



Phenotypic flexibility of body composition associated with seasonal acclimatization in passerine birds

Eric T. Liknes¹, David L. Swanson*

Department of Biology, University of South Dakota, 414 E. Clark St., Vermillion, SD 57069, USA

ARTICLE INFO

Article history:

Received 1 March 2011

Accepted 22 June 2011

Available online 1 July 2011

Keywords:

Birds

Phenotypic flexibility

Body composition

Exercise organs

Nutritional organs

Seasonal acclimatization

ABSTRACT

Improved winter cold tolerance is widespread among small birds overwintering in cold climates and is associated with improved shivering endurance and elevated summit metabolic rate (M_{sum}). Phenotypic flexibility resulting in elevated M_{sum} could result from either increased skeletal muscle mass (perhaps with support from similar adjustments in “nutritional organs”) and/or cellular metabolic intensity. We investigated seasonal changes in body composition of three species of passerine birds resident in cold winter climates, all of which show large seasonal variations in M_{sum} (> 25%); white-breasted nuthatch (*Sitta carolinensis*), black-capped chickadee (*Poecile atricapillus*), and house sparrow (*Passer domesticus*). All three species displayed significant winter increases in pectoralis and heart masses, and supracoracoideus mass also increased in winter chickadees. Gizzard mass increased in winter for all three species, but masses of other nutritional organs did not vary consistently with season. These data suggest that winter increases in pectoralis and heart masses are important contributors to elevated thermogenic capacity and cold tolerance, but seasonal variation in nutritional organ masses, other than gizzard, which is likely associated with dietary changes, are not universally associated with seasonal phenotypes. The winter increases in pectoralis and heart masses are consistent with data from other small passerines showing marked seasonal changes in cold tolerance and support the Variable Maximum Model of seasonal phenotypic flexibility, where physiological adjustments that promote improved cold tolerance, also result in elevated M_{sum} .

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Because organisms do not live in static environments, they must have the capacity to adjust to meet the demands of their changing environments. Phenotypic flexibility refers to phenotypic changes that are reversible, temporary, and repeatable, such as acclimation and acclimatization (Piersma and Lindstrom, 1997; Piersma and Drent, 2003). Physiological traits exhibit flexibility with climate, daily and annual cycles, aging, and behaviors (e.g., reproduction or migration). Therefore, phenotypic flexibility of physiological traits may be expected to be most apparent in organisms that live in variable environments or that seasonally engage in energetically demanding behaviors such as long-distance migration. While several studies have explicitly addressed mechanisms underlying phenotypic flexibility of physiological traits in birds during migration (Battley et al., 2000; McWilliams and Karasov, 2001; Guglielmo and Williams, 2003; Landys-Ciannelli

et al., 2003; Bauchinger and Biebach, 2005; Vézina et al., 2007), fewer studies have examined phenotypic flexibility associated with winter acclimatization or cold acclimation in small birds (Liu and Li, 2006; Vézina et al., 2006; Zheng et al., 2008).

Permanent resident birds of temperate climates provide a natural experiment in phenotypic flexibility. While molt and reproduction may represent periods of potentially high energetic demands for small birds at higher latitudes (Drent and Daan, 1980; Dawson et al., 1983a; Murphy and King, 1984), energetic demands in winter are apparently even greater, at least for small birds in cold climates (Weathers, 1999; Cooper, 2000; Doherty et al., 2001; Swanson, 2010). Birds in cold winter climates must maintain high metabolic rates for prolonged periods in the face of extreme cold, decreased access to food, and fewer hours of daylight in which to forage (Lima, 1986, 1988). This results in the formation of what can be termed “seasonal phenotypes,” which may involve a combination of phenotypically flexible responses to proximate (weather) and ultimate (climate) factors (Swanson and Olmstead, 1999; Piersma and Drent, 2003; Vézina and Williams, 2005).

The winter phenotype in small birds wintering in cold climates is characterized by a marked improvement in cold tolerance and an elevated summit metabolic rate (M_{sum} = maximum thermoregulatory

* Corresponding author. Tel.: +1 605 677 6175.

E-mail addresses: etliknes@northern.edu (E.T. Liknes), david.swanson@usd.edu (D.L. Swanson).

¹ Present address: Department of Biology, Northern State University, 1200S. Jay St., Aberdeen, SD 57401, USA.

metabolic rate; Dawson and Marsh, 1989, Marsh and Dawson, 1989; Swanson, 2010). Such increments of cold tolerance and M_{sum} may be associated with elevated fattening (Dawson et al., 1983b; Blem, 1990; Rogers and Smith, 1993), increased shivering endurance (Dawson and Marsh, 1989), changes in catabolic enzyme activities and substrate metabolism (Marsh and Dawson, 1982, Carey et al., 1989; Marsh et al., 1990; Swanson, 1991; O'Connor, 1995a), pectoralis muscle hypertrophy (Swanson, 1991; O'Connor, 1995a; Cooper, 2002), and increased blood oxygen carrying capacity and oxygen extraction efficiency (Swanson, 1990; Cooper and Same, 2000; Arens and Cooper, 2005a). The relative contributions of the above mechanisms to elevated cold tolerance and M_{sum} in winter appear to be neither consistent nor universal among small birds, and the functional significance of seasonal changes in these parameters is uncertain in many cases. Therefore, a definitive mechanistic explanation for the marked winter increases in cold tolerance and M_{sum} in small birds remains elusive.

M_{sum} is phenotypically correlated with basal metabolic rate (BMR) in temperate-zone birds (Dutenhoffer and Swanson, 1996; Rezende et al., 2002). Because of the high energetic costs associated with elevated thermogenesis in winter, energy processing capacity is also likely to improve in winter birds (Dykstra and Karasov, 1992; Konarzewski and Diamond, 1994). Increases in masses of “nutritional” organs (i.e., stomach, intestine, liver, and kidney) facilitate increased energy intake and expenditure and may contribute to an increased basal metabolic rate (Piersma et al., 1996a). Indeed, seasonal changes in muscle and visceral organ masses are often correlated with elevated metabolic rates in winter acclimatized (Swanson, 1991; O'Connor, 1995b; Liu and Li, 2006, Vézina et al., 2006) and migrating (Piersma et al., 1996a; Vézina et al., 2007) birds.

Black-capped chickadees (*Poecile atricapillus*), white-breasted nuthatches (*Sitta carolinensis*), and house sparrows (*Passer domesticus*) are permanent residents over most of their ranges, which include cold, temperate-zone climates (AOU, 1998). Large winter increments of basal and summit metabolism have been previously documented in all three of these species (Hart, 1962; Cooper and Swanson, 1994; Liknes and Swanson, 1996; Arens and Cooper, 2005b). Such increases in metabolic rates could result from elevated cellular metabolic intensity or from increments of tissue masses. In this study, our objectives were to determine: (1) whether summer to winter variation in organ masses occurs in small birds occupying cold winter climates, (2) whether seasonal changes in masses of certain organs consistently contribute to seasonal phenotypes, and (3) whether seasonal variation in organ masses, if present, is consistent with previously documented patterns of seasonal change in M_{sum} and BMR for these species.

2. Materials and methods

2.1. Birds

We captured wild, free-living adult birds by mist-net before 12:30 CST in Union and Clay counties, South Dakota (approximately 42°45'N, 97°W) in summer (mid-May to early September) and winter (mid-December to early March) from 1998 to 2002 to determine seasonal variation in masses of the flight muscles (pectoralis and supracoracoideus), the combined leg musculature, heart, and “nutritional organs” (liver, proventriculus, gizzard, intestine, and kidney). At capture, we weighed birds to the nearest 0.1 g using an Ohaus portable balance (Model LS200, Pine Brook, New Jersey) and recorded visible fat score (Helms and Drury, 1960). Only birds with completely ossified skulls (aged as adults; Pyle, 1997) were used for body composition measurements. We determined gender by plumage characteristics and

examination of gonads after dissection. We used approximately equal numbers of males and females for all species-season groups except summer nuthatches, where males predominated (male:female ratios—chickadees 21:21 in winter, 12:10 in summer; sparrows 15:13 in winter, 9:11 in summer; nuthatches 14:17 in winter, 16:3 in summer).

After capture, we transported birds to the laboratory where they were caged at room temperature (20–25 °C) for 1–5 h in small flight cages (40 cm × 26 cm × 22 cm). We fed birds mealworms (*Tenebrio* spp.), sunflower seeds, and water ad libitum during this holding period. The birds that we used for organ mass measurements were part of a larger study examining the effects of cold exposure on substrate metabolism (Liknes, 2005), so following the holding period, we subjected birds to 60-min metabolic trials at thermoneutral (30 °C in air) or cold (6 to –3 °C in 79% helium/21% oxygen) temperatures. For the cold-treatment birds, we exposed individual birds to a single temperature within this range, with the temperature varying depending on season and species (i.e., colder temperatures for winter birds and for larger species). A subset of birds was held for 1–3 h in flight cages without metabolic trials as controls. Animal handling protocols were approved by the University of South Dakota Animal Care and Use Committee and conformed to the Ornithological Council's Guidelines for the Use of Wild Birds in Research.

2.2. Tissue and organ masses

Immediately upon completion of the metabolic trials or after the 1–3 h holding period (for controls), we euthanized birds by cervical dislocation and rapidly excised tissues on ice, measuring wet masses to 0.01 g before flash-freezing tissues in liquid nitrogen with subsequent storage at –70 °C. We removed gut contents prior to weighing stomach and intestine, but did not identify dietary items because birds were allowed to feed in the laboratory prior to euthanasia. Complete dissection of each bird took approximately 45 min and all dissections were completed within 9 h of capture. We dissected birds between 1200–1730 CST in winter and 1200–1900 CST in summer. We measured dry mass after tissues were freeze-dried in a Lyph-Lock 4.5 L lyophilizer (Labconco Corporation, Kansas City, Missouri). For paired muscles, we usually only freeze-dried one of the pair, so dry mass data for these muscles represent values for one muscle only. We calculated tissue water content as the difference between wet mass and dry mass. We defined carcass mass as the mass of the skeleton and remaining tissues after removal of the muscles and organs measured in this study, as well as gonads, skin and plumage.

2.3. Statistical analyses

We present data as means ± SD. We compared body mass between genders and seasons for each species by student's *t*-test or Mann–Whitney tests if sample variances were not homogeneous. For individual species, we conducted seasonal comparisons of mean muscle and organ wet masses by three-way ANCOVA, with season, sex, and treatment (thermoneutral, cold exposure and controls) as independent variables to account for differences in treatment histories prior to dissection, and carcass mass as the covariate. In a few cases where the assumptions of normality and/or homogeneity or variances were not met, we *ln* transformed data and repeated the ANCOVA on transformed data. In a few cases, *ln* transformation did not satisfy ANCOVA assumptions. However, ANCOVA is robust with respect to small deviations from homogeneity of variances and deviations from normality (Zar, 1996). If main effects of gender and treatment, interaction terms, or the covariate term were not significant, we simplified the model by removing non-significant variables and re-ran the

simplified ANCOVA (or ANOVA if the covariate was not significant) until only significant variables (and gender or treatment effects if interaction terms involving gender or treatment were significant but main effects were not) remained in the model. We used the Tukey test for pairwise multiple comparisons if the ANCOVA gave significant results. We compared percent water content of tissues by three-way ANOVA, with season, gender, and treatment as independent variables.

We were not able to obtain dry mass data for all individual birds because a number of tissues were used for other experiments (Liknes, 2005). Thus, sample sizes were smaller for dry mass comparisons than for wet mass comparisons and in some cases did not provide sufficient samples sizes for three-way ANCOVA. Consequently, we pooled data for sexes and treatments and compared summer vs. winter values for dry organ masses by *t*-test (or Mann–Whitney *U*-test if parametric assumptions were not met). We felt justified in pooling data because gender and treatment were often not significant effectors of wet organ masses in nuthatches and sparrows and we used similar ratios of males to females in seasonal comparisons for all species except summer nuthatches where males predominated in the sample. However, given that male nuthatches average larger than females (Pyle, 1997), winter increments in this species are likely conservative, so unequal weighting of seasonal means by differential sex ratios did not confound analyses. For dry mass comparisons, we also pooled proventriculus and gizzard together into “stomach,” as proventriculus dry mass was very small and difficult to measure.

We conducted comparisons of seasonal variation in the relationship between pectoralis and supracoracoideus masses (the principal flight muscles) by comparing linear regressions of the residuals of wet pectoralis mass vs. wet carcass mass against residuals of wet supracoracoideus mass vs. wet carcass mass. We compared slopes of these lines (summer and winter) for each species by *t*-test (Zar, 1996). All ANCOVA, ANOVA, *t*-tests, Mann–Whitney tests, and linear regressions were performed with SAS statistical software (SAS Institute Cary, North Carolina) or Sigma-Stat, version 3.5 (Systat Software, Inc., Chicago, Illinois).

3. Results

Body mass (M_b) for black-capped chickadees was significantly greater in males than in females at both seasons (winter $U=400.0$, $P<0.001$; summer $t_{19}=5.057$, $P<0.001$) and significantly greater in winter than in summer for both genders (females $t_{28}=2.736$, $P=0.011$; males $U=195.5$, $P=0.010$). Mean M_b values for chickadees were: winter females, 12.06 ± 0.53 g ($n=21$); winter males, 13.50 ± 1.04 g ($n=21$); summer females, 11.50 ± 0.47 g ($n=9$); summer males, 12.55 ± 0.47 g ($n=12$). Male house sparrows had significantly greater M_b than females in winter ($t_{26}=3.554$, $P=0.001$), but not in summer, and winter M_b was significantly greater than in summer for males ($t_{22}=3.209$, $P=0.004$), but not for females. House sparrow mean M_b values were: winter females, 26.04 ± 1.52 g ($n=13$); winter males, 28.72 ± 2.32 g ($n=15$); summer females, 26.17 ± 1.85 g ($n=11$); summer males, 26.0 ± 1.30 g ($n=9$). M_b in white-breasted nuthatches did not vary by gender in either season, so we pooled values for seasonal comparisons. Winter nuthatches (20.64 ± 0.94 g, $n=31$) had significantly ($t_{48}=2.625$, $P=0.012$) larger M_b than summer nuthatches (19.64 ± 1.78 g, $n=19$).

3.1. Wet muscle masses

All three study species showed significant increases in pectoralis mass in winter relative to summer. Season, gender, and treatment all significantly influenced pectoral muscle mass in chickadees, with

mean pectoralis mass 12.3% greater in winter than in summer ($F_{1,62}=24.79$, $P<0.001$), males with 15.3% greater pectoralis mass than females ($F_{1,62}=9.61$, $P=0.003$), and cold-exposed birds having 6.7% lower pectoralis mass than warm-exposed birds ($F_{2,57}=5.07$, $P=0.009$). Nuthatches and sparrows also exhibited significant winter increases in pectoralis mass of 11% ($F_{1,49}=13.90$, $P<0.001$) and 8% ($F_{1,47}=6.02$, $P=0.018$; Fig. 1). No other significant main effects of treatment or gender on pectoralis mass occurred in nuthatches or sparrows, nor were any interaction terms significant.

Supracoracoideus wet mass was significantly greater in winter than in summer for black-capped chickadees (9.9%, $F_{1,61}=5.98$, $P=0.018$), but did not vary significantly on a seasonal basis for house sparrows or white-breasted nuthatches. The only other significant effect on supracoracoideus mass was the season*gender interaction for nuthatches ($F_{1,49}=5.44$, $P=0.024$), where females had larger muscles than males in summer, but males had larger muscles than females in winter.

Last squares regression of residuals from wet carcass mass vs. pectoralis mass against those from wet carcass mass vs. supracoracoideus mass showed that slopes differed significantly between summer and winter for sparrows ($t_{42}=2.02$, $P=0.01$) but not for chickadees or nuthatches (Fig. 2). Sparrows showed a steeper slope in winter than in summer, which indicates that

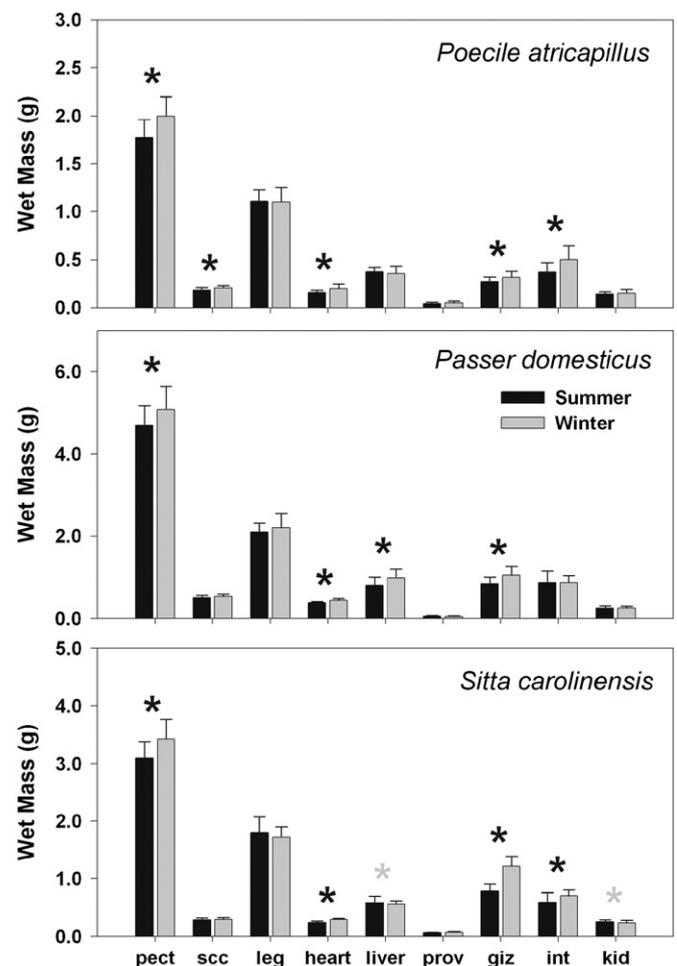


Fig. 1. Seasonal changes in wet body composition (mean \pm SD) in black-capped chickadees (*Poecile atricapillus*; $n=22$ summer, 42 winter), house sparrows (*Passer domesticus*; $n=20$ summer, 28 winter), and white-breasted nuthatches (*Sitta carolinensis*; $n=20$ summer, 37 winter). Mean seasonal values include all birds (treatment groups pooled). Organ abbreviations are pect (pectoralis), scc (supracoracoideus), leg (mixed leg muscle), prov (proventriculus), giz (gizzard), int (intestine), and kid (kidney). * (Black) Winter significantly greater than summer; * (Grey) Summer significantly greater than winter.

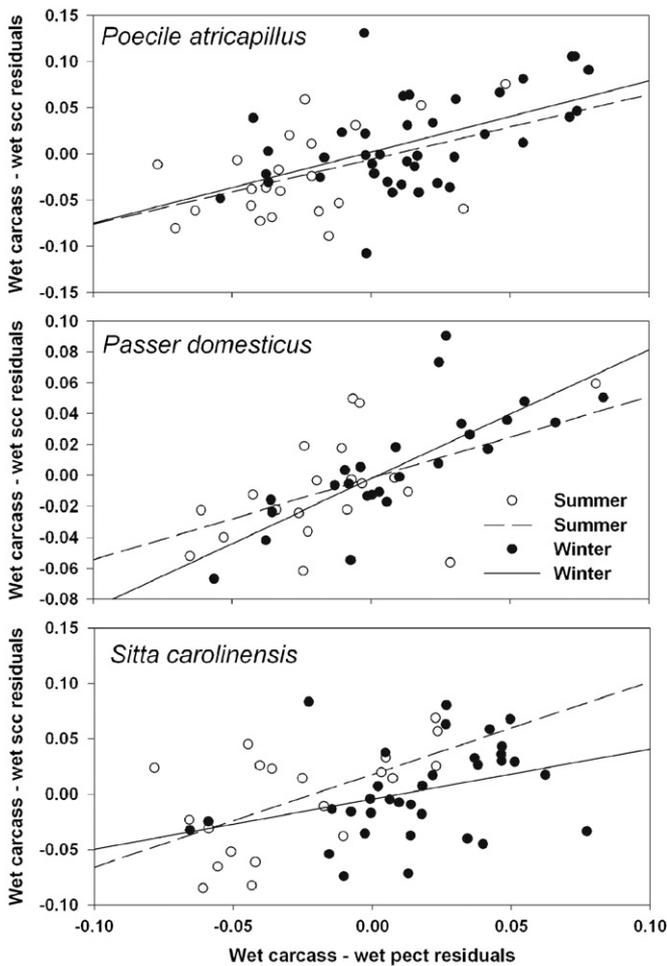


Fig. 2. Seasonal changes in the relationship of supracoracoideus and pectoralis residuals (g) from regressions of muscle mass against carcass mass in black-capped chickadees (*Poecile atricapillus*), house sparrows (*Passer domesticus*), and white-breasted nuthatches (*Sitta carolinensis*).

supracoracoideus mass increases with body size at a faster rate than pectoralis mass in winter, such that larger birds have a higher ratio of supracoracoideus mass to pectoralis mass.

Leg muscle wet mass did not vary significantly with season or treatment in any of the three species (Fig. 1). Chickadees, however, did show a significant gender effect ($F_{1,55}=9.08$, $P=0.004$), with males having 10.3% larger leg muscle mass than females. No significant interaction terms were detected for leg muscle for any species. The increase in heart wet mass in winter relative to summer was significant for all three species (Fig. 1). Statistics for these comparisons were: chickadees (29.2%, $F_{1,62}=6.80$, $P=0.012$); sparrows (14.2%, $F_{1,47}=22.15$, $P<0.001$); and nuthatches (24.1%, $F_{1,49}=47.77$, $P<0.001$). No significant gender or treatment main effects or interactions on heart mass were present for any of the three species.

3.2. Wet nutritional organ masses

Proventriculus wet mass showed no significant seasonal variation for any of the three study species (Fig. 1). Female nuthatches had significantly larger proventriculus mass than males ($F_{1,48}=6.42$, $P=0.015$), but gender did not influence proventriculus mass in chickadees or sparrows. Treatment did not significantly affect proventriculus mass in any of the three species. However, the season*treatment ($F_{2,52}=5.75$, $P=0.006$) and season*gender ($F_{1,59}=6.25$, $P=0.016$) interactions were

significant for chickadees, with proventriculus mass greater in warm-exposed birds than in cold-exposed birds in winter but the opposite pattern in summer, and female proventriculus mass greater than that in males in summer, but not in winter. No other interaction terms were significant for any of the three species.

All three species demonstrated significant winter increases in gizzard wet masses (Fig. 1), with masses increasing by 17.4% for chickadees ($F_{1,59}=6.56$, $P=0.013$), 19.2% for sparrows ($F_{1,47}=12.65$, $P<0.001$), and 50.8% for nuthatches ($F_{1,48}=87.69$, $P<0.001$). No significant treatment or gender effects for gizzard wet mass were observed for any of the three species and the only significant interaction term was season*gender for chickadees ($F_{1,59}=5.03$, $P=0.029$), where gizzard mass in males was greater than in females in winter, but not in summer.

Intestine wet mass was significantly greater in winter than in summer for chickadees (26.4%, $F_{1,62}=14.03$, $P<0.001$) and nuthatches (18.3%, $F_{1,46}=8.31$, $P=0.006$), but not for sparrows (Fig. 1). Treatment also exerted a significant effect on intestine wet mass in chickadees ($F_{2,60}=3.20$, $P=0.048$), with cold-exposed birds having greater masses than controls. The season*gender ($F_{1,46}=4.90$, $P=0.034$) and treatment*gender ($F_{2,39}=6.60$, $P=0.003$) interaction terms were also significant for sparrows, with intestine mass greater in females than in males in summer but not in winter, and cold-exposed males with larger intestines than control males or thermoneutral females. Nuthatches displayed several significant interaction terms, including season*gender ($F_{1,49}=15.52$, $P<0.001$), with intestine wet mass in females greater than in males in summer but not in winter, season*treatment ($F_{2,39}=7.47$, $P=0.002$), with control birds having smaller intestines than cold or thermoneutral birds in summer but not in winter, and treatment*gender ($F_{2,39}=5.02$, $P=0.012$), with females having larger intestines than males in cold and thermoneutral groups but not in controls. No other significant main effects or interactions were detected for intestine wet mass.

The three species showed different seasonal trends for liver wet mass. Sparrows exhibited a significant winter increase (22%, $F_{1,46}=10.76$, $P=0.002$), nuthatches a significant summer increase ($F_{1,49}=5.08$, $P=0.029$), and chickadees no significant seasonal variation. Gender also significantly affected liver wet mass in nuthatches, with females having 19.4% larger livers than males ($F_{1,49}=6.65$, $P=0.003$). Treatment also showed a significant effect on liver mass in nuthatches ($F_{2,44}=5.70$, $P=0.006$), with cold-exposed birds having 13.6% greater liver wet mass than warm-exposed birds. The only significant interaction term for liver wet mass in any of the study species was season*gender in house sparrows ($F_{1,46}=5.96$, $P=0.019$), where females had larger livers than males in summer but not in winter.

Kidney wet mass was significantly greater in summer than in winter for nuthatches ($F_{1,49}=6.09$, $P=0.018$), but did not significantly vary with respect to season in chickadees or sparrows (Fig. 1). Treatment also significantly affected kidney wet mass in nuthatches ($F_{2,45}=3.58$, $P=0.036$), with control birds having smaller kidneys than cold- or thermoneutral-exposed birds. No other main effects or interactions were significant for kidney wet mass for any of the three species.

3.3. Water content

Water content did not vary significantly between seasons for any tissue and ranged from 70% to 80% for most tissues (Fig. 3). Carcass water content ranged from 64–67% for all species.

3.4. Dry muscle masses

Dry masses of skeletal muscles and heart followed similar patterns of seasonal mass changes as wet muscle masses. Pectoralis

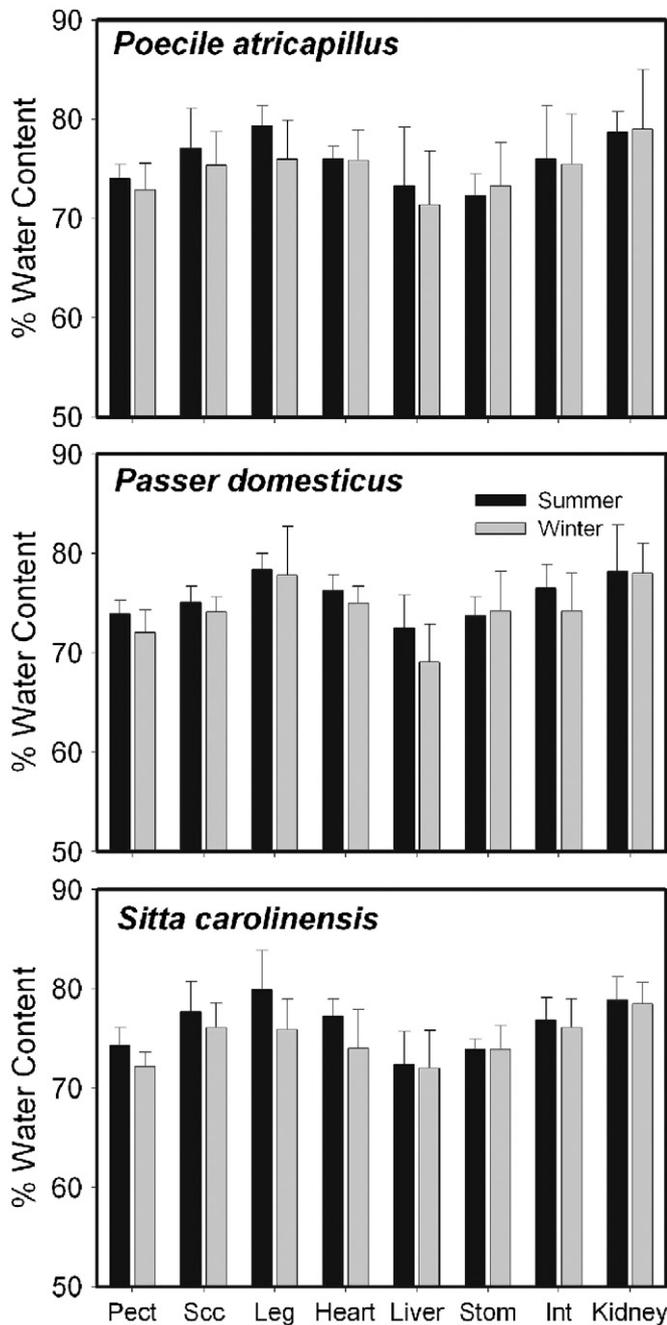


Fig. 3. Seasonal variation in organ water content (mean \pm SD) in black-capped chickadees (*Poecile atricapillus*), house sparrows (*Passer domesticus*), and white-breasted nuthatches (*Sitta carolinensis*). No significant seasonal differences were detected. Tissue abbreviations are the same as in Fig. 1, except Stom=stomach.

dry mass increased significantly in winter for all three species, with increments of 18.4% in chickadees ($U_{38}=250.0$, $P=0.002$), 17.1% in sparrows ($t_{27}=2.62$, $P=0.014$), and 15.0% in nuthatches ($U_{25}=138.0$, $P=0.008$). Similarly, supracoracoideus dry mass increased in winter for all species, significantly so only for house sparrows (7.8%, $U_{25}=129.0$, $P=0.046$), but winter increments approached significance for both chickadees (19.0%, $P=0.066$) and nuthatches (14.8%, $P=0.056$). Leg muscle dry mass displayed no significant seasonal variation for any of the three species. Heart dry mass displayed significant winter increases for all three species. Winter increments of heart dry mass were 31.2% for chickadees ($t_{38}=3.38$, $P=0.002$), 14.3% for sparrows ($U_{25}=34.0$, $P=0.034$), and 34.1% for nuthatches ($U_{24}=139.0$, $P<0.001$).

3.5. Dry nutritional organ masses

As for muscles, lean dry nutritional organ masses followed similar patterns of mass changes as did wet organ masses. Stomach dry mass increased significantly in winter for both house sparrows (28.1%, $t_{20}=2.45$, $P=0.024$) and white-breasted nuthatches (50.9%, $t_{22}=6.30$, $P<0.001$), but not for black-capped chickadees. Intestine dry mass was higher in winter than in summer for chickadees (33.9%, $t_{37}=2.59$, $P=0.014$) and nuthatches (21.1%, $t_{25}=2.29$, $P=0.031$), but not for sparrows. Liver dry mass increased significantly in winter only for house sparrows (28.8%, $U_{31}=198.0$, $P=0.019$). Kidney dry mass showed no significant seasonal variation for any of the three species.

4. Discussion

4.1. Muscle masses

Because the pectoralis muscle is the primary thermogenic organ in birds (Carey et al., 1978; Marsh and Dawson, 1982), and thermogenic capacity increases in winter for all three study species, it is not surprising that winter increments of pectoralis mass were a consistent component of the winter phenotype in these birds. Several previous studies documented a positive seasonal correlation between thermogenic capacity and pectoralis mass in small birds (Swanson, 1991; O'Connor, 1995b; Cooper, 2002). Myostatin, a prominent inhibitor of muscle growth in birds and mammals, may play a regulatory role in seasonal changes in pectoralis mass and thermogenic capacity, as gene expression of both myostatin and its metalloproteinase activator TLL-1 were reduced in winter relative to summer house sparrows (Swanson et al., 2009). This seasonal effect appears generally limited to the pectoralis muscle; however, as supracoracoideus masses did not consistently increase in winter for all three species in this study and leg muscle masses did not increase in winter for any species.

Our study population of house sparrows appears to show relatively small winter increments of M_{sum} (11%; Swanson and Liknes, 2006) compared to other populations from Ontario (43%; Hart, 1962) and Wisconsin (30%; Arens and Cooper, 2005b), but the winter increase in M_{sum} closely matches the increase in pectoralis mass (8% wet, 18% dry). These results are similar to previous studies that document a winter increase in pectoralis mass that also approximates the winter increase in M_{sum} (Swanson, 1991; O'Connor, 1995a; Cooper, 2002).

Because birds shiver isometrically (Hohtola, 1982), shivering capacity might be limited by the smallest muscle of an antagonistic pair. In volant birds, the supracoracoideus, which powers the flight upstroke, is much smaller than the pectoralis, which powers the flight downstroke and is, therefore, potentially limiting to thermogenesis by flight muscles (Marsh and Dawson, 1989). However, significant winter increases in supracoracoideus wet mass occurred only for chickadees (10%) and significant winter increases in dry mass only for sparrows (8%). Moreover, the percent winter increases in supracoracoideus mass were similar to those for pectoralis and mass-specific metabolic intensity of the supracoracoideus, as measured by citrate synthase activity, was not preferentially elevated in winter relative to pectoralis for these study species (Liknes, 2005). Thus, greater increments of aerobic capacity in supracoracoideus relative to pectoralis do not appear to contribute to winter increases in thermogenic capacity in small birds. Nevertheless, these three species showed a general trend toward winter increases in supracoracoideus mass, which could contribute to overall winter increases in thermogenic capacity. In addition, if birds can morphologically "lock" the shoulder joint in position while

shivering, then supracoracoideus size may not function to limit isometric shivering capacity and winter increases in the size of pectoralis and supracoracoideus could both contribute to winter increments of thermogenic capacity.

Leg muscles are the principal muscles involved in shivering for precocial chicks of Galliform birds, where leg muscles are larger and, for some species, more aerobic than pectoral muscles (Marjoniemi and Hohtola, 1999). In contrast, the pectoralis plays the prominent role in shivering thermogenesis in altricial pigeon (*Columba livia*) nestlings (Marjoniemi and Hohtola, 1999), nestling and adult red-winged blackbirds (*Agelaius phoeniceus*; Olson, 1994), and other passerines (Marsh and Dawson, 1989), where the pectoralis is largely aerobic. Because leg muscles may play a limited, but supporting, role in thermogenesis in passerine birds (Marsh and Dawson, 1989; Carey et al., 1989; Olson, 1994), leg muscle hypertrophy in winter, similar to that for pectoralis, might also contribute to winter increases in M_{sum} . This does not seem to be the case for these three species, as leg muscle mass did not vary significantly with season. This is perhaps not surprising because leg muscles tend to be less aerobic than flight muscles in passerines (Carey et al., 1978, 1989; Dawson and Olson, 2003), and leg muscle masses were only 39–57% of flight muscle masses in these species, which use leg muscles principally for perching or arboreal locomotion.

Significant winter heart hypertrophy occurred in all three species in this study, which suggests that elevated capacity of the cardiovascular system to deliver blood to working muscles contributes to the winter phenotype in small birds generally. Such an increase in heart muscle mass with elevated thermogenic demands is consistent with similar increases in heart mass associated with winter acclimatization and migration (Piersma et al., 1999; Liu and Li, 2006; Zheng et al., 2008) and also accompanies increases in hematocrit or oxygen carrying capacity of the blood in winter (deGraw et al., 1979; Swanson, 1990) and migration (Morton, 1994; Piersma et al., 1996b). This suggests that heart mass is commonly correlated with elevated capacities for highly aerobic endurance activity in birds (Chappell et al., 1999; Hammond et al., 2000). Elevated winter capacity for pumping blood may act in concert with higher oxygen carrying capacities and higher ventilatory oxygen extraction efficiencies (EO_2) during cold exposure to enhance oxygen delivery to shivering muscles in winter birds. Both house sparrows and black-capped chickadees can modify EO_2 (calculated from measures of oxygen consumption and minute volume) during cold exposure on a seasonal basis, with higher EO_2 in winter than in summer for both species (Cooper and Same, 2000; Arens and Cooper, 2005a, 2005b).

4.2. Nutritional organ masses

Changes in gizzard and intestine masses in birds are correlated with changes in diet (Ankney and Scott, 1988; Brugger, 1991; Geluso and Hayes, 1999; Dekinga et al., 2001; Piersma et al., 2004), but may be associated with changes in energetic demands (Dykstra and Karasov, 1992; Piersma et al., 1996a; but see Vézina et al., 2006). Winter gizzard hypertrophy was significant for all three species in this study, but masses of other nutritional organs did not vary consistently with season. Winter gizzard hypertrophy may be largely influenced by seasonal changes in diet. The greatest winter increment in gizzard mass in our study occurred in nuthatches (51%, compared to 17–19% for chickadees and sparrows). This is consistent with seasonal variation in diet in the three species, with nuthatches switching from a diet with no seeds in summer to a diet comprised of 68% seeds in winter (Odum, 1942; Martin et al., 1951; Grubb and Pravosudov, 2008). In contrast, black-capped chickadees switch from a diet of 10–20%

plant matter in summer to about 50% plant matter in winter (Smith, 1993) and house sparrows maintain a high percentage of plant material (> 90%) in their diet throughout the year, although the arthropod content is slightly higher in summer diets (Kalmbach, 1940 in Martin et al., 1951; Lowther and Cink, 2006).

Seasonal variation in proventriculus mass was not apparent for any of the study species, so was not related to the winter phenotype. Intestine mass did not reflect a straightforward relationship with an assumed increase in energy assimilation capacity in winter or with dietary changes for the three study species. Intestine mass increased in winter for chickadees and nuthatches, but was seasonally stable for sparrows. Gender differences were also evident in sparrows and nuthatches, with summer females exhibiting greater intestine masses than winter females and males at both seasons, perhaps reflecting specific breeding requirements for females of these species. The absence of consistent winter increases in intestine mass in this study contrasts with results from Eurasian tree sparrows (*Passer montanus*), which exhibit consistent winter increases in intestine mass (Liu and Li, 2006; Zheng et al., 2008).

Liver mass increased significantly in winter only for house sparrows and nuthatches showed a winter decrease in liver wet mass. Thus, elevated liver mass is also not consistently associated with winter increases in the capacity for energy assimilation in these species. This is in contrast to studies of seasonal acclimatization in some other passerines, which showed winter increases in liver mass (Coleman and Robson, 1975; Swanson, 1991; Liu and Li, 2006; Zheng et al., 2008). Our results suggest that winter increases in liver mass are not a universal component of the winter phenotype in small birds. Changes in kidney mass were not correlated with winter acclimatization in any of the three study species. The lack of significant winter increments of kidney mass is consistent with other studies of small birds (Casotti, 2001; Liu and Li, 2006; Zheng et al., 2008).

In conclusion, the consistent winter increases in pectoralis and heart masses suggest that hypertrophy of these organs is a common contributor to the winter phenotype in small birds, whereas seasonal phenotypic flexibility of nutritional organ masses is not universally associated with acclimatization. The winter hypertrophy of heart and pectoralis muscles is consistent with data from previous studies of small birds. Two non-mutually exclusive hypotheses have been developed to mechanistically describe seasonal metabolic phenotypes in small birds, the Variable Fraction Model and the Variable Maximum Model (Liknes et al., 2002). The Variable Fraction Model predicts that increased cold tolerance (i.e., shivering endurance) of winter birds results from these birds being able to sustain higher fractions of M_{sum} than summer birds. In this model, M_{sum} may not vary seasonally, but physiological adjustments that promote improvements in fuel delivery and substrate metabolism act to enhance endurance at submaximal levels of heat production, thereby promoting elevated shivering endurance and cold tolerance. The Variable Maximum Model contends that the primary mechanism promoting increased cold tolerance in winter birds is an increase in M_{sum} (i.e., thermogenic capacity), such that sustaining even a constant fraction of M_{sum} would elevate shivering endurance and cold tolerance. The primary physiological adjustments underlying this model would act to increase overall organismal aerobic capacity by winter increases in muscle size or cellular aerobic capacity. The consistent winter hypertrophy of flight muscles and heart in the three species in this study should act to increase M_{sum} , but not the fraction of M_{sum} , which can be sustained. Thus, these data are more consistent with the Variable Maximum Model than the Variable Fraction Model as the principal mechanism promoting winter metabolic acclimatization in small birds (Liknes et al., 2002).

Acknowledgments

Thanks to Karen Olmstead for statistical assistance and to Christy Delancy, Blake Greenfield, Travis Hills, Sherri McGregor, and Holly Retzer, and for technical assistance. We also thank Mark Dixon for help with statistics and SAS. We thank two anonymous reviewers, whose comments greatly improved the manuscript. This study was partially funded by NSF-EPSCoR 0091948 and by grants from the USD Office of Research to ETL and DLS, Sigma Xi, and Frank M. Chapman Memorial Fund to ETL. DLS was also supported by NSF IOS 1020218. Birds were collected under federal (PRT-7774790) and state (16) collecting permits to ETL.

References

- American Ornithologists' Union, 1998. Checklist of North American Birds, seventh ed. American Ornithologists' Union, Washington DC.
- Ankney, C.D., Scott, D.M., 1988. Size of digestive organs in breeding brown-headed cowbirds, *Molothrus ater*, relative to diet. *Can. J. Zool.* 66, 1254–1257.
- Arens, J.R., Cooper, S.J., 2005a. Seasonal and diurnal variation in metabolism and ventilation in house sparrows. *Condor* 107, 433–444.
- Arens, J.R., Cooper, S.J., 2005b. Metabolic and ventilatory acclimatization to cold stress in house sparrows (*Passer domesticus*). *Physiol. Biochem. Zool.* 78, 579–589.
- Battley, P.F., Piersma, T., Dietz, M.W., Tang, S., Dekinga, A., Hulsman, K., 2000. Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proc. R. Soc. B* 267, 191–195.
- Bauchinger, U., Biebach, H., 2005. Phenotypic flexibility of skeletal muscles during long-distance migration of garden warblers: muscle changes are differentially related to body mass. *Ann. N.Y. Acad. Sci.* 1046, 271–281.
- Blem, R.C., 1990. Avian energy storage. *Curr. Ornithol.* 7, 59–113.
- Brunner, K.E., 1991. Anatomical adaptation of the gut to diet in red-winged blackbirds (*Agelaius phoeniceus*). *Auk* 108, 562–567.
- Carey, C., Dawson, W.R., Maxwell, L.C., Faulkner, J.A., 1978. Seasonal acclimatization to temperature in cardueline finches. II. Changes in body composition and mass in relation to season and acute cold stress. *J. Comp. Physiol.* 125, 101–113.
- Carey, C., Marsh, R.L., Bekoff, A., Johnston, R.M., Olin, A.M., 1989. Enzyme activities in muscles of seasonally acclimatized house finches. In: Bech, C., Reinertsen, R.E. (Eds.), *Physiology of Cold Adaptation in Birds*. Plenum, New York, pp. 95–104.
- Casotti, G., 2001. Effects of season on kidney morphology in house sparrows. *J. Exp. Biol.* 204, 1201–1206.
- Chappell, M., Bech, C., Buttemer, W., 1999. The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* 202, 2269–2279.
- Coleman, J.D., Robson, A.B., 1975. Variation in body weight, fat-free weights and fat deposition of starlings in New Zealand. *Proc. N. Z. Ecol. Soc.* 22, 7–13.
- Cooper, S.J., 2000. Seasonal energetics of mountain chickadees and juniper titmice. *Condor* 102, 635–644.
- Cooper, S.J., 2002. Seasonal metabolic acclimatization in mountain chickadees and juniper titmice. *Physiol. Biochem. Zool.* 75, 386–395.
- Cooper, S.J., Swanson, D.L., 1994. Seasonal acclimatization of thermoregulation in the black-capped chickadee. *Condor* 96, 638–646.
- Cooper, S.J., Same, D.R., 2000. Ventilatory accommodation under cold stress in seasonally acclimatized black-capped chickadees. *Am. Zool.* 40, 980A.
- Dawson, W.R., Marsh, R.L., Yacoe, M.E., 1983a. Metabolic adjustments of small passerine birds for migration and cold. *Am. J. Physiol.* 245, R755–R767.
- Dawson, W.R., Marsh, R.L., Buttemer, W.A., Carey, C., 1983b. Seasonal and geographic variation of cold resistance in house finches. *Physiol. Zool.* 56, 353–369.
- Dawson, W.R., Marsh, R.L., 1989. Metabolic acclimatization to cold and season in birds. In: Bech, C., Reinertsen, R.E. (Eds.), *Physiology of Cold Adaptation in Birds*. Plenum, New York, pp. 83–94.
- Dawson, W.R., Olson, J.M., 2003. Thermogenic capacity and enzymatic activities in the winter-acclimatized dark-eyed junco (*Junco hyemalis*). *J. Therm. Biol.* 28, 497–508.
- deGraw, W.A., Kern, M.D., King, J.R., 1979. Seasonal changes in blood composition of captive and free-living white-crowned sparrows. *J. Comp. Physiol.* 129, 151–162.
- Dekinga, A., Dietz, M.W., Koolhaas, A., Piersma, T., 2001. Time course and reversibility of changes in the gizzards of red knots alternately eating hard and soft food. *J. Exp. Biol.* 204, 2167–2173.
- Doherty Jr., P.F., Williams, J.B., Grubb Jr., T.C., 2001. Field metabolism and water flux of Carolina chickadees during breeding and nonbreeding seasons: a test of the "peak-demand" and "reallocation" hypotheses. *Condor* 103, 370–375.
- Drent, R.H., Daan, S., 1980. The prudent parent: energetic adjustments in avian breeding. *Ardea* 68, 225–252.
- Dutenhoffer, M.S., Swanson, D.L., 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. *Physiol. Zool.* 69, 1232–1254.
- Dykstra, C.R., Karasov, W.H., 1992. Changes in gut structure and function of house wrens (*Troglodytes aedon*) in response to increased energy demands. *Physiol. Zool.* 65, 422–442.
- Geluso, K., Hayes, J.P., 1999. Effects of dietary quality on basal metabolic rate and internal morphology of European starlings (*Sturnus vulgaris*). *Physiol. Biochem. Zool.* 72, 189–197.
- Grubb Jr., T.C., Pravosudov, V.V., 2008. White-breasted nuthatch (*Sitta carolinensis*). In: Poole, A. (Ed.), *The Birds of North America Online*. Cornell Laboratory of Ornithology, Ithaca, NY.
- Guglielmo, C.G., Williams, T.D., 2003. Phenotypic flexibility of body composition in relation to migratory state, age, and sex in the western sandpiper (*Calidris mauri*). *Physiol. Biochem. Zool.* 76, 84–98.
- Hammond, K., Chappell, M., Cardullo, R., Lin, R., Johnsen, T., 2000. The mechanistic basis of aerobic performance variation in red junglefowl. *J. Exp. Biol.* 203, 2053–2064.
- Hart, J.S., 1962. Seasonal acclimatization in four species of small wild birds. *Physiol. Zool.* 35, 224–236.
- Helms, C.W., Drury Jr., W.H., 1960. Winter and migratory weight and fat field studies on some North American buntings. *Bird-Banding* 31, 1–40.
- Hohtola, E., 1982. Thermal and electromyographic correlates of shivering thermogenesis in the pigeon. *Comp. Biochem. Physiol.* 73A, 159–166.
- Kalmbach, E.R., 1940. The economic status of the English sparrow in the United States. *U.S. Dept. Agric. Tech. Bull.* 711.
- Konarzowski, M., Diamond, J., 1994. Peak sustained metabolic rate and its individual variation in cold-stressed mice. *Physiol. Zool.* 67, 1186–1212.
- Landys-Ciannelli, M.M., Piersma, T., Jukema, J., 2003. Strategic size changes of internal organs and muscle tissue in the bar-tailed godwit during fat storage on a spring stopover site. *Funct. Ecol.* 17, 151–159.
- Liknes, E.T., 2005. Seasonal Acclimatization Patterns and Mechanisms in Small, Temperate Resident Passerines: Phenotypic Flexibility of Complex Traits. Ph.D. Dissertation, University of South Dakota, Vermillion, South Dakota.
- Liknes, E.T., Scott, S.M., Swanson, D.L., 2002. Seasonal acclimatization in the American goldfinch revisited: to what extent do metabolic rates vary seasonally? *Condor* 104, 548–557.
- Liknes, E.T., Swanson, D.L., 1996. Seasonal variation in cold tolerance, basal metabolic rate, and maximal capacity for thermogenesis in white-breasted nuthatches *Sitta carolinensis* and downy woodpeckers *Picoides pubescens*, two unrelated arboreal temperate residents. *J. Avian Biol.* 27, 279–288.
- Lima, S.L., 1986. Predation risk and unpredictable feeding conditions: determinants of body mass in birds. *Ecology* 67, 377–385.
- Lima, S.L., 1988. Initiation and termination of daily feeding in dark-eyed juncos: influences of predation risk and energy reserves. *Oikos* 53, 3–11.
- Liu, J.-S., Li, M., 2006. Phenotypic flexibility of metabolic rate and organ masses among tree sparrows *Passer montanus* in seasonal acclimatization. *Acta Sin.* 52, 469–477.
- Lowther, P.E., Cink, C.L., 2006. House Sparrow (*Passer domesticus*). In: Poole, A. (Ed.), *The Birds of North America Online*. Cornell Laboratory of Ornithology, Ithaca, NY.
- Marjonniemi, K., Hohtola, E., 1999. Shivering thermogenesis in leg and breast muscles of Galliform chicks and nestlings of the domestic pigeon. *Physiol. Biochem. Zool.* 72, 484–492.
- Marsh, R.L., Dawson, W.R., 1982. Substrate metabolism in seasonally acclimatized American goldfinches. *Am. J. Physiol.* 242, R563–R569.
- Marsh, R.L., Dawson, W.R., 1989. Avian adjustments to cold. In: Wang, L.C.H. (Ed.), *Advances in Comparative and Environmental Physiology 4: Animal Adaptation to Cold*. Springer-Verlag, New York, pp. 205–253.
- Marsh, R.L., Dawson, W.R., Camilliere, J.J., Olson, J.M., 1990. Regulation of glycolysis in the pectoralis muscles of seasonally acclimatized American goldfinches exposed to cold. *Am. J. Physiol.* 258, R711–R717.
- Martin, A.C., Zim, H.S., Nelson, A.C., 1951. *American Wildlife and Plants: A Guide to Wildlife Food Habits*. McGraw-Hill, New York.
- McWilliams, S.R., Karasov, W.H., 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. *Comp. Biochem. Physiol.* A 128, 577–591.
- Morton, M.L., 1994. Hematocrits in montane sparrows in relation to reproductive schedule. *Condor* 96, 119–126.
- Murphy, M.E., King, J.R., 1984. Sulfur amino acid nutrition during molt in the white-crowned sparrow. 1. Does dietary sulfur amino acid concentration affect the energetics of molt as assayed by metabolized energy? *Condor* 86, 314–323.
- O'Connor, T.P., 1995a. Seasonal acclimatization of lipid mobilization and catabolism in house finches (*Carpodacus mexicanus*). *Physiol. Zool.* 68, 985–1005.
- O'Connor, T.P., 1995b. Metabolic characteristics and body composition in house finches: effects of seasonal acclimatization. *J. Comp. Physiol.* B 165, 298–305.
- Odum, E.P., 1942. Annual cycle of the black-capped chickadee. *Auk* 59, 499–531.
- Olson, J.M., 1994. The ontogeny of shivering thermogenesis in the red-winged blackbird (*Agelaius phoeniceus*). *J. Exp. Biol.* 191, 59–88.
- Piersma, T., Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. *TREE* 18, 228–233.
- Piersma, T., Bruinzeel, L., Drent, R., Kersten, M., Van Der Meer, J., Wiersma, P., 1996a. Variability in basal metabolic rate of a long-distance migrant shorebird (red knot, *Calidris canutus*) reflects shifts in organ sizes. *Physiol. Zool.* 69, 191–217.
- Piersma, T., Everaarts, J.M., Jukema, J., 1996b. Build-up of red blood cells in refuelling bar-tailed godwits in relation to individual migratory quality. *Condor* 98, 363–370.

- Piersma, T., Gudmundsson, G.A., Lillendahl, K., 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiol. Biochem. Zool.* 72, 405–415.
- Piersma, T., Lindstrom, A., 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. *TREE* 12, 134–138.
- Piersma, T., Gessaman, J.A., Dekinga, A., Visser, G.H., 2004. Gizzard and other lean mass components increase, yet basal metabolic rates decrease, when red knots *Calidris canutus* are shifted from soft to hard-shelled food. *J. Avian Biol.* 35, 99–104.
- Pyle, P., 1997. Identification guide to North American birds. Part I. Columbidae to Ploceidae. Slate Creek Press, Bolinas, California.
- Rezende, E.L., Swanson, D.L., Novoa, F.F., Bozinovic, F., 2002. Passerines versus nonpasserines: so far, no statistical differences in the scaling of avian energetics. *J. Exp. Biol.* 205, 101–107.
- Rogers, C.M., Smith, J.N.M., 1993. Life-history theory in the nonbreeding period: trade-offs in avian fat reserves? *Ecology* 74, 419–426.
- Smith, S.M., 1993. Black-capped chickadee (*Poecile atricapillus*). In: Poole, A. (Ed.), *The Birds of North America Online*. Cornell Laboratory of Ornithology, Ithaca, NY.
- Swanson, D.L., 1990. Seasonal variation of vascular oxygen transport in the dark-eyed junco. *Condor* 92, 62–66.
- Swanson, D.L., 1991. Substrate metabolism under cold stress in seasonally acclimatized dark-eyed juncos. *Physiol. Zool.* 64, 1578–1592.
- Swanson, D.L., 2010. Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr. Ornithol.* 17, 75–129.
- Swanson, D.L., Liknes, E.T., 2006. A comparative analysis of thermogenic capacity and cold tolerance in small birds. *J. Exp. Biol.* 209, 466–474.
- Swanson, D.L., Olmstead, K., 1999. Evidence for a proximate influence of winter temperature on metabolism in passerine birds. *Physiol. Biochem. Zool.* 72, 566–575.
- Swanson, D.L., Sabirzhanov, B., VandeZande, A., Clark, T.G., 2009. Down-regulation of myostatin gene expression in pectoralis muscle is associated with elevated thermogenic capacity in winter acclimatized house sparrows (*Passer domesticus*). *Physiol. Biochem. Zool.* 82, 121–128.
- Vézina, F., Jalvingh, K.M., Dekinga, A., Piersma, T., 2006. Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size. *J. Exp. Biol.* 209, 3141–3154.
- Vézina, F., Jalvingh, K.M., Dekinga, A., Piersma, T., 2007. Thermogenic side effects to migratory disposition in shorebirds. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, 1287–1297.
- Vézina, F., Williams, T.D., 2005. Interaction between organ mass and citrate synthase activity as an indicator of tissue maximal oxidative capacity in breeding European starlings: implications for metabolic rate and organ mass relationships. *Funct. Ecol.* 19, 119–128.
- Weathers, W.W., 1999. Winter and breeding-season energetics of nonmigratory white-crowned sparrows. *Auk* 116, 842–847.
- Zar, J.H., 1996. *Biostatistical Analysis*, third ed. Prentice-Hall, Upper Saddle River, NJ.
- Zheng, W.-H., Li, M., Liu, J.-S., Shao, S.-L., 2008. Seasonal acclimatization of metabolism in Eurasian tree sparrows (*Passer montanus*). *Comp. Biochem. Physiol.*, A 151, 519–525.