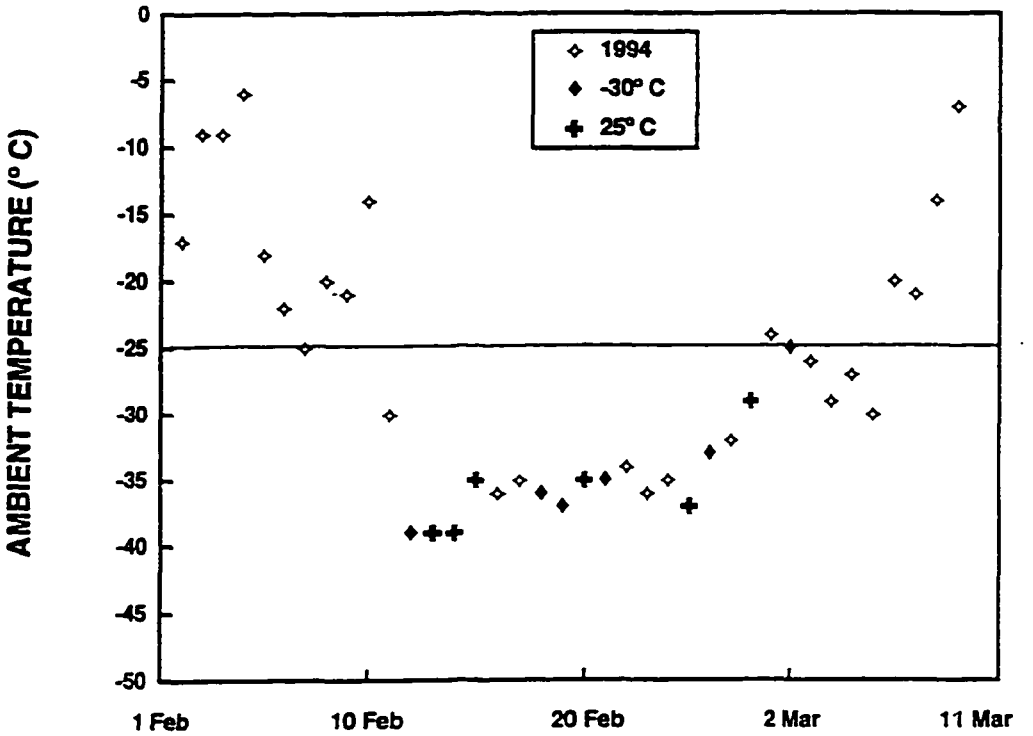
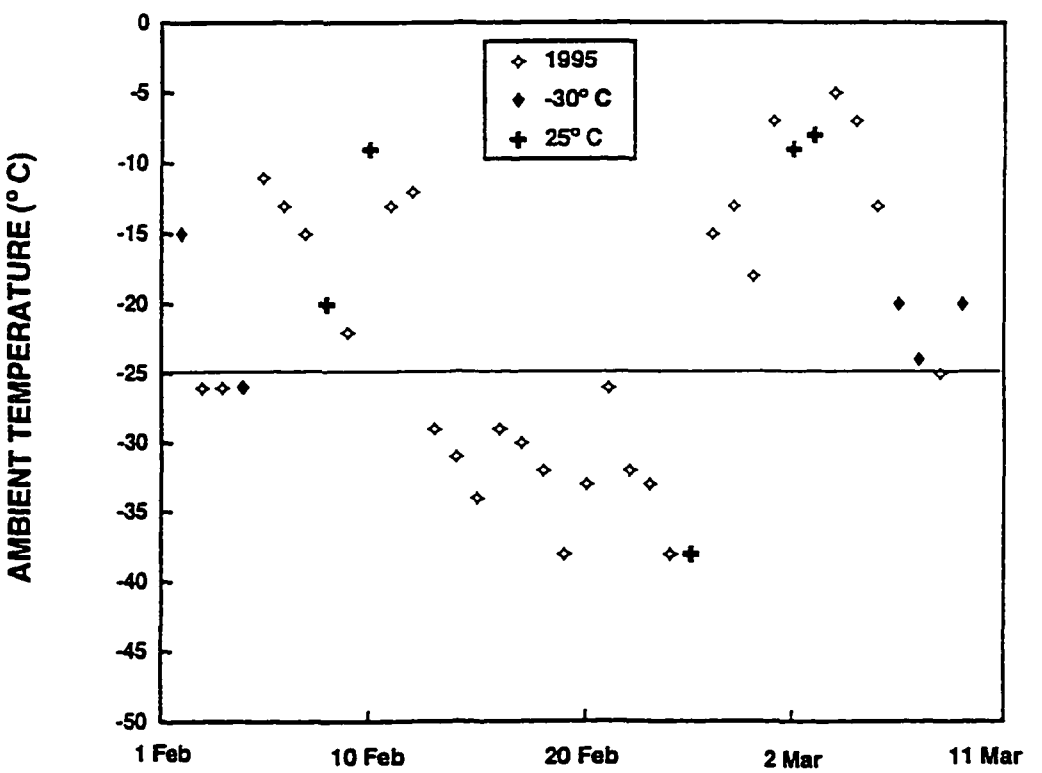


Figure 5. Minimum daily temperature during the time of metabolic experiments (1 February to 10 March) in 1994 (upper panel) and 1995 (lower panel). Filled diamonds and crosses represent days on which trials were conducted at -30° C or at 25° C, respectively. Solid line at -25° C is a point of reference. Dates and temperatures of metabolic trials were randomly assigned prior to testing.





Chapter Two

Adipose lipoprotein lipase activity and winter fattening in the Black-capped Chickadee (*Parus atricapillus*) in Interior Alaska†

---

† Susan Sharbaugh, with Pierre Deviche and Marilyn Ramenofsky (co-authors), prepared for submission to Journal of Comparative Physiology

Abstract

Black-capped Chickadees (*Parus atricapillus)* depend on lipid stores to fuel metabolism during nocturnal fasts. During winter, fat stores accumulated during the day are nearly exhausted by the next morning. At high latitudes (e.g., 64° N), fat to fuel an 18 hour nocturnal fast must be laid down during the 6 hours of daylight available for foraging. Birds residing at this latitude display an unusually high level of activity of the enzyme critical for fat deposition, adipose lipoprotein lipase (ALPL). Seasonal variation in enzyme activity positively correlates with changes in daily fattening rates from August until April. There are two unusual characteristics of ALPL in Blac-capped Chickadees in interior Alaska. There is no daily oscillation in enzyme activity and enzyme activity is very labile over a narrow range of temperatures (28 -37° C), although enzyme affinity does not vary with temperature. We speculate that nocturnal hypothermia, which reduces overnight energy expenditure, inactivates a large pool of ALPL. Reactivation of the enzyme upon rewarming in the morning allows for immediate fat deposition with the first meal. To delay fattening on winter mornings while waiting for new enzyme synthesis may be too energetically costly in time-limited environments.

Introduction

Lipid depots are an essential source of fuel used by birds for overwintering and migration (Pond 1981, Griminger 1986, Blem 1990). Patterns and rates of fat deposition have been studied on both seasonal (White and West 1977, Dawson and Marsh 1986, Scott et al. 1994) and diurnal (Lehikoinen 1987, Haftorn 1989) scales. Winter fattening is a common phenomenon in small birds in the temperate zone (King and Farner 1966). Daily cycles of fat deposition and use are of greater amplitude at higher latitudes during the winter, but fat stores generally provide only enough fuel for an overnight fast and a few daylight hours (Blem 1976).

Traditionally, the amount and rate of fat deposition during winter or pre-migration have been thought to be limited by the amount of food available, the rate of nutrient absorption, and the rate of catabolism of body stores (daily energy expenditure) (Lindstrom 1991, Klaassen et al. 1997). An additional limitation may be the ability of adipose tissue to sequester circulating free fatty acids.

Fat deposition in animals depends on the presence of the enzyme adipose lipoprotein lipase (ALPL), which catalyzes the hydrolysis of triacylglycerols in circulating lipoproteins into fatty acids and glycerol (Eckel 1987). This hydrolysis is the rate limiting step in moving free fatty acids from the bloodstream into adipocytes (Robinson and Wing 1970). Thus, increasing ALPL activity facilitates a higher rate of lipid deposition in adipose tissue (Ramenofsky, 1990).

In interior Alaska (64° N), the Black-capped Chickadee (*Parus atricapillus*) (hereafter chickadee) is the smallest (12 g) overwintering passerine. Chronic sub-freezing temperatures (Figure 1) and short winter photoperiods constitute a

physiological and behavioral challenge to these birds. In early winter, foraging time is approximately 6 hours (Kessel 1976). Each day, birds must obtain enough energy to meet immediate needs and store fuel for an 18 hour nocturnal fast. Black-capped Chickadees at middle latitudes (42° N) show both a seasonal and daily cycle of fattening (Chaplin 1974). Their fat stores are the largest in midwinter, and the daily fluctuation in fat is also greatest at this time.

The objective of this study was to determine whether constraints of high latitude winter (short days and low temperature) would alter these patterns. We measured daily fattening rates of wild birds (August through April) and ALPL activity in captive acclimatized birds (December through April). We hypothesized that fattening rate would increase with decreasing daylength and that ALPL would play an important role in rapid fattening, with high fattening rates being correlated with high level of ALPL activity. In addition, we predicted that levels of ALPL activity will be reduced at night, a time of lipid utilization, when compared to activity levels during the day, a time of feeding and fattening. We also postulated that if environmental conditions constitute a constraint, then chickadees living at high latitude may be physiologically different than other resident species because of their small body size. This hypothesis was tested by comparing ALPL activity of chickadees with that of Common Redpolls (*Carduelis flammea*) (hereafter redpoll), a larger (16 g), high latitude winter resident, and of Dark-eyed Juncos (*Junco hyemalis*) (hereafter junco), a 20 g migratory passerine which breeds in interior Alaska.

Material and Methods

Capture of birds

We collected fat samples from both captive and free-living birds. We captured birds at feeders by mist-net and Potter traps around Fairbanks, Alaska (64° 54'N) in September 1993 and October 1994. We measured body mass to the nearest 0.1 gram with a Pesola spring scale and then determined wing chord and furcular fat score (Helms and Drury 1960). Age and stage of molt were based on plumage characteristics (Pyle 1987). We placed a unique color combination of plastic leg bands on the birds brought into captivity.

We transported birds to the Animal Quarters Facility of the Institute of Arctic Biology (IAB), University of Alaska Fairbanks (UAF). Ten birds lived in each of two large (4 x 3 x 2.5 m) outdoor aviaries. We constructed the aviaries on a cement pad (7 x 6 m) which was covered by a 5 to 3.5 m high slanted metal roof. This roof protected the area from snow but was tall enough to allow natural light into the aviaries, so that birds were exposed to ambient light and temperature. We fed the birds an *ad libitum* diet consisting of sunflower seeds, suet, peanuts and peanut butter mixed with commercial avian vitamin supplement (Avi-Con, Vet-A-Mix, Inc.). Mealworms ($2 \cdot bird^{-1}$) were placed in the aviaries daily. We provided fresh water in the summer, and snow was available at other times.

We captured juncos (n = 35) using Potter traps at established feeding stations on the UAF campus in the spring and fall of 1992. We measured them as described above, transported them to IAB, and then killed them by decapitation.

We used Potter traps to capture redpolls (n = 8) at established feeding stations near Fairbanks in March of 1994. We measured the birds as above, biopsied them as described below, and released them at the site of capture.

Collection of Fat Samples

We obtained fat from captive chickadees approximately every three weeks between late November and early April in 1992 and 1993, sampling between 1200 -1300h AST and 0000 - 0100h AST in 1992 and between 1200 - 1300h AST in 1993. To collect fat samples, we wetted the feathers covering the furcula, made a small incision in the skin, and then pulled a piece of fat (7 - 15 mg) through the incision with forceps and snipped it off with scissors. Samples were blotted and placed on ice. We sealed the incision with Vetbond® (3M). All birds recovered from surgery with no apparent detrimental effects. We collected junco fat by removing all fat from the furcular fossa immediately after decapitation. We sampled redpolls in the field as described for captive chickadees. Samples remained on ice until all birds were processed.

We homogenized the samples with a ground glass tissue grinder in 1 ml of cold 0.25 M sucrose-1 mM EDTA medium, pH 7.4. We centrifuged the homogenates at 12,500 rpm for 15 minutes at 4° C. We removed the lipid layer and froze the fat-free supernatants at -70° C for later determination of enzyme activity.

We validated the assay and determined the Michaelis-Menten constants (K_m) (defined as the substrate concentration at which the reaction rate is half of its maximal value; calculated as $K_m = V_{max}/2$ (Stryer 1988)) using fat taken from free-living chickadees (n = 7) mist-netted in March 1997. We transported the

birds to IAB and killed them by exsanguination. We removed all of the furcular fat and the leg fat depot and processed samples as described above. We pooled these samples before freezing.

Adipose Lipoprotein Lipase Assay

We determined ALPL activity (the release of free fatty acids) following a modification of the methods of Schotz et al. (1970). We incubated triplicate samples (0.1 ml) in a shaking water bath at 37° C for 30 minutes with an emulsion (0.1 ml) that consisted of non-radioactive triolein (Aspec) and ^{14}C-labeled triolein (1.8 μCi, NEN) in 3:1 ratio, lysolethicin (Sigma Chemical Co.), fatty acid poor bovine serum albumin (Sigma Chemical Co.), and fasted human serum in Tris buffer (Sigma Chemical Co.). One tube of each sample triplicate contained 0.1 ml of 2M NaCl. NaCl inhibition of ALPL activity was used to distinguish between ALPL and other lipase activity (Nilsson-Ehle 1987). The reaction was stopped by adding 3.25 ml of extraction medium (chloroform, methanol, heptane (all from Sigma Chemical Co.) in a 2.3:2.5:1.8 ratio). We added carbonate borate buffer (1.05 ml) to separate the free fatty acids into an upper layer. We centrifuged the samples, placed 1 ml from the upper layer into a scintillation vial, and counted it in a scintillation counter. Samples used to determine daily and seasonal changes in enzymatic activity were from individual captive chickadees. We used pooled samples from wild chickadees to determine thermal dependency of reaction velocity and K_m. All redpoll values are from pooled samples. All junco values are from individual birds.

Because Schotz et al.'s (1970) method was developed for mammals, we determined whether optimal conditions for measuring ALPL activity were

similar in birds and mammals. For this, enzyme activity was measured as a function of: (1) duration of incubation (15 -60 minutes), (2) NaCl concentration (0 - 0.67 M NaCl) in incubation medium, and (3) incubation temperature (25°, 32°, 37°, and 42° C).

We also determined enzyme activity as a function of substrate concentration, 0.087, 0.175, 0.35, 0.7, 1.05, 1.40 and 1.75 μMol triolein (total cold and ^{14}C labeled) at 28°, 35°, 36°, 37°, and 42° C. Standard assay conditions used a substrate concentration of 0.7 μMol triolein.

To standardize amount of enzyme activity among samples, we determined protein content by Lowry assay (Lowry et al. 1951). Rates are reported as μMol free fatty acids released·hour of incubation^{-1}·mg of protein^{-1}.

Fattening Rates

We calculated daily rates of fattening from data collected from 1990 through 1993 at an established banding station near UAF. Fattening rate is the change in furcular fat score per hour. This rate is the slope of the regression line fitted to fat scores of birds caught over the day against time of capture expressed as hours past civil twilight (1 lux). We grouped the data as follows: August 21 - September 20, September 21 - October 20, October 21 - November 20, November 21 - December 20, December 21 - January 20, January 21 - March 20, and March 21 - April 20, to represent seasonal change in daily fattening rate.

Data Analysis

We tested all data sets for normality and equality of variance. Data that did not comply with normality or equal variance criteria were analyzed by non-

parametric procedures. Means are reported ± standard deviation (SD). A Student's t-test or Mann-Whitney U test was used to test for statistical significance between two means. One-way analysis of variance (ANOVA) or Kruskal-Wallis comparisons of ranks were used to test multiple (>2) means. When necessary, multiple comparisons were made with Scheffe's test of multiple contrasts (ANOVA) or a non-parametric equivalent of Tukey's multiple pairwise comparison tests (Kruskal-Wallis). Significance level was set at 0.05.

Results

Fattening rates

Furcular fat scores of chickadees during January 1991 and 1992 increased linearly as a function of time past civil twilight (Figure 2), coefficient of determination (r^2) = 0.71, $P < 0.001$. Rates of fattening varied with photoperiod (Figure 3). Highest rates of fattening occurred during the winter months with lower fattening rates in the fall and spring. All monthly fattening rates were significantly different than 0 ($0.001 \leq P \leq 0.05$). Maximal fattening (fat score = 5) did not occur in spring or fall.

ALPL Activity

ALPL activity (μMol FFA·h^{-1}·mg $protein^{-1}$ throughout) in chickadee samples collected at night from February through April 1994 (15.5 ± 7.6, n = 14) did not differ from those collected during the day (16.3 ± 11.7, n = 14). Activity level was higher in chickadees (15.9 ± 9.6, n = 28) than in juncos (1.9 ± 1.4, n = 35, $P < 0.0001$).

In chickadees, ALPL activity was higher in Dec-Jan (27.3 $\pm$ 19.2, n = 14) than in April (12.7 $\pm$ 7.5, n = 15) (ANOVA, F = 3.015, P = 0.04; Scheffe's Multiple Contrasts, P< 0.05) (Figure 4). Enzymatic activity levels in February (17.5 $\pm$ 12.1, n = 14) and March (17.4 $\pm$ 11.7, n = 10) were not different from each other and intermediate to those of Dec-Jan and April. ALPL activity in juncos was similar in spring (1.6 $\pm$ 1.1, n = 23) and fall (2.6 $\pm$ 1.6, n = 12).

Redpoll ALPL activity increased linearly as a function of incubation temperature: $y = 1.099x - 24.88$, $r^2 = 0.96$, P = 0.007. ALPL activity in juncos did not increase with temperature, $y = 0.02x + 1.4$, $r^2 = 0.2$, P = 0.57 (Ramenofsky and Gray, unpub. data). As mentioned earlier, incubation temperature influenced chickadee ALPL activity level non-linearly (Figure 5).

The reaction rate under standard conditions (37° C, 0.7 μMol triolein) was linear for 45 minutes ($r^2 = 0.99$, P < 0.005). NaCl inhibited ALPL activity at concentrations of 0.67 M. Peak activity occurred at 37° C (Table 1).

Michaelis-Menten constants (K_m) did not vary as a function of incubation temperature, $y = -0.002x + 0.134$ ($r^2 = 0.29$, P = 0.20) (Figure 6). Peak activity from 28° - 36° C was described by the equation $y = 0.39x + 12.73$ ($r^2 = 0.58$, P = 0.45). There is a 1.7X increase in activity between 36° and 37° C and a subsequent 2.1X decrease from 37° to 42° C . Highest activity occurred at 37° C when substrate concentration exceeded 0.4 μMol triolein (Figure 7).

Discussion

High metabolic rates and a high surface to volume ratio of small passerines overwintering at high latitude combine to present a substantial physiological challenge in an environment characterized by low ambient temperatures and

limited duration of daylight available to feed. Lipid depots are essential for meeting these energy expenditures, especially while fasting through the long winter night. At high latitudes, where nocturnal roosting lasts approximately 18 hours, the role of fat is magnified (Blem 1990). Chickadees have about 6 hours of daylight to meet their daily energetic needs and store enough energy to fuel the nocturnal fast. We have shown that their hourly rate of daily fattening is extremely rapid and is highest when photoperiod is shortest.

The rate of fat deposition depends on amount of energy that a bird gains from the food it eats (daily metabolized energy intake, DME) and the amount of energy it expends during the day (daily energy expenditure, DEE) (Lindstrom 1991). If DME > DEE, then the surplus can be stored as fat. DME can be constrained metabolically by digestive processes (when food is not limiting) or by the time available to obtain food (Lindstrom 1991). With decreasing daylength and increasing thermal demand, chickadees in winter can reduce the amount of time and energy searching of food by using a cache (Smith 1991). Birds begin caching insects and seeds on winter territories in late summer when food is abundant (Kessel 1976). Finding sufficient food is the first step in increasing DME over DEE, the second challenge is to maximize the energy intake from this food. Adaptations and adjustments in gut capacity, digesta retention time, nutrient absorption rate, and rates of hydrolysis by digestive enzymes all influence energy extraction efficiency (Karasov 1996, Murphy 1996).

Black-capped Chickadees in the interior of Alaska have made additional adjustments at the following step of energy extraction, use at the target tissue. By increasing the activity of an enzyme that is crucial for fat deposition, they have increased their ability to rapidly convert circulating triacylglycerols into fat

depots. Rapid fattening rates correlate with comparatively high levels of enzyme activity. Seasonal change in levels of fat deposition are accompanied with a seasonal change in ALPL activity. Lower ALPL activity levels with the same energy intake in midwinter could result in a lower rate of deposition. This would give the bird a smaller fat store at dusk, which may be insufficient to fuel its metabolic costs overnight. High ALPL activity levels may also increase the clearance rate of triacylglycerols from circulation. If plasma levels of nutrients modulate foraging, quick clearance rates may increase feeding behavior.

Another subarctic winter resident, the Common Redpoll, does not have this high ALPL activity, although it experiences the same climatic conditions. This difference reflects the difference in nocturnal strategy employed by chickadees and redpolls in winter. Redpolls do not depend on fat as their main source of fuel overnight, instead they go to roost with seeds stored in a diverticulated esophagus (a "crop") (White and West 1977). Fat stores vary little from dusk until dawn, while crops are empty in the morning (White and West 1977). Parids lack this anatomical feature.

Adipose lipoprotein lipase is present in a wide variety of species (including humans, sheep, pigeons, rainbow trout) (Cryer 1987). Optimal conditions for the ALPL activity assay in chickadees were identical to those previously published for mammals (Hietanen and Greenwood 1977) and juncos (Ramenofsky and Gray, unpub. data). Although optimal conditions were the same, activity of chickadee ALPL was different from that of the rat and junco in two ways: the lack of a diurnal rhythm in ALPL activity and thermal sensitivity over a very narrow range of temperatures.

These modifications may enhance the chickadee's ability to begin fat deposition immediately after leaving the roost. We found no difference between diurnal and nocturnal enzyme activity. This is counterintuitive, because night is a time of lipid utilization, and Koltar and Borensztajan (1977) have shown a diurnal cycle of enzyme activity that persists in fasted rats. This steady level of activity in chickadees may be explained by the thermal sensitivity of ALPL in these birds. Peak ALPL activity occurs at 37° C, but there is a rapid decrease in activity over a narrow range of decreasing temperatures. This positive thermal modulation (Hochachka and Somero 1973) may allow birds to inactivate a large pool of enzyme during nocturnal hypothermia (Chaplin 1974, Reinertsen and Haftorn 1983, Sharbaugh 1997), and then reactivate it during pre-dawn arousal. Nocturnal hypothermia was defined by the above investigators as a core body temperature of < 35° C. The apparent half-life of cellular ALPL at 37° C is 60 minutes (Ong et al. 1988). Rather than resynthesize ALPL in response to increased insulin from the first meal (Ong et al. 1988), chickadees could begin hydrolyzing triacylglycerols immediately from this reactivated pool. Eckel (1987) has stated that increased ALPL activity in response to feeding depends on new enzyme synthesis. In cultured adipocytes, ALPL synthesis takes from 30 minutes to 4 hours (Eckel 1987). With only 6 hours of foraging time, a delay of even 30 minutes may be critical for chickadees to meet energy requirements.

Differential activity of lipoprotein lipase has also been proposed in another instance of high energy demand in small birds. During migration, small passerines use increased levels of very low density lipoproteins (VLDL) to deliver fatty acids to flight muscles (Jenni-Eiermann and Jenni 1992). Increased

activity of muscle lipoprotein lipase would enhance the uptake of FFAs during endurance locomotion (Nilson et al. 1980).

ALPL inactivation over a very narrow range of temperatures has not been found in other species. Alaskan Common Redpolls, which do not use nocturnal hypothermia (Sharbaugh unpub. data), display a linear increase in enzyme activity with temperature (Figure 5). By combining two alterations to basic avian physiology (nocturnal hypothermia and positive thermal modulation over a very narrow temperature range), chickadees gain a distinct advantage for survival in extreme conditions.

Heterothermy of Black-capped Chickadees may also explain the broad temperature range of stable enzyme affinity. This characteristic is found in ectotherms that are exposed to a wide array of ambient temperatures (Hochachka and Somero 1973). With unchanging enzyme affinity over a range of temperatures, chickadees can modulate body temperature to conserve energy and efficiently deposit fat stores.

Foraging time may be the crucial limitation for chickadees overwintering in interior Alaska. As we hypothesized, fattening rates increased with decreasing photoperiod. ALPL activity also varied seasonally. The two aspects of ALPL, which we did not predict, illustrate modifications that increase the efficiency with which chickadees have responded to scarce resource (time). The thermal sensitivity of chickadee ALPL allows immediate fattening during a period of limited foraging time. Although this may represent only a small advantage, "...it is through the summation of small advantages that selection leads to successful organisms." (King and Murphy 1985, p.960).

Acknowledgments

Funding for this project was provided by an American Heart Association Fellowship to S. M. S. and a National Institutes of Health Biomedical Research Pilot Grant to P.D. and S.M.S. Sigma Xi Grants in Aid of Research provided funding for travel to the University of Washington to S.M.S. for instruction in assay techniques. A Graduate School Research Fellowship from the University of Washington provided funding to M. R. All birds were collected with the necessary state and federal permits (Scientific collecting permits: AK 96-52 and USFWS PRT-692453). All animal care and surgical procedures were approved by the IAB Institutional Animal Care and Use Committee (Protocol # 96-26). Use of fasted human serum in the ALPL assay was approved by the UAF Human Subjects Committee (Protocol # 93 - 6). Thanks are due to Drs. Jim Sedinger, Dale Feist and Brian Barnes for helpful comments on earlier drafts of the manuscript.

Literature Cited

Berthold P 1996 Control of Bird Migration. Chapman and Hall, London

Blem CR 1976 Patterns of lipid storage and utilization in birds. American Zoologist 16:671-684

Blem CR 1990 Avian energy storage. In: Power DM (ed), Current Ornithology. Vol.7. Plenum Publishing Corp., pp. 59-113

Chaplin SB 1974 Daily energetics of the Black-capped Chickadee, *Parus atricapillus*, in winter. Journal of Comparative Physiology 89:321-330

Cryer A 1987 Comparative biochemistry and physiology of lipoprotein lipase. In: Borensztajn J (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago. pp 277-329

Dawson WR and RL Marsh 1986 Winter fattening in the American goldfinch and the possible role of temperature in its regulation. Physiological Zoology 59(3):357-368

Eckel RH 1987 Adipose tissue lipoprotein lipase. In: Borensztajn J (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago. pp 277-329

Griminger P 1986 Lipid metabolism. In: Sturkie PD (ed), Avian Physiology. Springer-Verlag, New York, pp 345-358

Haftorn S 1989 Seasonal and diurnal body weight variations in titmice, based on analyses of individual birds. Wilson Bulletin 101:217-235

Hammond KA, M Konarzewski, RM Torres, and J Diamond. 1994. Metabolic ceilings under a combination of peak energy demands. Physiological Zoology 67:1479-1506

Helms CW and WH Drury 1960 Winter and migratory weight and fat field studies on some North American Buntings. Bird Banding 31:1-40

Hietanen E and MRC Greenwood 1977 A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats. Journal of Lipid Research 18:480-490

Hochachka PW and GN Somero 1973 Strategies of Biochemical Adaptation. WB Saunders Company, Philadelphia

Jenni-Eiermann S and L Jenni 1992 High plasma triglyceride levels in small birds during migratory flight: a new pathway for fuel supply during endurance locomotion at very high mass-specific metabolic rates. Physiological Zoology 65:112-123

Karasov WH 1996 Digestive plasticity in avian energetics and feeding ecology. In: Carey C (ed), Avian Energetics and Nutritional Ecology. Chapman and Hall, New York. pp.61-84

Kessel B 1976 Winter activity patterns of Black-capped Chickadees in interior Alaska. Wilson Bulletin 88:36-61

King JR and DS Farner 1966 The adaptive role of winter fattening in the White-crowned Sparrow with comments on its regulation. American Naturalist 914:403-418

King JR and ME Murphy 1985 Periods of nutritional stress in the annual cycles of endotherms: fact or fiction? American Zoologist 25:955-964

Klaassen M, A Lindstrom and R Zijlstra 1997 Composition of fuel stores and digestive limitations to fuel deposition rate in the long-distance migratory thrush nightingale, *Luscina luscinia*. Physiological Zoology 70:125-133

Kotlar TJ and J Borensztajn 1977 Oscillatory changes in muscle lipoprotein lipase activity of fed and starved rats. American Journal of Physiology 233:E316-E319

Lehikoinen E 1987 Seasonality of the daily weight cycle in wintering passerines and its consequences. Ornis Scandinavica 18:216-226

Lindstrom A 1991 Maximum fat deposition rates in migrating birds. Ornis Scandinavica 22:12-19

Lowry RN, NJ Rosebrough, AL Farr, and RJ Randall 1951 Protein measurements with the Folin phenol reagent. Journal of Biological Chemistry 193:265-275

Murphy ME 1996 Nutrition and metabolism. In: Carey C (ed), Avian Energetics and Nutritional Ecology. Chapman and Hall, New York. pp.31-60

Nilsson-Ehle J 1987 Measurements of lipoprotein lipase activity. In: Borensztajn J (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago. pp 277-329

Ong JM, TG Kirchgessner, MC Schotz and PA Kern 1988 Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated rat adipocytes. Journal of Biological Chemistry 263:12933-12938

Pond CM 1981 Storage. In: Townsend CR and Calow P (eds.) Physiological Ecology. An Evolutionary Approach to Resource Use. Sinauer Associates, Sunderland MA pp 190-219

Ramenofsky M 1990 Fat storage and fat metabolism in relation to migration. In: Gwinner E (ed) Bird Migration. Physiology and Ecophysiology. Springer-Verlag, Berlin pp 214-231

Reinertsen RE and S Haftorn 1983 Nocturnal hypothermia and metabolism in the Willow Tit *Parus montanus* at 63°N. Journal of Comparative Physiology 151:109-118

Robinson DS and DR Wing 1970 Regulation of adipose tissue clearing factor lipase activity. In: Jeanrenaud B and D Hepp (eds) Adipose Tissue. Regulation and Metabolic Functions. Academic Press New York pp 41-46

Schotz M, A Garfinkel, RJ Huebolter, and JE Stewart 1970 A rapid assay for lipoprotein lipase. Journal of Lipid Research 11:68-69

Scott I, PI Mitchell and PR Evans 1994 Seasonal changes in body mass, body composition and food requirements in wild migratory birds. Proceedings of the Nutrition Society 53:521-531

Smith S. M. 1991 The Black-capped Chickadee. Behavioral Ecology and Natural History. Cornell University Press, Ithaca, New York

White CM and GC West 1977 The annual lipid cycle and feeding behavior of Alaskan redpolls. Oecologia 27:227-238

Table 1. Black-capped Chickadee adipose lipoprotein lipase activity (μMol free fatty acids (FFA) released·unit time^{-1}·mg protein^{-1}) as a function of duration of incubation, NaCl concentration in the incubation medium, and incubation temperature. Data are means of three pooled samples assayed in triplicate (± standard deviations).

A.	Duration of incubation (min)	μMol FFA·mg protein^{-1}
	15	9.8 ± 1.6
	30	22.0 ± 0.5
	45	34.1 ± 2.4
	60	38.3 ± 5.0

B.	NaCl (M)	μMol FFA·h^{-1}·mg protein^{-1}
	0	47.0 ± 1.1
	0.16	53.9 ± 2.1
	0.32	52.4 ± 0.9
	0.5	47.1 ± 1.3
	0.67	6.3 ± 0.3

C.	Temperature (°C)	μMol FFA·h^{-1}·mg protein^{-1}
	28	18.3 ± 2.1
	35	23.9 ± 0.5
	36	27.3 ± 1.8
	37	34.2 ± 2.1
	42	21.4 ± 2.4

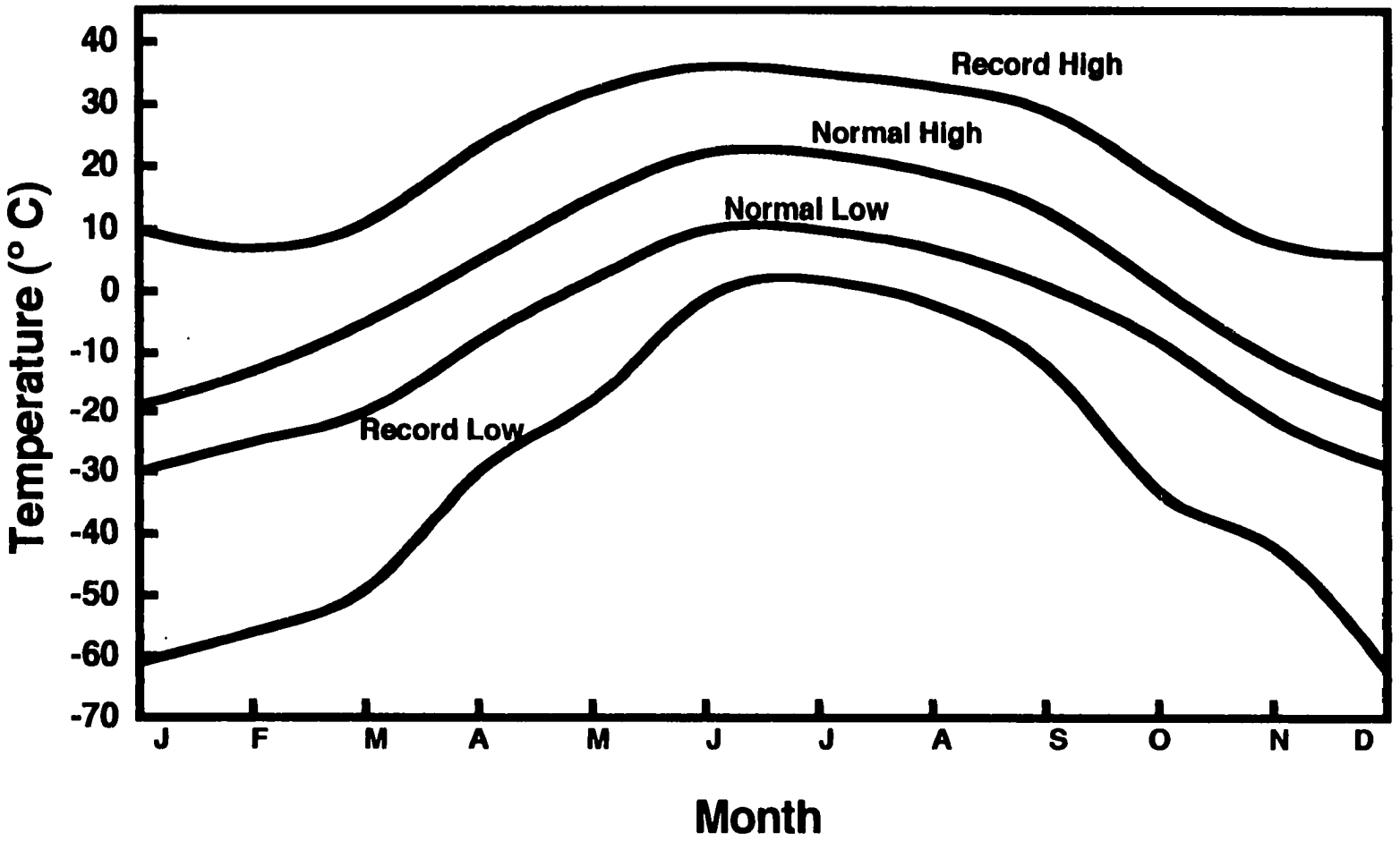


Figure 1. Monthly Record High, Normal Low, Normal High and Record Low temperatures (°C) in Fairbanks, AK (64° N) from 1941 to present. Data were obtained from the National Weather Service, Fairbanks, AK. Letters on the x-axis correspond to the months of the year.

Figure 2. Changes in winter fat levels of individual wild Black-capped Chickadees (n = 22 individuals) measured in January 1991 and 1992 in interior Alaska as a function of time past civil twilight.

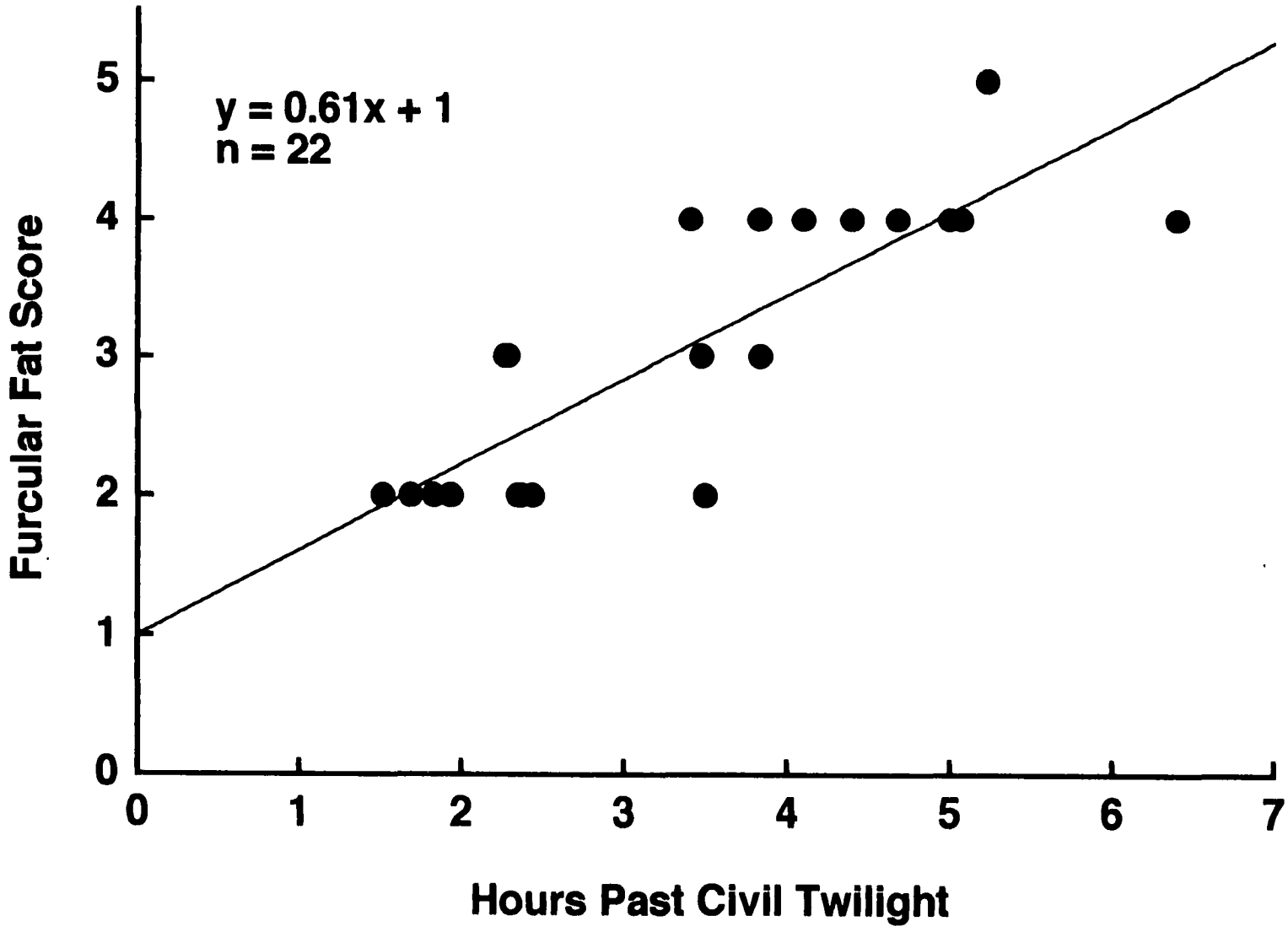


Figure 3. Seasonal change in daily fattening rate and associated changes in photoperiod in wild Black-capped Chickadees in interior Alaska. All fattening rates are significantly different from 0. Error bars are standard deviation. Sample sizes for each interval are as follows: September, n = 22; October, n = 57; November, n = 34; December, n = 33; January, n = 44; February, n = 25; April, n =10. Although individual birds were measured multiple times throughout the years, only one value per bird was used in each interval. Data were collected from 1990 - 1993.

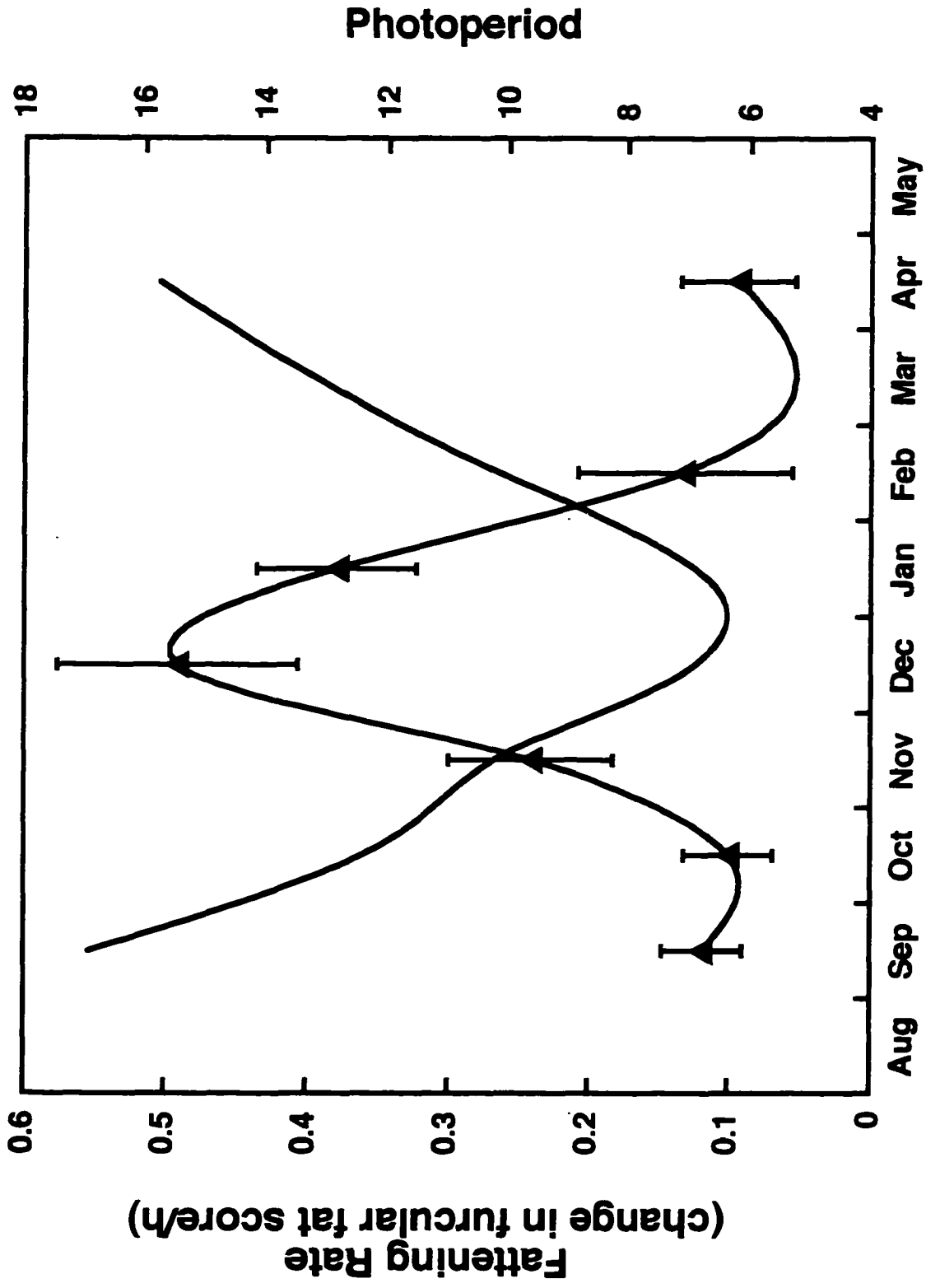


Figure 4. Seasonal *in vitro* adipose lipoprotein lipase activity of winter acclimatized Black-capped Chickadees. Each bar represents the mean (± s.d.) of individuals measured in that time period, December-January (n = 14), February (n = 14), March (n = 10), and April (n = 10). Activity in December-January and April (starred bars) were significantly different from each other, ($P < 0.05$).

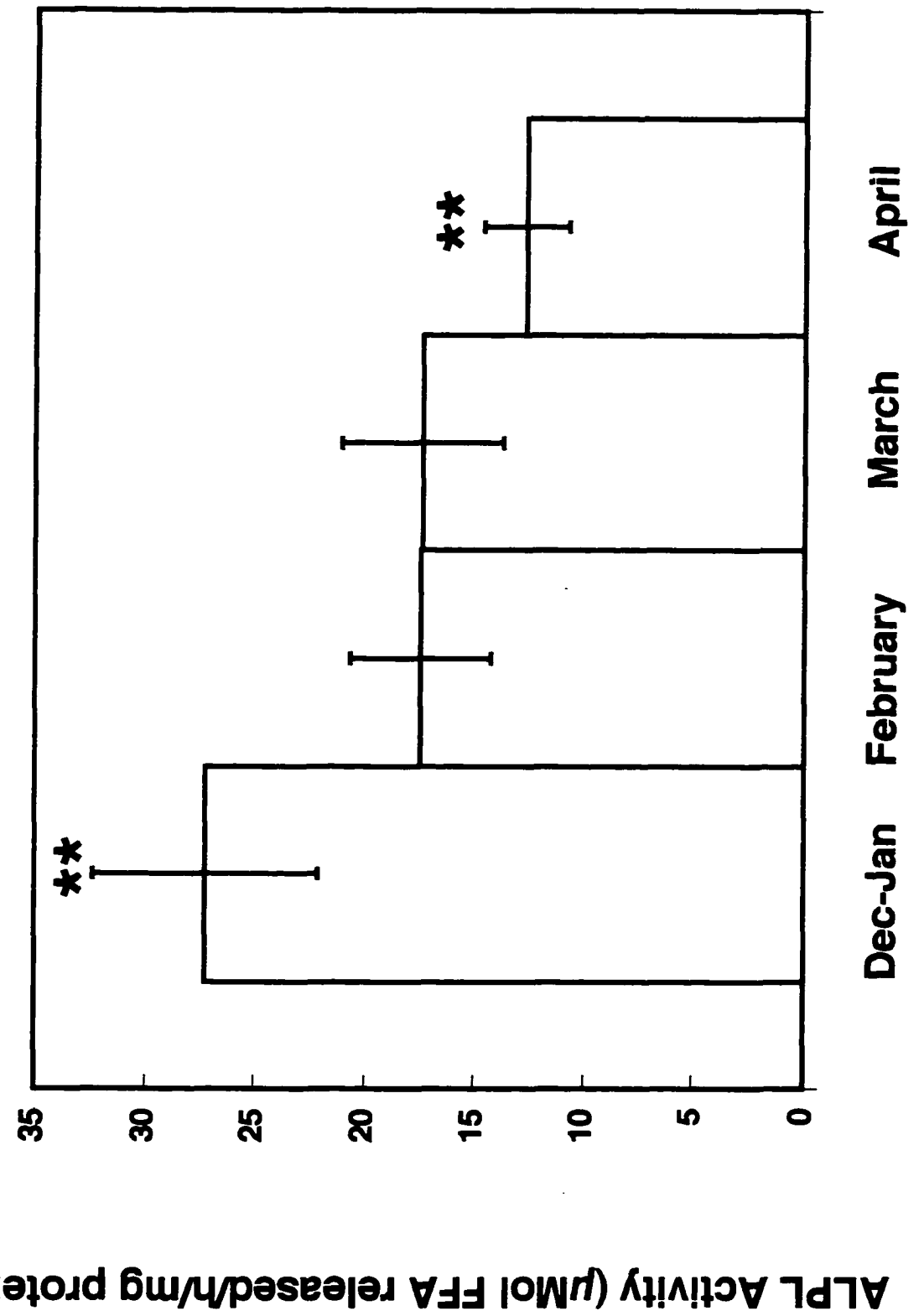
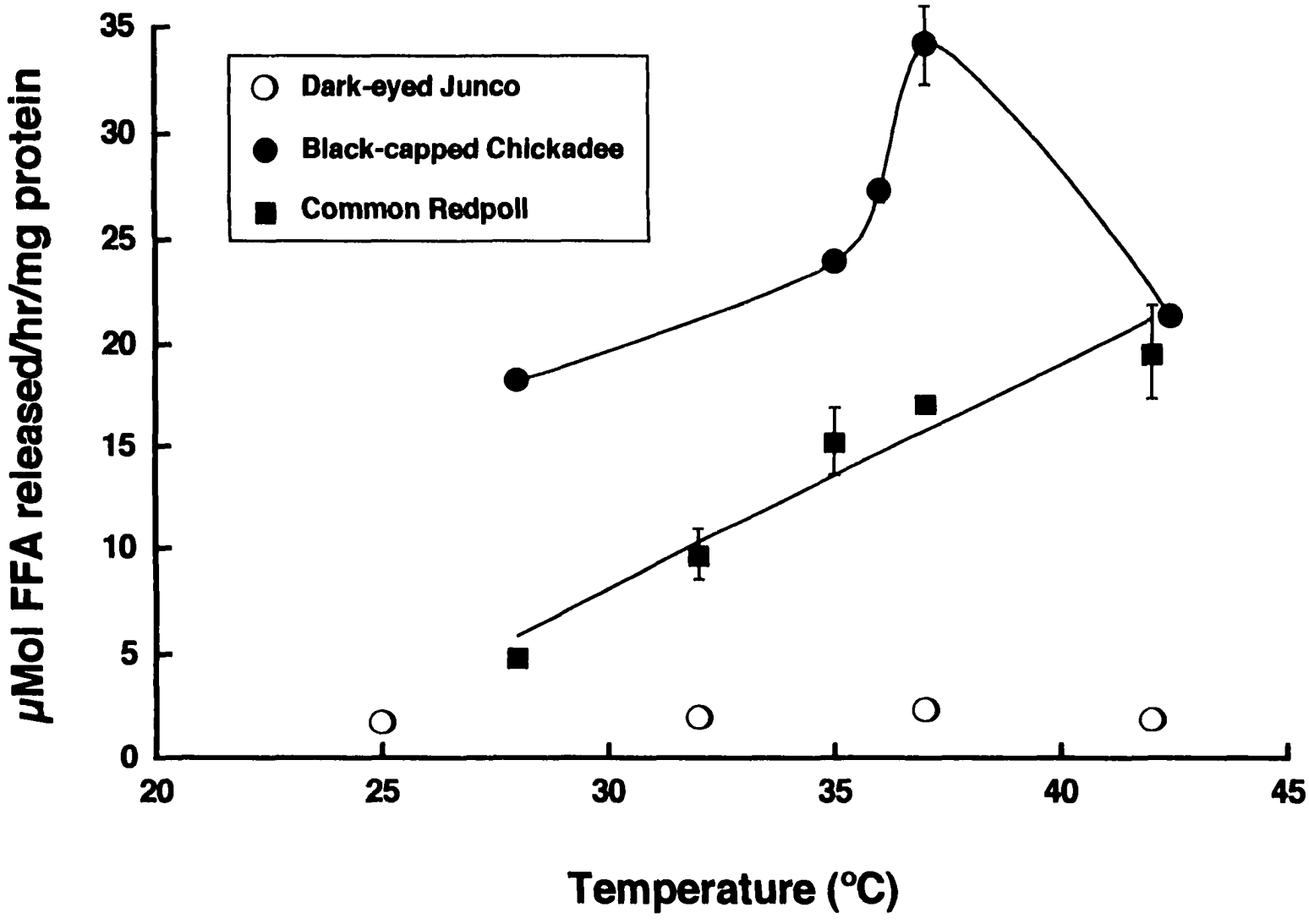


Figure 5. *In vitro* adipose lipoprotein lipase activity (mean ± s.d.) of winter acclimatized Black-capped Chickadees, wild Common Redpolls and wild Dark-eyed Juncos. Values for junco values were obtained from Ramenofsky and Gray (unpub. data) (error bars not available).



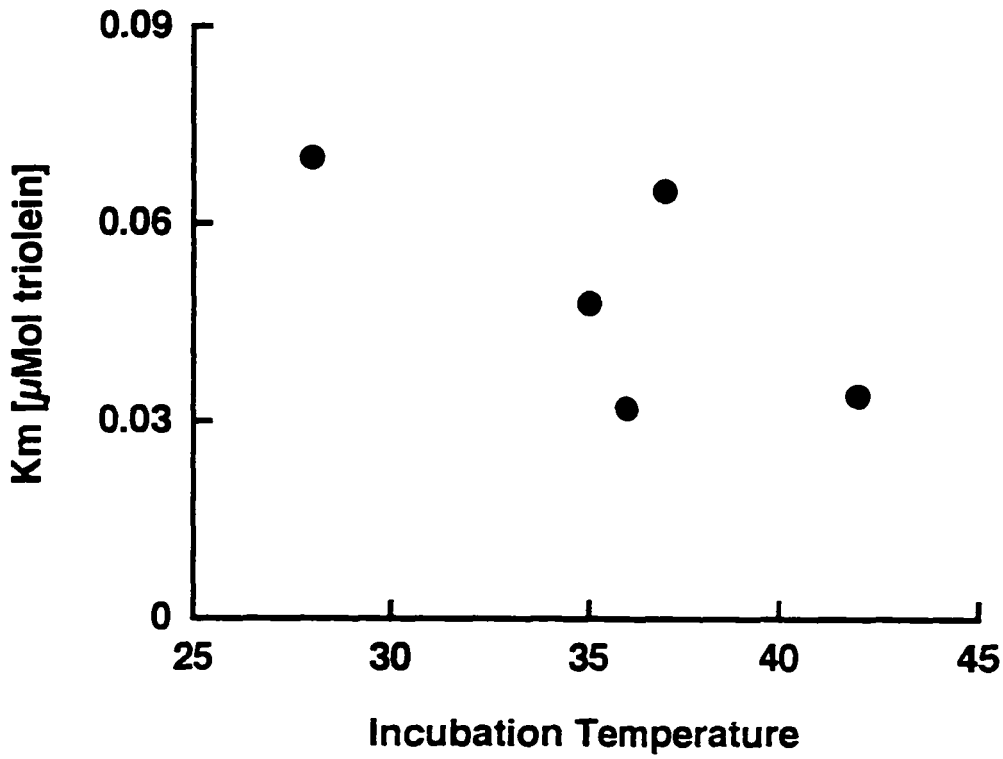
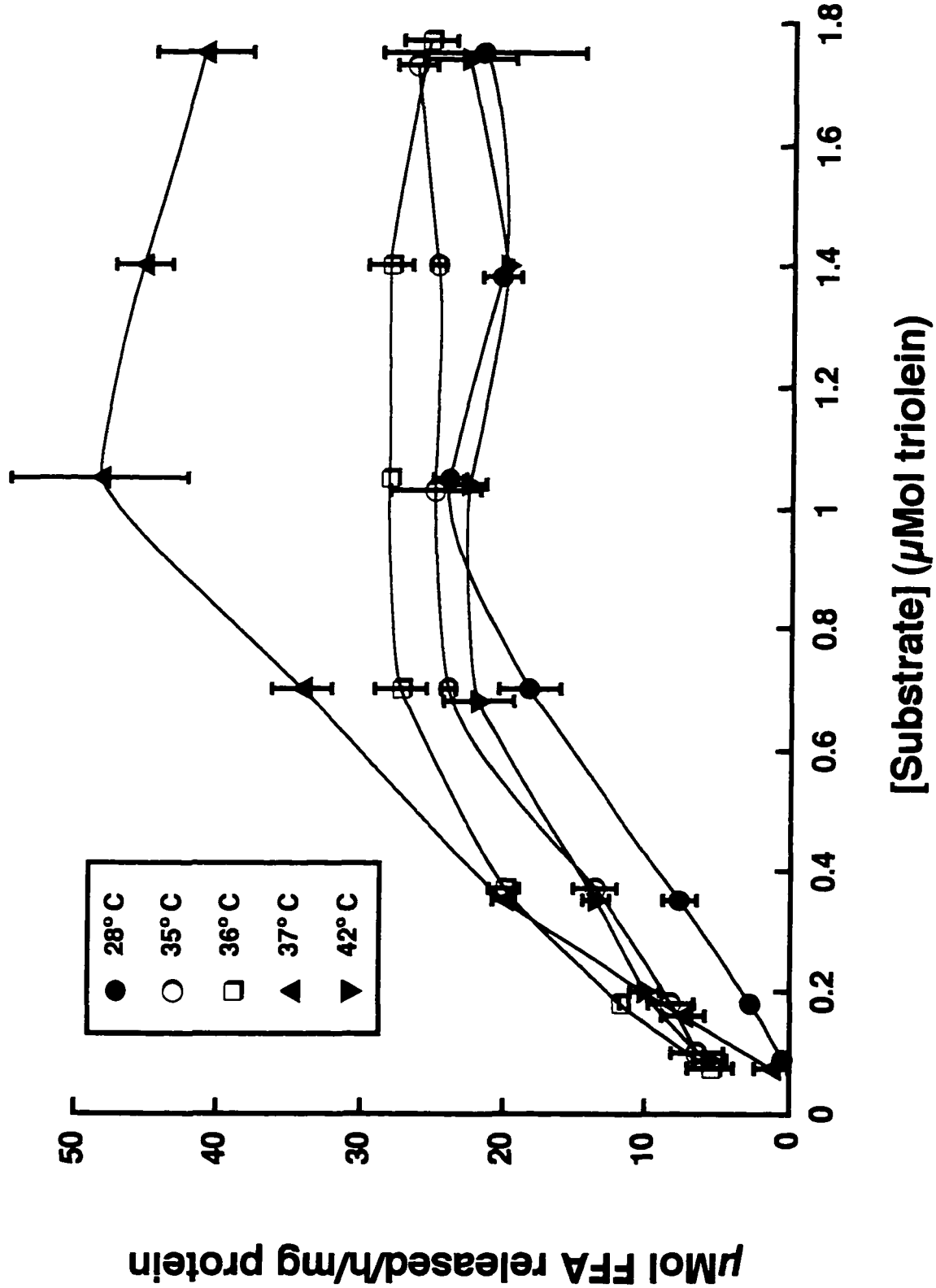


Figure 6. Apparent Michaelis-Menten constants (Km) for winter acclimatized Black-capped Chickadee adipose lipoprotein lipase incubated at 28° C, 35° C, 36° C, 37° C and 42° C.

Figure 7. *In vitro* adipose lipoprotein lipase activity (means ± s.d.) of wild Black-capped Chickadees vs. substrate concentration [μMol triolein] incubated at 28° C, 35° C, 36° C, 37° C and 42° C. Standard assay substrate concentration of 0.7 μMols triolein. Three pooled samples were assayed in triplicate at each substrate concentration at each temperature.



Chapter Three

Latitudinal Variation in Adipose Lipoprotein Lipase Activity in Winter Acclimatized Black-capped Chickadees (*Parus atricapillus*)†

---

† Prepared for submission to The Condor

Abstract. Black-capped Chickadees (*Parus atricapillus*) are resident passerines that have a broad geographic distribution. Local populations experience a wide variety of environmental conditions from the mild maritime climate of the mid-latitude Pacific coast to extreme continental fluctuations of interior Alaska. Environmental conditions are hypothesized to influence phenotypes. I examined differences in body mass, wing length, and the activity and temperature sensitivity of the rate-limiting enzyme in fat deposition, adipose lipoprotein lipase (ALPL) in three local populations: western Washington (47° N), northwestern New York (42° N), and interior Alaska (64° N). Birds from Washington were significantly lighter and had shorter wings than birds from the other two regions. ALPL activity in winter-acclimatized birds was highest in Alaska, although all populations exhibited activity higher than previously reported for passerines during pre-migratory fattening (another time of increased lipid deposition). ALPL thermal sensitivity was highest in the two populations (Alaska and New York) that use nocturnal hypothermia for energy conservation. Chickadees that experience harsh winter conditions have anatomical and biochemical modifications that increase their probability of overwinter survival.

INTRODUCTION

It has been hypothesized that the physiological limits of individuals to environmental factors define a species' range (Root 1988). Black-capped Chickadees (*Parus atricapillus*) (hereafter chickadees) are widespread across a broad climatic range. A common resident passerine, this bird inhabit deciduous forests from the Atlantic to Pacific Oceans, north to the Brooks Range of Alaska (Irving 1960) and south to the Great Basin area of northern Arizona and Utah (Smith 1991). Nine subspecies have been identified (AOU 1957), although recent mtDNA work has shown that all continental subspecies share the same haplotype (Gill et al. 1993). Populations experience a wide variety of climatic conditions, from the mild, equitable maritime climate of the Pacific coast to the highly seasonal, continental climate of interior Alaska. These differing climates presumably exert their greatest influence during the winter (Weathers 1979, Root 1988, Weathers and Sullivan 1993). The chickadee's small body size (10 -12 g) and concomitant high mass-specific metabolic rate make the winter season (low temperature and short photoperiod) an energetically demanding period (Reinertsen 1996). Seasonal acclimatization in parids has been well studied (Budd 1973, Chaplin 1974, 1976, Cooper and Swanson 1994, Grossman and West 1977, Kessel 1976, Reinertsen and Haftorn 1986, Rising and Hudson 1974, Sharbaugh in prep). One important aspect of winter-acclimatization is a seasonal increase and the daily amplitude of change in fat reserves. Lipid depots are critical for the overwinter survival of small passerines (Blem 1976, 1990, King and Farner 1966). During winter, chickadees depend on fat reserves deposited during the day to survive nocturnal fasts (Chaplin 1974). When adequate food is available, the rate of daily fat deposition is limited by the activity of the enzyme, adipose

lipoprotein lipase (ALPL) (Eckel 1987). Recent work in Alaska has shown unusually high levels of ALPL activity in winter-acclimatized chickadees (Sharbaugh 1997). In addition, in this population the enzyme is thermally sensitive over a very narrow range of temperatures. Enzyme activity increases as much as 7 times as incubation temperature rises from 35 to 37° C (Sharbaugh 1997). This temperature sensitivity may reflect the heterothermic nature of chickadees. These birds use nocturnal hypothermia, reducing body temperature as much as 10° C overnight, as a means of energy conservation when exposed to subfreezing temperatures (Chaplin 1974, Cooper and Swanson 1994, Sharbaugh 1997). In contrast, the vast majority of passerines exhibit a daily rhythm in body temperature of only 1-3° C (Prinzinger et al. 1991).

Chickadees in interior Alaska experience severe winter conditions of chronic sub-freezing temperatures and short photoperiods. Do populations that experience less severe winters also exhibit high ALPL activity? Is the enzyme as sensitive to temperature in these other populations? To answer these questions, fat samples were collected from winter-acclimatized chickadees in interior Alaska, northwestern New York and western Washington. Birds from these areas were selected to represent three very different winter conditions. Given the lack of genetic diversity among chickadee populations throughout continental North America, I hypothesized no biochemical differences among populations.

METHODS AND MATERIALS

Black-capped Chickadees

I captured birds at feeders between 1200 - 1400h using mist-nets and Potter traps around Fairbanks, Alaska (64° 54'N, February 1996, n = 6), Ithaca, New York (42° 47'N, January 1996, n = 10), and western Washington (47° 56'N, January 1996, n = 12). I measured body mass to the nearest 0.1 gram with a Pesola spring balance. Wing chord and furcular fat score (Helms and Drury 1960) were also determined.

Collection of Fat Samples

I then anesthetized the birds using Metofane® (methoxyflurane) (Pitman-Moore, Inc.). To collect fat samples, I wetted the feathers covering the furcula and made a small incision in the skin covering the furcular (claviculo-coracoid) fossa. I pulled a piece of fat (7 - 15 mg) through the incision with forceps and snipped it off with scissors. Samples were blotted and placed on ice. I sealed the incision with Vetbond® (3M). All birds recovered with no apparent detrimental effects. The samples remained on ice until all birds were processed (no longer than two hours).

We homogenized samples with a ground glass tissue grinder in 1 ml of cold 0.25 M sucrose-1 mM EDTA medium, pH 7.4. Homogenates were then centrifuged at 12,500 rpm for 15 minutes at 4° C. I removed the lipid layer and froze the fat-free supernatants at -70° C for later determination of adipose lipoprotein lipase (ALPL) activity.

Adipose Lipoprotein Lipase Assay

I determined ALPL activity following a modification of the methods of Schotz et al. (1970). Triplicate samples (0.1 ml) were incubated in a shaking water bath at 37° C for 30 minutes with an emulsion (0.1 ml) that consisted of non-radioactive triolein (Aspec) and ^{14}C-labeled triolein (1.8 μCi, NEN) in 3:1 ratio, lysolethicin (Sigma Chemical, Co.), fatty acid poor bovine serum albumin (Sigma Chemical, Co.), and fasted human serum in Tris buffer (Sigma Chemical, Co.). One tube of each sample triplicate contained 0.1 ml of 2M NaCl. NaCl inhibition of ALPL activity was used to distinguish between ALPL and other lipase activity (Nilsson-Ehle 1987). To stop the reaction, I added 3.25 ml of an extraction medium (chloroform, methanol, heptane in a 2.3:2.5:1.8 ratio). I added carbonate borate buffer (1.05 ml) to separate the free fatty acids into an upper layer. I centrifuged the samples for 15 minutes at 2500 RPM, removed 1 ml of the upper layer, which was counted by a scintillation counter. I used pooled samples to determine peak enzyme activity (at standard assay conditions) and thermal sensitivity of the enzyme.

To determine thermal sensitivity, I held all assay conditions constant except incubation temperature. I ran assays at incubation temperatures of 28°, 35°, 36°, 37°, and 42° C. A copper-constantan thermocouple read on a Bailey Model Bat 12 digital reader continuously measured waterbath temperature (± 0.1 °C).

I determined optimal assay conditions for Black-capped Chickadees previously (Sharbaugh 1997) and they were identical to those published for mammals (Hietanen and Greenwood 1977) and other passerines (Ramenofsky and Gray, unpub. data).

To standardize amount of enzyme activity among samples, I determined protein content by Lowry assay (Lowry et al. 1951). Rates are reported as μMol free fatty acids released·h of incubation^{-1}·mg of protein^{-1}.

Data Analysis

I tested all data sets for normality and equal variance. Data that did not comply with normality or equal variance criteria were analyzed using non-parametric procedures. Means are reported $\pm$ standard deviation (SD). Differences between two means were determined with Student's t-test or Mann-Whitney U tests. Differences among several (>2) means were tested with one-way analysis of variance (ANOVA) or Kruskal-Wallis comparisons of ranks. When necessary, multiple comparisons were made with Tukey's test of multiple contrasts (ANOVA) or a non-parametric equivalent of Tukey's multiple comparison tests (Kruskal-Wallis). Statistical significance was set at 0.05.

RESULTS

Birds from Washington (10.8 ± 0.7 g, n = 10) were significantly lighter than those from New York (11.9 ± 0.3 g, n = 14) and Alaska (12.1 ± 0.4 g, n = 6) (ANOVA followed by the Tukey test, $P < 0.001$); (Figure 2). The body mass of chickadees from New York and Alaska did not differ.

Chickadees from Washington had significantly shorter wings (60 ± 1 mm, n =10) than those from both New York (64 ± 1 mm, n=14) and Alaska (65 ± 3 mm, n =6) (ANOVA followed by the Tukey test, $P < 0.001$); (Figure 2). Wing length did not differ significantly between New York and Alaska birds.

Furcular fat score did not differ among the three samples (Kruskal-Wallis, H = 3.99, n.s.). The mode in all groups was 3, the range in New York was 3 - 4, in Washington, 2.5 - 4 and all birds in Alaska were scored as 3's.

ALPL activity (μMol FFA$\cdot h^{-1}\cdot$mg protein^{-1} throughout) under standard assay conditions (incubation temperature = 37° C) in Alaskan birds (147.7 $\pm$ 0.3) was significantly higher than in the samples from New York (40.3 $\pm$ 4.9) and Washington (19.7 $\pm$ 5.6) (ANOVA followed by the Tukey test, P = 0.005). Activity did not differ between samples from New York and Washington birds (Figure 3).

In all instances, enzyme activity did not vary with temperature over the range of 28° - 35° C. In the birds from Alaskan and New York, there was a dramatic increase in activity from 35° - 37° C (7X and 3X, respectively) (Figure 3). Birds from Washington maintained the same activity level across all tested temperatures ($y = 0.4x + 4.7$, $r^2 = 0.55$, P =0.15).

DISCUSSION

Black-capped Chickadees exhibit regional differences in body size across their geographic distribution. These differences follow the same pattern seen in other widely distributed passerines, e.g., American Goldfinch, *Carduelis tristis* (Dawson and Marsh 1986), House Finch, *Carpodacus mexicanus* (O'Connor 1996), House Sparrow, *Passer domesticus* (Kendeigh and Blem 1974), and White-crowned Sparrow, *Zonotrichia leucophrys* (King and Farner 1966). Populations residing at lower latitudes or warmer regions are generally smaller than conspecifics at higher latitudes (or relatively colder habitats). Washington birds had a smaller body mass and smaller wings than both New York and Alaskan populations.

Wing chord can be used as a general indicator of body size. In intraspecific comparisons, longer wings represent an increase in length in skeletal components and a concomitant increase in fat-free body mass (James 1970, Bryant 1989). James (1970) used wing chord to assess size variation in Carolina Chickadees (*Parus carolinensis*) throughout their geographic range.

At 12 grams, the Black-capped Chickadee is the smallest overwintering passerine in the far north. Small body size is a disadvantage at low temperatures due to increased surface to volume ratio and limited ability to store fat (decreased fasting duration). It could be that northern populations are at the lower limit of body size for overwinter survival at high latitudes. From the ratio of heat gain (M_b) to the coefficient of heat loss (C), Yarbrough (1971) has calculated that overwintering passerines in cold-temperate to subarctic regions can not be smaller than approximately 10 g. Anecdotal evidence supports this idea. Small-bodied Washington chickadees (9 - 10 g) brought to interior Alaska were unable to survive overwinter in outdoor flight aviaries when provided with *ad libitum* food and insulated roost boxes (Sharbaugh, unpub data). These birds were found dead and frozen in their nestboxes following the first -25° C night of the winter. Larger chickadees from Washington (11 - 12 g) maintained weight and survived the Alaskan winter (Sharbaugh, unpub data). Larger size would also be an advantage to chickadees in upstate New York.

Although body size varied regionally, level of fattening, relative to body size, was similar in each area. At the time of sampling, ambient temperature varied substantially among regions: +10° C in Washington, -7° C in New York and -24° C in Alaska. Photoperiod was more similar, 9.5/14.5 (Light/Dark) in New York and Washington and 8/16 (L/D) in Alaska. If photoperiod is the

ultimate cue controlling winter fattening, similar levels of fat deposition would be the end result. A similar pattern has been reported for another broadly distributed bird, the American Goldfinch (Dawson and Marsh 1986): birds in Texas deposited as much fat as conspecifics in Michigan, although ambient temperatures differed greatly between the two areas. The authors' explanation was that the record minimum temperature in both areas was not different. The degree of winter fattening in these two species, therefore, may be influenced by long-term climatic conditions.

Rogers et al. (1994) describe three patterns of winter fattening: responders (respond to the proximate environment), predictors (anticipate long-term environmental conditions) and responder-predictors (a combination of both types of regulation). Under this model, chickadees and goldfinches would be classified as predictors. Small-bodied, non-migratory chickadees may hedge their bets in winter by fattening sufficiently to give them enough reserves to survive a sudden dip in temperature. Social interactions and the predictability of food resources may also influence the extent of fattening (Ekman and Lilliendahl 1993). In Willow Tits (*Parus montanus*), subordinate flock members (with unpredictable access to food) carry more fat than dominant birds (their position in the flock guarantees priority access to food). But, in this study, all flock members fattened sufficiently to ensure overnight survival. Modifications occur after this basic level of fattening is reached.

Assuming that all birds maximize their food intake during the day, ALPL activity may explain in part how Alaskan birds achieve the same level of lipid deposition with one and a half hours less daylight and a larger thermal gradient than birds at lower latitudes experience. Enzyme activity in Alaska was much

higher than in both middle latitude populations. Increasing ALPL activity would increase the number of triacylglycerols hydrolyzed, resulting in more free fatty acids available for deposition (Ramenofsky 1990). This would allow for increased deposition in less time. It is also important to point out that even the lowest level of chickadee ALPL activity (Washington birds, 19.7 μMol FFA·h^{-1}·mg protein^{-1}) is 10X the enzyme activity in pre-migratory Dark-eyed Juncos (*Junco hyemalis*) (1.9 μMol FFA·h^{-1}·mg protein^{-1}) (Sharbaugh 1997) and Garden Warblers (*Sylvia boren*) (1.7 μMol FFA·h^{-1}·mg protein^{-1}) (Ramenofsky unpub. data). This suggests that chickadees have a basic mechanism that allows them to fatten rapidly. This existing system is then modified by Alaskan chickadees.

Increased activity may be a by-product of higher enzyme efficiency. Protein levels in Alaskan chickadee samples were half of the levels in the other populations. Further investigation is necessary to determine whether there is a seasonal increase in efficiency of the enzyme or an increase in the amount of enzyme.

ALPL activity varies with season in the Alaskan population (Sharbaugh 1997). Highest levels of activity coincide with the fastest daily fattening rates during the days of lowest temperature and shortest daylength, (December/January). Because the New York and Washington populations were sampled only once, we do not know whether their enzyme activity varies with environmental conditions. Repeated measures of these birds throughout the winter would help determine if ambient conditions or photoperiod influence enzyme activity.

An intriguing biochemical difference among these populations is the varied degree of thermal sensitivity of ALPL. In both Alaskan and New York

samples there is a dramatic increase in enzyme activity from 36 ° to 37° C. This rapid increase suggests the enzyme has undergone a conformational change (Hochachka and Somero 1973). Both populations use nocturnal hypothermia as a means of energy conservation during the winter (Chaplin 1974, Sharbaugh 1997). This reduction in body temperature may in turn inactivate a pool of ALPL that is reactivated upon rewarming at dawn. There would be no delay in fat deposition required by new enzyme synthesis after foraging begins in the morning. In a time limited environment (short daylength of winter), immediate fat deposition would be an advantage for survival. It is the daily fat deposition that fuels nocturnal energy expenditure.

Reinertsen and Haftorn (1983) have shown that the depth of nocturnal hypothermia in Willow Tits (*Parus montanus*) in Norway depends on ambient temperature. With relatively warmer winter temperatures, Washington chickadees may use nocturnal hypothermia infrequently. ALPL in this population may not be as sensitive to temperature as in the New York or Alaskan populations. Seasonal changes in the thermal behavior of enzymes have been observed in other heterothermic organisms (Pehowich and Wang 1984). Very minor alterations in the primary structure of an enzyme can produce changes in thermal sensitivity (Somero 1995). Exposure to a specific threshold temperature may be necessary for the production of a temperature sensitive isozyme. This could be an internal cue, lowered core temperature during bouts of nocturnal hypothermia or an external cue, low ambient temperatures.

Although I found differences in the body size (a very plastic characteristic), Black-capped Chickadees across their range are biochemically conservative with respect to the level of ALPL activity. An enzyme that is crucial

for overwinter survival shows the same general trend in activity (higher than other passerines) in all climatic regions. Enzyme activity and thermal sensitivity may be modulated by environmental conditions, increasing with decreasing temperature and photoperiod. This suggests that the basic biochemical machinery was in place when these birds expanded their range northward after the retreat of the Wisconsinian ice sheet 10,000 years ago (Gill et al. 1993). Slight modification of already existing mechanisms has allowed these small-bodied birds to survive in environments too hostile for other small passerines.

ACKNOWLEDGMENTS

Funding for this project was provided by an American Heart Association Fellowship awarded to the author. Sigma Xi Grants in Aid of Research provided travel funding to the author for instruction in assay techniques at the University of Washington. Many thanks to Dr. Andre Dhondt for access to birds and laboratory space at the Cornell Laboratory of Ornithology; Drs. Marilyn Ramenofsky and John Wingfield for access to and assistance at Friday Harbor Labs; and Gene Nelson, Sally, Burley and Juanita Sharbaugh for the use of their feeders. All birds were collected with the necessary state and federal permits (Scientific collecting permits: AK 96-52, NY LCP95-709, WA WM-0005 and USFWS PRT-692453). Surgical procedures were approved by the Institute of Arctic Biology Institutional Animal Care and Use Committee (Protocol # 96-26). Thanks are due to Drs. Pierre Deviche, Jim Sedinger, Dale Feist and Brian Barnes for helpful comments on earlier drafts of the manuscript.

LITERATURE CITED

American Ornithologists' Union (A. O. U.). 1957. Check-list of North American Birds, 5th ed. The Lord Baltimore Press, Inc., Baltimore, MD.

Blem C.R. 1976. Patterns of lipid storage and utilization in birds. American Zoologist 16:671-684.

Blem C.R. 1990. Avian energy storage. In: Power D.M. (ed), Current Ornithology. Vol.7. Plenum Publishing Corp., pp. 59-113.

Bryant D. M. 1989. House Martins. In: Newton I. (ed), Lifetime Reproduction in Birds. Academic Press, San Diego CA, pp. 89-107.

Budd S. M. 1973. Thermoregulation, bioenergetics, and endocrinology of cold acclimatization of Black-capped Chickadees, *Parus atricapillus*. PhD diss., Cornell University, Ithaca, New York.

Chaplin S. B. 1974. Daily energetics of the Black-capped Chickadee, *Parus atricapillus*, in winter. Journal of Comparative Physiology 89: 321-330.

Chaplin S. B. 1976. The physiology of hypothermia in the Black-capped Chickadee, *Parus atricapillus*. Journal of Comparative Physiology 112: 335-344.

Cooper S. J. and D. L. Swanson. 1994. Seasonal acclimatization of thermoregulation in the Black-capped Chickadee. Condor 96:638-646.

Cryer A 1987 Comparative biochemistry and physiology of lipoprotein lipase. In: Borensztajn J (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago. pp 277-329.

Dawson W. R. and R. L. Marsh. 1986. Winter fattening in the American goldfinch and the possible role of temperature in its regulation. Physiological Zoology 59:357-368.

Eckel R. H. 1987. Adipose tissue lipoprotein lipase. In: Borensztajn J. (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago. pp 277-329.

Ekman J. B. and K. Lilliendahl. 1993. Using priority to food access: fattening strategies in dominance-structured Willow Tit (*Parus montanus*) flocks. Behavioural Ecology 4:232-238.

Gill F. B., A. L. Mostrom, and A. L. Mack. 1993. Speciation in North American chickadees: I. Patterns of mtDNA genetic divergence. Evolution 47:195-212.

Grossman A. F. and G. W. West. 1977. Metabolic rate and temperature regulation of winter acclimatized Black-capped Chickadees, *Parus atricapillus*, of interior Alaska. Ornis Scandinavica 3:127-138.

Hochachka P. W. and G. N. Somero. 1973. Strategies of Biochemical Adaptation. W. B. Saunders Company, Philadelphia.

Irving L. 1960. Birds of Anaktuvuk Pass, Kobuk, and Old Crow. A study in arctic adaptation. U.S. National Museum, Washington, D.C..

James F. C. 1970. Geographic size variation in birds and its relationship to climate. Ecology 51:365-390.

Kendeigh S. C. and C. R. Blem. 1974. Metabolic adaptation to local climate in birds. Comparative Biochemistry and Physiology 48A:175-187.

Kessel B. 1976. Winter activity patterns of Black-capped Chickadees in interior Alaska. Wilson Bulletin 88:36-61.

King J. R. and D. S. Farner. 1966. The adaptive role of winter fattening in the White-crowned Sparrow with comments on its regulation. American Naturalist 914:403-418.

O'Connor T. P. 1996. Geographic variation in metabolic seasonal acclimatization in House Finches. Condor 98:371-381.

Pehowich D. J. and L. C. H. Wang. 1984. Seasonal changes in mitochondrial succinate dehydrogenase activity in a hibernator, *Spermophilus richardsonii.* Journal of Comparative Physiology B 154:495-501.

Prinzinger R., A. Preßmar and E. Schleucher. 1991. Body temperature in birds. Comp. Biochem. Physiol. 99A:499-506.

Ramenofsky M. 1990. Fat storage and fat metabolism in relation to migration. In: Gwinner E. (ed) Bird Migration. Physiology and Ecophysiology. Springer-Verlag, Berlin. pp 214-231.

Reinertsen R. E. 1996. Physiological and ecological aspects of hypothermia. Pp. 125-157 in Carey C. (ed) Avian Energetics and Nutritional Ecology. Chapman and Hall, New York.

Reinertsen R. E. and S. Haftorn. 1983. Nocturnal hypothermia and metabolism in the Willow Tit *Parus montanus* at 63°N. Journal of Comparative Physiology 151:109-118.

Rising J. D. and J. W. Hudson. 1974. Seasonal variation in the metabolism and thyroid activity of the Black-capped Chickadee (*Parus atricapillus*). Condor 76:198-203.

Rogers C.. M., V. Nolan, Jr. and E. D. Ketterson. 1994. Winter fattening in the dark-eyed junco: plasticity and possible interaction with migration trade-offs. Oecologia 97:526-532.

Root T. 1988. Energy constraints on avian distributions and abundances. Ecology 69:330-339.

Schotz M., A. Garfinkel, R. J. Huebolter, and J. E. Stewart 1970 A rapid assay for lipoprotein lipase. Journal of Lipid Research 11:68-69.

Sharbaugh S. M. 1997. Physiological Adaptations for Overwintering by Black-capped Chickadees (*Parus atricapillus*) in Interior Alaska. PhD diss., University of Alaska Fairbanks, Fairbanks, Alaska.

Smith S. M. 1991. The Black-capped Chickadee. Behavioral Ecology and Natural History. Cornell University Press, Ithaca, New York.

Somero G. N. 1995. Proteins and temperature. Annual Review of Physiology 57:43-68.

Weathers, W. W. 1979. Climatic adaptation in avian standard metabolic rate. Oecologia 42:81-89.

Weathers W. W. and K. A. Sullivan. 1993. Seasonal patterns of time and energy allocation by birds. Physiological Zoology 66:511-536.

Yarbrough, C. G. 1971. The influence of distribution and ecology on the thermoregulation of small birds. Comparative Biochemistry and Physiology 39A: 235-266.

Figure 1. Distribution of the Black-capped Chickadee in North America. Dashed lines mark the northern and southern boundaries of the range. Stars indicate location where samples were collected.

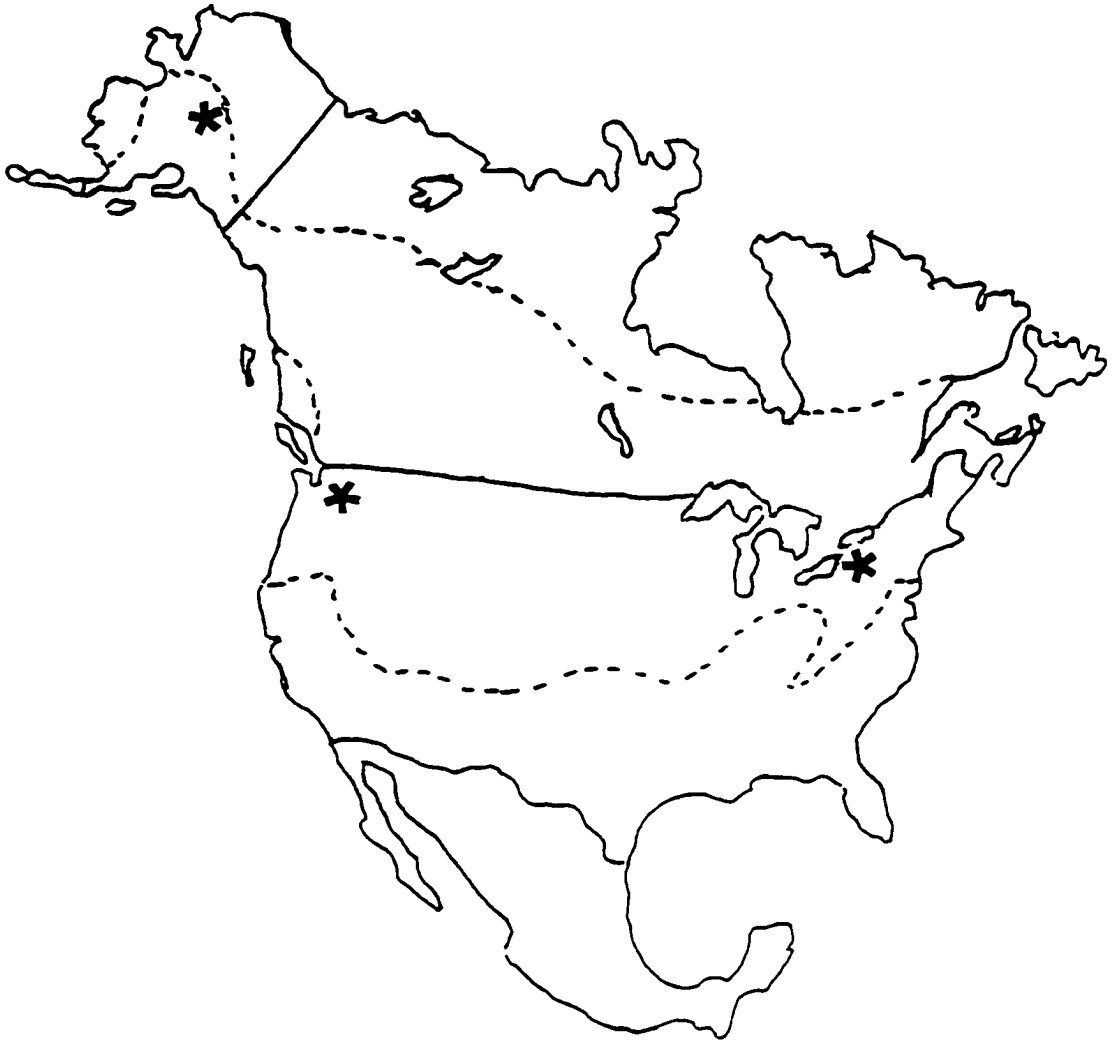
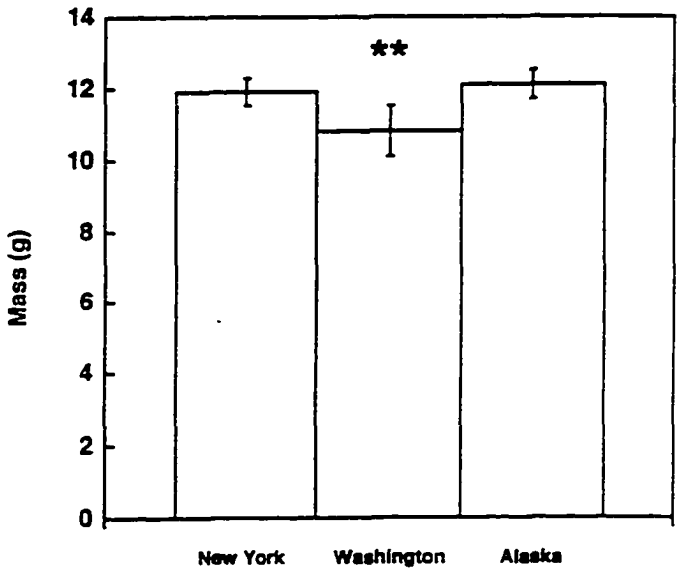


Figure 2. Mass (mean ± SD) (upper panel) and wing length (mean ± SD) (lower panel) of winter acclimatized Black-capped Chickadees captured in western Washington (n = 10) and northwestern New York (n = 14) in January 1996 and interior Alaska (n = 6) in February 1996. Stars indicate a significantly different population. Washington birds were significantly lighter and had significantly shorter wings than both New York and Alaska birds ($P < 0.001$ and $P < 0.001$, respectively).



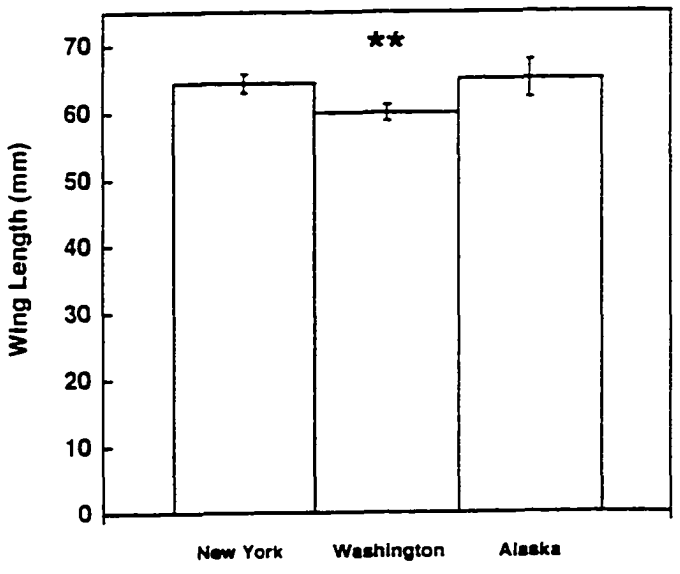
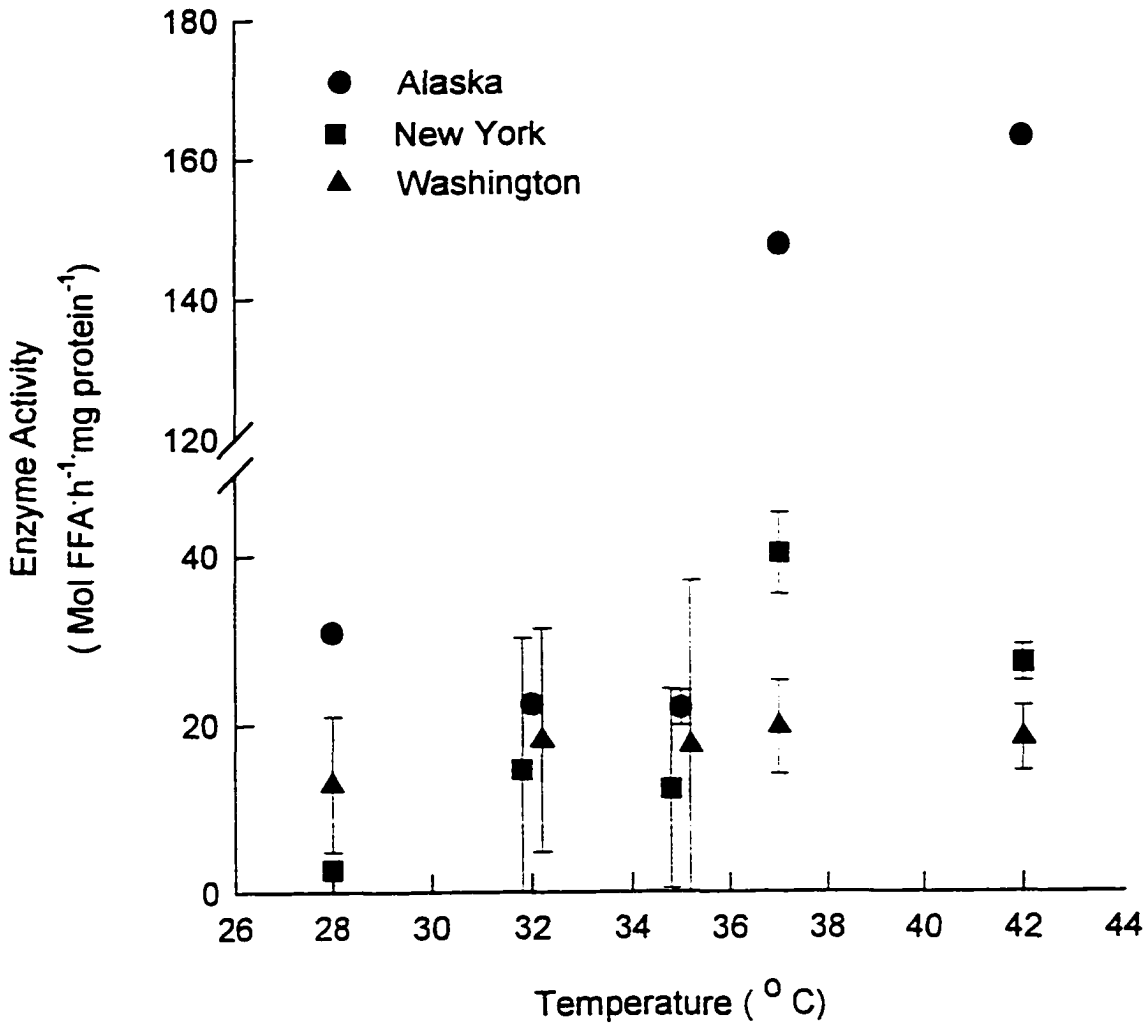


Figure 3.

In vitro adipose lipoprotein lipase activity (μMol free fatty acids released·h $^{-1}$·mg protein^{-1}) in pooled samples from winter acclimatized Black-capped Chickadee populations in western Washington, northwestern New York and interior Alaska incubated at 28°, 32°, 35°, 37° and 42° C.



SUMMARY AND CONCLUSIONS

The Black-capped Chickadee has proven to be a new model for examining the physiological adaptations for overwintering in the sub-arctic. This subspecies (*Parus atricapillus turneri*) follows the pattern of seasonal acclimatization first reported for conspecifics at lower latitudes: increased thermogenic capacity and efficiency and increased lipid reserves on a daily and seasonal basis. In addition, this northern population has gone on to modify these basic processes in response to more extreme climatic conditions.

Alaskan Black-capped Chickadees, like populations in temperate areas (Chaplin 1974, Cooper and Swanson 1994), do use nocturnal hypothermia as a means of energy conservation. But, unlike populations at lower latitudes, sub-arctic birds retain the ability to reduce their body temperature at night through the summer. The short sub-arctic summer combined with fluctuations in the severity of the climate may favor birds that do not lose their cold acclimatization during summer. These findings give us additional clues to how northern birds survive the long winter night. In order to fully understand the dynamics of overnight energy expenditure, we need to know to what temperatures the birds are exposed overnight. Where do these birds roost? What makes a good roost site? With this information, we can calculate a more realistic energy budget.

When we examine the results of the investigation of seasonal acclimatization of standard metabolic rate (SMR), we find that Alaskan Black-capped Chickadees show no seasonal change in SMR. This result is similar to the New York population but different than the South Dakota population. This dichotomy reflects the current state of the literature. The role of SMR in seasonal acclimatization is yet undefined.

However, when we compare SMR between winters, a different pattern emerges. Birds measured in one winter had significantly lower SMRs than those measured in the subsequent winter. Decreasing SMRs in winter have been reported in only two other species, King Penguin chicks, *Aptenodytes patagonicus* (Barre 1986) and Alaskan Willow Ptarmigan, *Lagopus lagopus* (West 1972), both high latitude residents. Lowering SMR during winter may reduce the daily energy expenditure (DEE) of these birds. The heterothermic nature of chickadees may allow a slight reduction in body temperature that would result in a reduction of DEE. If DEE is lowered, these birds may be able to divert metabolized energy into fat stores or limit utilization of fat stores if food is unavailable. This is important because depositing lipid reserves is imperative for small birds in the winter (King and Farner 1966). Fat deposited daily supplies the energy necessary to survive a nocturnal fast (Blem 1990). Controlled experiments need to be performed to completely explain the role of variation in ambient conditions on SMR. Is it the fluctuation in temperature or absolute temperature that has the greatest impact?

The role of lipid reserves in winter-acclimatization is clear. Black-capped Chickadees lose ≈8% of their body mass in fat overnight and replace these stores during the following day (Chaplin 1974). This daily cycle of winter fattening is observed in the interior Alaska population of Black-capped Chickadees. There is also a seasonal cycle of fattening with birds carrying more fat by dusk in winter than in other seasons. The increased activity of the enzyme necessary for fat deposition, adipose lipoprotein lipase (ALPL) (Cryer 1987), may aid Black-capped Chickadees in rapidly depositing fat during the cold, dark winter in Alaska.

ALPL activity in winter-acclimatized Alaskan birds was 20 - 30 times higher than activity levels previously reported for passerines during premigratory fattening (Ramenofsky, unpub. data). Activity level also varies with season, being highest when the daily rate of fattening is the highest (winter). Maximizing ALPL activity during the time when foraging time is the most limiting and the need for a large fat reserve is the greatest increases the efficiency of fat deposition. Hydrolyzation of triacylglycerols by ALPL is the rate-limiting step in the movement of free fatty acids (FFA) from the bloodstream into the cell (Eckel 1987). Increased ALPL activity results in an increase in the number and rate of FFAs entering the adipocyte (Ramenofsky 1990). This activity varies both with season and latitude. When comparing winter ALPL activity among three regions, interior Alaska (64° N), western Washington (47° N) and upstate New York (42° N), there were distinct geographic differences. ALPL activity levels were highest in the Alaska birds. The two lower-latitude populations showed similar activity levels. ALPL activity in all populations of Black-capped Chickadees is consistently higher than levels previously reported in other passerines. What we do not know is whether birds from Washington or New York would increase ALPL activity levels if exposed to Alaskan winters. More information about the biochemistry of chickadee ALPL is necessary to determine the mechanism behind this increased activity. What is the effect of plasma insulin (levels of this hormone increase after the ingestion of food) on ALPL activity? What role does ambient temperature play? Increasing or decreasing levels of circulating corticosterone (a "stress hormone") or thyroxine may signal changes in ambient conditions and may influence ALPL synthesis. Photoperiod may also be an important variable, changing levels of melatonin

may also have an effect. It would also be instructive to know how the activity of muscle lipoprotein lipase (MLPL) varies. This enzyme hydrolyzes triacylglycerols at muscle cells. There FFAs are used for fuel, not storage. Are ALPL levels low when MLPL levels are high? Do these tissues "compete" for circulating triacylglycerols? My thesis has examined only a few pieces of the puzzle of the biochemical aspects of winter fattening. It is an area ripe for investigation at the cellular and molecular level.

Although I have discussed these areas of investigation separately, they are by no means independent. It is the integration of many physiological and behavioral processes that result in a successful phenotype. And these processes work at vastly different scales, from the whole animal to the biochemical level. It is the challenge of the modern scientist to recognize that a specific physiological mechanism or single aspect of behavior is only a small part of a very complex, constantly changing system. Each element must ultimately be examined in context.

My thesis has shown that, in general, Black-capped Chickadees overwintering in interior Alaska display the same physiological adaptations as conspecifics at lower latitudes. But as climate increases in severity, small modifications of this basic pattern illustrate the inherent flexibility in the system. By fine-tuning a few details, (increased activity in a common, yet essential, enzyme, extending the use of an existing energy saving mechanism, and modulating the cost of existence metabolism), these birds can survive in a climate too harsh for most passerines.

Literature Cited

Barre H. 1986. Metabolic and insulative changes in winter- and summer-acclimatized king penguin chicks. Journal of Comparative Physiology B 154: 317 - 323.

Blem C.R. 1990. Avian energy storage. In: Power D. M. (ed), Current Ornithology. Vol. 7. Plenum Publishing Corp., pp. 59-113.

Chaplin S. B. 1974. Daily energetics of the Black-capped Chickadee, *Parus atricapillus,* in winter. Journal of Comparative Physiology 89: 321-330.

Cooper S. J. and D. L. Swanson. 1994. Seasonal acclimatization of thermoregulation in the Black-capped Chickadee. Condor 96: 638-646.

Cryer A. 1987. Comparative biochemistry and physiology of lipoprotein lipase. In: Borensztajn J. (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago. pp 277-329.

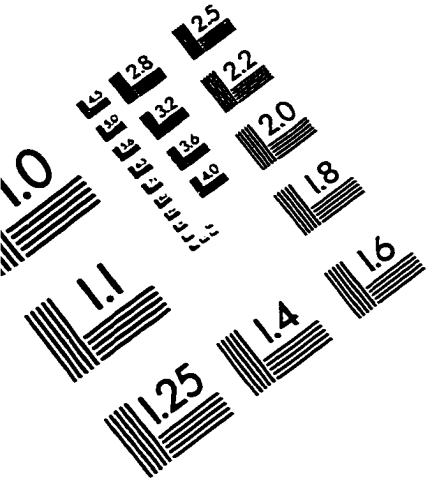
Eckel R. H. 1987. Adipose tissue lipoprotein lipase. In: Borensztajn J. (ed), Lipoprotein Lipase. Evener Publishers, Inc., Chicago. pp 277-329.

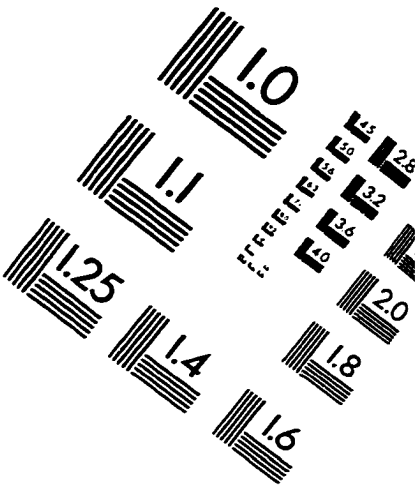
King J. R. and D. S. Farner 1966. The adaptive role of winter fattening in the White-crowned Sparrow with comments on its regulation. American Naturalist 914: 403-418.

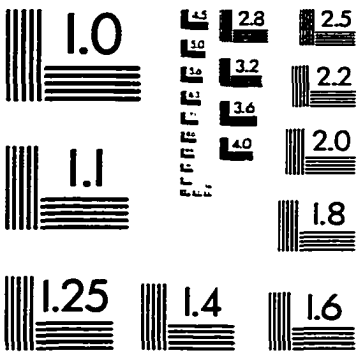
Ramenofsky M. 1990. Fat storage and fat metabolism in relation to migration. In: Gwinner E. (ed) Bird Migration. Physiology and Ecophysiology. Springer-Verlag, Berlin pp 214-231.

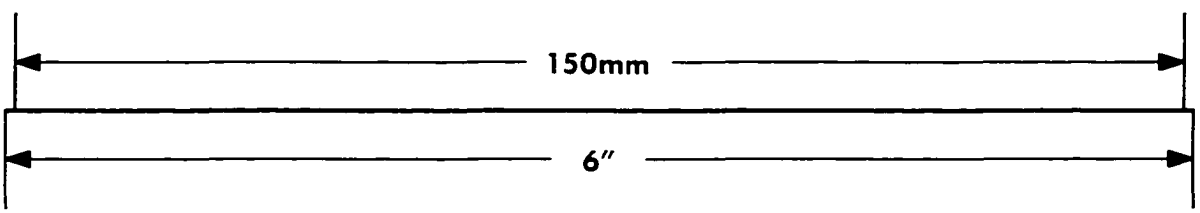
West G. C. 1972. Seasonal differences in resting metabolic rate of Alaskan ptarmigan. Comparative Biochemistry and Physiology A 42: 867-876.

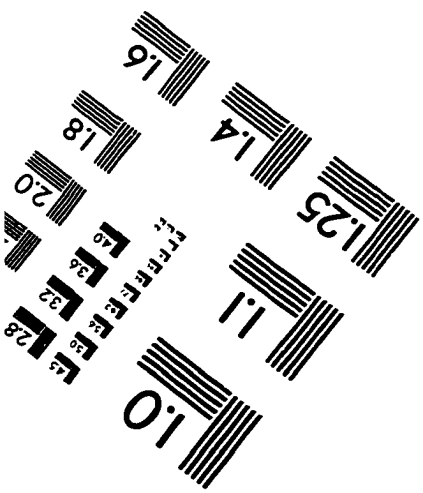
IMAGE EVALUATION TEST TARGET (QA-3)

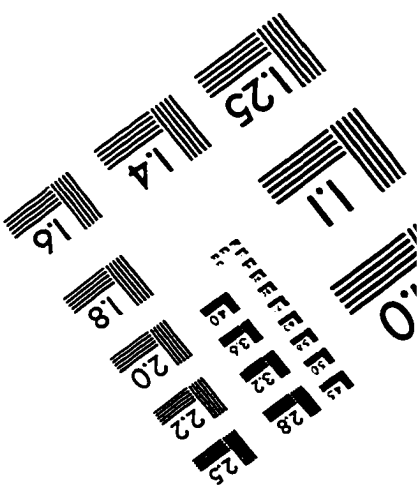












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989