Wintering Snow Buntings Elevate Cold Hardiness to Extreme Levels but Show No Changes in Maintenance Costs

Audrey Le Pogam[1,2,*]
Oliver P. Love[3]
Lyette Régimbald[1]
Karine Dubois[1,2]
Fanny Hallot[1,2]
Myriam Milbergue[1,2]
Magali Petit[1,2]
Ryan S. O'Connor[1,2]
François Vézina[1,2]
[1]Département de biologie, chimie et géographie, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, Québec G5L 3A1, Canada; [2]Groupe de recherche sur les environnements nordiques BORÉAS, Centre d'Études Nordiques, Centre de la Science de la Biodiversité du Québec, 300 allée des Ursulines, Rimouski, Québec G5L 3A1, Canada; [3]Department of Integrative Biology, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B 3P4, Canada

Accepted 8/23/2020; Electronically Published 10/13/2020

ABSTRACT

Resident temperate passerines adjust their phenotypes to cope with winter constraints, with peak performance in metabolic traits typically occurring during the coldest months. However, it is sparsely known whether cold-adapted northern species make similar adjustments when faced with variable seasonal environments. Life in near-constant cold could be associated with limited flexibility in traits underlying cold endurance. We investigated this by tracking individual physiological changes over five consecutive winters in snow buntings (*Plectrophenax nivalis*), an Arctic-breeding migratory passerine typically confronted with nearly constant cold. Buntings were held in an outdoor aviary and exposed to seasonal temperature variation typical of temperate zone climates. We measured phenotypic changes in body composition (body, fat, and lean mass, pectoralis muscle thickness), oxygen transport capacity (hematocrit), metabolic performance (basal metabolic rate [BMR] and summit metabolic rate [M_{sum}]), thermogenic endurance (time to reach M_{sum}), and cold tolerance (temperature at M_{sum}). Snow buntings showed flexibility in functions underlying thermogenic capacity and cold endurance comparable to that observed in temperate resident passerines wintering at similar latitudes. Specifically, they increased body mass (13%), fat mass (246%), hematocrit (23%), pectoralis muscle thickness (8%), and M_{sum} (27%). We also found remarkable cold tolerance in these birds, with individuals reaching M_{sum} in helox at temperatures equivalent to less than −90°C in air. However, in contrast with resident temperate passerines, lean mass decreased by 12%, and there was no clear increase in maintenance costs (BMR). Our results show that the flexibility of traits underlying thermal acclimatization in a cold-adapted northern species is comparable to that of temperate resident species living at lower latitudes and is therefore not limited by life in near-constant cold.

Keywords: summit metabolic rate (M_{sum}), basal metabolic rate (BMR), fat mass, lean mass, hematocrit, snow bunting, cold acclimatization, winter.

Introduction

Organisms face multiple proximate (i.e., energetic) costs within and across life stages induced by environmental constraints that ultimately (i.e., in a fitness sense) shape life-history variation within and across species. Organisms can respond to environmental variability across a diversity of timescales via multiple nonexclusive mechanisms, including phenotypic flexibility (Piersma and van Gils 2011), phenotypic (developmental) plasticity (Pigliucci 2005), and contemporary evolution (Grant and Grant 2002). Within studies of short-term flexible responses to environmental challenges, more and more integrative ecologists are examining underlying mechanisms, such as gene transcription (Barrio et al. 2016; Bengston et al. 2018) and physiological central regulation (Karasov and Martinez del Rio 2007; Evans et al. 2017; Bennett et al. 2019), to quantify the details of how and why organisms respond to environmental variability. Many of these investigations have centered on the seasonal regulation of metabolic responses to extreme environments (e.g., cold, deserts; Sharbaugh 2001; Smit and McKechnie 2010; McKechnie et al. 2015), with small, short-lived, high-metabolic-demand endothermic species such as mammals and birds living in extreme environments making compelling models (McKechnie and Wolf 2019). Within these studies, a focus on the central regulation and outputs of whole-animal metabolism has been an especially fruitful area of investigation (Swanson and Liknes 2006; White and Kearney 2013; Swanson et al. 2017*b*), although there remains a diversity of unanswered questions.

Winter cold acclimatization is a well-documented process in small resident bird species, particularly in those living in

---

*Corresponding author; email: audreylp@wanadoo.fr.

Physiological and Biochemical Zoology 93(6):417–433. 2020. © 2020 by The University of Chicago. All rights reserved. 1522-2152/2020/9306-9115$15.00. DOI: 10.1086/711370

temperate areas (Cooper and Swanson 1994; Swanson and Liknes 2006; McKechnie 2008; Petit et al. 2013; McKechnie et al. 2015). Typically, these birds increase the size of their fat reserves in winter (Blem 1976; Lehikoinen 1987; Gosler 1996; Cooper 2007) and enlarge several internal organs (Swanson 2010; Petit et al. 2014; Barceló et al. 2017), including the heart and lungs (Zheng et al. 2008; Liknes and Swanson 2011; Petit et al. 2014) and the pectoralis muscles (O'Connor 1995; Cooper 2002; Petit et al. 2013; Swanson and Merkord 2013; Swanson and Vézina 2015; but see Swanson et al. 2014; Barceló et al. 2017; Milbergue et al. 2018). Cold acclimatization also triggers an increase in oxygen carrying capacity (e.g., hematocrit; Swanson 1990*b*; O'Connor 1996) to support metabolic activities, such as shivering thermogenesis (Carey and Morton 1976; Swanson 1990*a*; Petit and Vézina 2014), and leads to adjustments in metabolic performance. Summit metabolic rate (M_{sum}) is a measure of maximal shivering heat production and is considered an index of cold endurance, while basal metabolic rate (BMR) is interpreted as an index of physiological maintenance costs (Swanson et al. 2017*b*). Both M_{sum} and BMR are commonly found to increase as temperatures decline in the fall (McKechnie and Swanson 2010; Swanson 2010; for reviews, see Petit et al. 2013; McKechnie et al. 2015), and the increase in M_{sum} has been associated with higher survival (Petit et al. 2017; Latimer et al. 2018).

Although winter cold acclimatization is well documented for temperate resident passerines that experience large seasonal thermal variation (Cooper and Swanson 1994; Swanson and Liknes 2006; McKechnie 2008; Petit et al. 2013; McKechnie et al. 2015), some species or populations of birds face cold temperatures almost year-round (Brooks 1968; Pohl and West 1973; White and West 1977; Sharbaugh 2001). These species, typically living above 50°N, can show phenotypic patterns differing from what is observed in temperate regions. For example, black-capped chickadees (*Poecile atricapillus*) living at 64°N in Alaska show no seasonal changes in resting metabolic rate (measured at −30° and 25°C) despite summer temperatures ranging from 10° to 20°C (Sharbaugh 2001). Similarly, willow tits (*Poecile montanus*) in Norway (63°N) maintain constant metabolic performance throughout summer and winter (Reinertsen and Haftorn 1983). This could be due to summers at these latitudes being short, relatively cold, and unpredictably variable in temperature (Martin and Wiebe 2004), thus promoting the maintenance of high cold endurance and therefore preventing clear seasonal phenotypic variation. However, whether species and/or populations experiencing cold most of the year are in fact able to adjust their phenotype similarly to those living in predictable seasonal environments at lower latitudes still remains to be examined.

The snow bunting (*Plectrophenax nivalis*) is one such remarkable case: except for a few weeks during breeding when temperatures rise above freezing in most of their range, buntings remain associated with snowy, cold, relatively harsh environments year-round (Montgomerie and Lyon 2011; Snell et al. 2018). Indeed, this small (30–50 g) circumpolar migratory songbird winters in open snowy plains of North America, Europe, and Asia (between 40°N and 60°N; Banks et al. 1991; Yesou 1998; Montgomerie and Lyon 2011; BirdLife International 2020; Snell et al. 2018). It then migrates through winter landscapes in the spring well before snowmelt (Laplante et al. 2019; McKinnon et al. 2019) and arrives on its Arctic breeding grounds up to 83.6°N (Cramp and Perrins 1994; BirdLife International 2020) while temperatures can still regularly reach −25° to −30°C (Meltofte 1983). Temperatures remain mostly above freezing during breeding, but highly unpredictable Arctic weather means that buntings can be faced with sudden snowstorms in the middle of an already short summer across their breeding range (Hussell 1972; Serreze and Barry 2005). In the fall, birds migrate south as winter begins in the Arctic, with first arrivals on their wintering grounds in late October (Macdonald et al. 2012; McKinnon et al. 2016; Snell et al. 2018). Therefore, snow buntings truly combine the "worst of both worlds" by having to migrate fairly long distances (>2,000 km; Johnson and Herter 1990; Macdonald et al. 2016) while enduring harsh, cold conditions across winter, migration, and arrival at their Arctic breeding grounds. Given that the species seems well adapted for life in nearly constant cold (Scholander et al. 1950*a*, 1950*b*; Vincent and Bédard 1976; Montgomerie and Lyon 2011), it constitutes an ideal model for examining whether such cold-adapted northern species are limited in their flexible response to seasonally variable environmental conditions, such as those of lower latitudes.

Here, we investigated wintering phenotypic adjustments in this cold specialist by exposing individuals to seasonal temperature variation typical of that experienced by temperate zone resident species (minimum: −26°C; maximum: 28°C). For this, we used a data set from five consecutive years from captive buntings held year-round in seminatural outdoor conditions within their wintering range (48°N). Specifically, we monitored fine adjustments in body mass (M_{b}), fat and lean mass, pectoralis muscle thickness, oxygen transport capacity (hematocrit), and metabolic performance (BMR and M_{sum}) from August to early March (i.e., the time during which snow buntings begin fattening for migration; Vincent and Bédard 1976). We predicted two potential outcomes given the life history of snow buntings. First, if flexibility of traits underlying cold endurance is a common attribute among birds irrespective of temperature variation experienced within their habitats, buntings should express a phenotypic pattern typical of cold acclimatization, as seen in resident temperate passerines during winter at this latitude (e.g., Petit et al. 2013)—namely, increases in body composition parameters (M_{b}, fat and lean mass), hematocrit, thermogenic capacity (M_{sum}), thermogenic endurance and cold tolerance (time to M_{sum} and temperature at M_{sum}, respectively), and maintenance energy expenditure (BMR), with trait values peaking at the coldest portion of winter (Petit et al. 2013, 2014). Alternatively, if life in near-constant cold is associated with limited flexibility in cold-endurance traits, birds would be expected to show no clear patterns from the end of summer to the peak of winter in a temperate environment, instead maintaining winter-level cold hardiness throughout the investigated period (Reinertsen and Haftorn 1983; Sharbaugh 2001).

Material and Methods

Ethics Statement

All bird handling was approved by the animal care committee of the Université du Québec à Rimouski (CPA-54-13-130 and CPA-71-17-195) and has been conducted under scientific (SC-48) and banding (10889E) permits from Environment and Climate Change Canada.

Captive Housing Conditions

From 2013 to 2018, we studied captive male snow buntings at the avian facilities of the Université du Québec à Rimouski, Canada (48°27′00″N, 68°30′45″W). Wild birds were captured during winters using walk-in traps in fields around Rimouski between January and March of each year. Birds were banded with a combination of four colored bands for identification, and we took morphometric measurements (length of head plus beak, tarsus, tail, and right wing) at capture to calculate a body size index (see below). Birds were maintained in an outdoor aviary (5.8 m wide × 5.3 m deep × 2.6–3.6 m high, with angled ceiling) and were exposed to the elements while being sheltered from direct precipitation. Once in the aviary, birds were fed ad lib. with a seed mix (crushed corn, wheat, sorghum, white millet, red millet, black oil sunflower; Armstrong, Hagersville, Ontario) and Mazuri small bird maintenance mini diet (562A; Mazuri, Richmond, IN) using several poultry feeders to guarantee access to food for all individuals. We also offered ice-free water ad lib. using heated bowls (1 L, Ukal, St. Hyacinthe, Quebec) and supplemented with electrolytes (0.17 g/L; Electrolytes Plus, Vetoquinol, Lavaltrie, Quebec) and vitamins (0.44 g/L; Poly-tonine A Complex, Vetoquinol). The size of our study group varied between 12 and 32 birds (average group size over 5 yr: 20.7 ± 0.7) and totaled 102 individual snow buntings because of the addition of new birds each winter to replace mortalities.

Tracking of Environmental Variables

Between 2013 and 2018, from November to March of each year, we measured air temperature (T_a) in the aviary using a temperature-sensitive data logger (iButton DS1922L, Maxim Integrated, San Jose, CA) suspended 155 cm above ground level in the shade. T_a was recorded every 10 min, and we extracted the average, minimum, and maximum T_a for each day. Missing data (9.5% of total data set) were obtained from regressions between iButton data and T_a data derived from the nearest weather station (48°30′50″N, 68°28′06″W; Government of Canada, http://climat.meteo.gc.ca; T_a average: $r^2 = 0.98$; T_a minimum: $r^2 = 0.97$; T_a maximum: $r^2 = 0.97$). Day length data (i.e., interval between sunrise and sunset, excluding twilight) were obtained from the National Research Council of Canada (https://www.nrc-cnrc.gc.ca).

Experimental Design and Sampling Schedule

Given that we were interested in tracking intraindividual phenotypic variation, we repeatedly measured each bird from the end of August to early March over 5 yr (range: 1–47 times depending on the individual; table A1). Measurements of metabolic performance, body composition, and hematocrit occurring on the same day were limited to four birds per day, resulting in an average of 21 d to measure all birds. These measurements were performed on a monthly basis except for 2016–2018 (table A1). However, from October 2014 to January 2016 and from November 2017 to January 2018, body composition and hematocrit measurements were obtained twice a month. Birds were thus measured a second time each month, all on the same day. Each measurement series (table A1) for a given month was conducted on similar dates each year.

Body Composition and Oxygen Carrying Capacity

For a given day of measure, our protocol involved the following steps. Birds were caught in the aviary between 0800 and 1100 hours using a handheld net, and a blood sample (<1% of M_b; McGuill and Rowan 1989) was immediately taken from the brachial vein. These samples were later centrifuged (10 min at 8,000 rpm; UNICO PowerSpin BX centrifuge C886, UNICO, Dayton, NJ) to obtain hematocrit data and plasma samples for parallel studies. The birds were then weighed (±0.01 g), and we noninvasively estimated (±0.01 g) the fat and lean components of their M_b using quantitative magnetic resonance (QMR; EchoMRI, Houston, TX; Guglielmo et al. 2011) beginning in November 2014. Three or four QMR measurements were taken on each bird (<12 min total in QMR machine), and average values were subsequently used for analyses. We also noninvasively estimated the thickness of pectoralis muscles by ultrasonography (Dietz et al. 2007; Swanson and Merkord 2013) using a LOGIQe ultrasound scanner fitted with a linear probe (12 MHz; GE Healthcare, Wauwatosa, WI). We placed the probe (enclosed in a latex condom containing ultrasonic gel to avoid soaking the feathers; Royer-Boutin et al. 2015) on the bird's left pectoralis at a 90° angle to the keel, 1–2 mm from the upper keel tip. This provided a cross-sectional image of the muscle, whereby a thickness measurement could be obtained from the base of the keel to the skin (at a 45° angle relative to the keel). Our approach followed the procedure described by Royer-Boutin et al. (2015) with the following improvement: keel height was also measured for each bird to control for variation in muscle thickness due to the positioning of the probe, knowing that both keel height and muscle thickness are at their tallest/thickest close to the furculum and values decrease toward the abdomen. As keel height is strongly correlated with muscle thickness ($n = 620$, $r^2 = 0.80$, $P < 0.001$), including keel height as a covariate in models statistically controls for any impact of probe position on muscle thickness. Three or four measurements of pectoralis muscle thickness and keel height were obtained for each bird and were averaged for analyses. Repeatability of ultrasound measurements were $r^2 = 0.97$ for pectoralis and $r^2 = 0.89$ for keel (but see Royer-Boutin et al. 2015). Birds were then moved to an indoor cage (117 cm wide × 31 cm deep × 39 cm high) with food and water provided ad lib. until they were either released in the aviary or used for metabolic performance measurements.

Metabolic Performance Measurements

On days of metabolic rate trials, two birds were taken from their cages for M_{sum} measurements at least 1 h after ultrasound measurements. Trials began between 1005 and 1450 hours (average: 1205 hours ± 1 h 2 min; duration: 1.97 ± 0.8 h), and measurements on the remaining two birds began between 1215 and 1730 hours (average: 1440 hours ± 56 min; duration: 1.80 ± 0.7 h). Order and timing of trials did not affect M_{sum}, T_{a} at M_{sum}, or time to M_{sum} values and were not considered further in analyses. Each trial began by measuring the birds' M_{b}. We then measured body temperature (T_{b}) with a thermocouple reader (Omega HH-25KC, NIST traceable, Omega, Montreal) using a copper constantan thermocouple inserted approximately 10 mm into the cloaca (mean T_{b} before $M_{\mathrm{sum:}}$ 41.2° ± 0.06°C). Birds were then placed in airtight stainless steel metabolic chambers (1.5 L) equipped with a perch and a copper constantan thermocouple connected to a Sable Systems TC-2000 thermocouple reader (Sable Systems, Las Vegas, NV) to continuously monitor chamber temperature. Chambers were placed inside a temperature-controlled cabinet (modified freezer), wherein we performed a sliding cold exposure protocol (Swanson et al. 1996). Using a flow rate of 1,200 mL/min controlled by mass flow valves (Sierra Instruments, Side-Trak, Monterey, CA) calibrated for air and helox with a Bubble-O-Meter (Dublin, OH), we first exposed the birds to dry, CO_2-free air for 10 min at −18°C (−15°C in Aug. and Sept.), during which oxygen readings on our analyzer (Servomex gas purity analyzer, model 4100, Servomex, Boston, MA) were adjusted to 20.95% for that same reference air. Chamber inflow was then switched to a helox gas mixture (21% oxygen, 79% helium) to increase heat loss (Rosenmann and Morrison 1974). A 5-min baseline in helox was then recorded before measuring the birds' oxygen consumption ($\dot{V}O_2$), starting at −18°C or −15°C, with temperature decreasing 3°C every 20 min until birds became hypothermic (visible as a steady decline in $\dot{V}O_2$ for several minutes) or the end of the program was reached. Birds with $T_{\mathrm{b}} < 37°C$ at the end of a trial were considered hypothermic (Swanson and Liknes 2006). Before January 2015, our program lasted 125 min, which allowed our freezer to reach temperatures less than −30°C (−27.05° ± 0.6°C in chambers). However, even though birds were exposed to helox, several snow buntings showed no signs of hypothermia at this temperature. Starting in January 2015, we therefore extended our program to 185 min and added dry ice in the cabinet as soon as the temperature in the cabinet reached −30°C, which allowed all birds to become hypothermic. Out of 326 individual measurements, 41 (12.6%) involved birds that were considered nonhypothermic by the end of the trial. However, as maximal $\dot{V}O_2$ can occur earlier than the typical decline in $\dot{V}O_2$ that indicates hypothermia (Dutenhoffer and Swanson 1996), a hypothermic state is not a prerequisite to confirm that M_{sum} has been attained. In this study, we decided to include all measurements in analyses because removing nonhypothermic birds had no influence on the final results. Birds were then placed back in their cage with food and water until BMR trials.

We measured BMR on all four birds simultaneously, starting between 1700 and 2200 hours (average duration: 13.7 ± 1.2 h). Metabolic chambers were placed in a PELT-5 (Sable Systems) temperature cabinet set at 25°C, a temperature within the thermoneutral zone of snow buntings (Scholander et al. 1950*a*). Throughout the night, birds received dry, CO_2-free air at a constant rate of 650 mL/min maintained by mass flow controllers. The air sent to the analyzer alternated automatically between reference air (10 min) and chamber air (40 min) using a multiplexer (Sable Systems MUX). After BMR measurements, birds were released back into the outdoor aviary.

For both M_{sum} and BMR measurements, before excurrent air from chambers entered the analyzer, it was scrubbed of water and CO_2 using columns of H_2O absorbent (indicating desiccant 6–8 mesh, Millipore, Etobicoke, Ontario, and indicating drierite 8 mesh, Hammond Drierite, Xenia, OH) and a column of CO_2 absorbent (Intersob 812 mesh, Intersurgical, Burlington, Ontario), respectively. Oxygen levels were recorded with a sampling interval of 5 s for M_{sum} and 20 s for BMR. Metabolic rate calculations were conducted using ExpeData software version 1.8.4 (Sable Systems). M_{sum} and BMR calculations were based on the highest and lowest averaged 10 min of $\dot{V}O_2$, respectively, using equation (10.1) from Lighton (2019). M_{sum} was calculated using the instantaneous measurement technique (Bartholomew et al. 1981), whereas BMR calculations assumed a steady state. As temperature changed automatically every 20 min during M_{sum} trials, all birds starting at −18°C ($n = 298$) experienced the same measurement sequence. Consequently, for these individuals, we could calculate thermogenic endurance (sensu Swanson and Liknes 2006), defined here as the time needed for a bird to reach its M_{sum} (hereafter, "time to M_{sum}"). We also extracted the metabolic chamber temperature at which each bird attained its M_{sum} (hereafter, "T_{a} at M_{sum}"). The duration of BMR trials ensured that birds were postabsorptive at the time of BMR measurement (which occurred after 6.28 ± 2.45 h of measurement on average). We converted $\dot{V}O_2$ to energy consumption assuming a respiratory quotient of 0.71, and we converted units to watts using a thermal equivalent of 19.8 kJ/L O_2 (Gessaman and Nagy 1988).

Statistical Analysis

Intraseasonal Variation in Phenotypic Traits. Our main objective was to obtain a detailed pattern of phenotypic variation in body composition, hematocrit, and metabolic performance in wintering outdoor captive snow buntings. Our analyses therefore focused on the period spanning from August to early March. August was our reference point for the summer phenotype, the time at which breeding in the Arctic is completed. Although March is still a winter month in Rimouski, with subzero temperatures and snow on the ground, it was considered the end of winter in this study because birds begin to fatten up at this time and change their phenotype for migration (Vincent and Bédard 1976; Laplante et al. 2019; Le Pogam et al. 2020).

We used linear mixed effects models to investigate patterns of variation in each parameter. Bird ID was included as a random variable to control for repeated measurements on the same bird. Models also included a fixed time variable called "month," which corresponds to the sequence of measurement, including one or two measures per month (e.g., Dec.-1, Dec.-2, Jan.-1, Jan.-2, etc.; table A1). The variable year was ultimately treated as a random parameter because we did not have measurements for all months in each of the 5 yr. Consequently, we could not include a month × year interaction term in our models, which would have been the variable required for interpreting year effects in seasonal phenotypic changes (because the variable year would pool values for all months). We nevertheless visually represented interannual variation for each parameter (gray points and lines in figs. 2, 3), and to do so, we conducted the same linear mixed effects models for each year separately and generated the associated least squares means used in the figures.

Although our captures were all conducted in the morning, we also considered the potential effect of daily fattening (e.g., Laplante et al. 2019) on our variables by including relative time of capture (e.g., the time between sunrise and capture/day length). However, this variable was never significant, so we did not consider it further. Because structurally large individuals can carry more fat reserves and more lean tissue than smaller individuals (Peig and Green 2009), we included a measure of structural body size as a covariate in models investigating variation in total, lean, and fat mass as well as in models for pectoralis muscle thickness. Least squares means extracted from models with a significant size covariate are therefore presented as size-independent variables. Our structural body size estimate was the first principal component obtained from a principal component analysis combining variation in head plus beak, tarsus, wing, and tail length (Rising and Somers 1989). For analyses investigating variation in pectoralis muscle thickness, we also included keel height as a covariate to control for variation in thickness due to probe positioning.

Seasonal variation in metabolic performance, thermogenic endurance, and cold tolerance was first analyzed considering whole $M_{\mathrm{sum},}$ time to $M_{\mathrm{sum},}$ T_a at $M_{\mathrm{sum},}$ and BMR before exploring mass-independent variation. This was done using the same approach as for the size effect on body composition. However, we used lean mass rather than total M_b as a covariate in models because changes in the amount and activity of lean tissue are considered the main driver of whole-animal metabolic rate in birds (Piersma et al. 1996, 2004; Swanson et al. 2017*a*). Least squares means extracted from models with a significant lean mass covariate are therefore presented as mass-independent variables. Note that time to M_sum and T_a at M_sum analyses were based only on data collected after we began using dry ice, and individuals starting at −15°C were removed for the time to M_sum analyses.

Relationships between Phenotypic Traits. This study also provided an opportunity to investigate how body composition parameters might covary over time and relate with metabolic performance. We thus extracted least squares means generated by the mixed effects models described above including year as a random parameter and used them in linear regressions where each point then represented the average value for a specific month (see Vézina et al. 2011). Therefore, this new set of data included 14 values for each parameter of body composition and hematocrit and eight values for each parameter of metabolic performance. In the specific cases where we compared least squares means of metabolic performance with least squares means of body composition, we kept only the eight values of body composition or hematocrit that matched the eight values of metabolic performance over time. Because the least squares means used came from our mixed effects models, they were all controlled for repeated measures (bird ID). Moreover, for lean, fat, and total mass and muscle thickness, least squares means were controlled for structural body size, while muscle thickness was also controlled for keel height. For metabolic performance, we used mass-independent M_sum and mass-independent BMR.

Normality of residuals was confirmed visually for all models. All analyses were conducted using JMP pro (12.0.1), and data are presented as mean ± SEM. Post hoc independent contrast analyses were used to investigate differences between selected months.

Results

Intraseasonal Variation in Abiotic Parameters and Phenotypic Traits

Temperature and Day Length. Over the course of the experiment, birds were exposed to mean daily T_a below 0°C from December to March (fig. 1), and T_a reached its coldest values in January (T_a average: $-8.9° \pm 0.7°\mathrm{C}$, T_a minimum: $-12.7° \pm 0.8°\mathrm{C}$). However, February was just as cold, with less than a 1°C difference (T_a average: $-8.7° \pm 0.7°\mathrm{C}$, T_a minimum: $-12.7° \pm 0.8°\mathrm{C}$). In August, mean T_a averaged $19.5° \pm 0.8°\mathrm{C}$ (T_a minimum: $15.3° \pm 0.8°\mathrm{C}$). Day length decreased 5.8 h between August (14.3 h) and December to reach the shortest day in December (8.4 h) before increasing 3.6 h between December and March (11.9 h).

Body Composition and Oxygen Carrying Capacity. Size-independent M_b (body size: $F_{1,91} = 10.7$, $P < 0.005$) varied over time (month: $F_{13,810} = 10.1$, $P < 0.0001$; fig. 2*A*). In early September, birds had a mass comparable to that observed in summer (Aug. vs. Sept.-1: $P = 0.5$). M_b then gradually increased until it culminated in late December at a level 13.2% higher than that in early September (Sept.-1 vs. Dec.-2: $F_{1,798} = 44.3$, $P < 0.0001$). Past this peak, size-independent M_b declined gradually to reach levels in March that were indistinguishable from those recorded in August (Aug. vs. Mar.: $P = 0.4$) or early September (Sept.-1 vs. Mar.: $P = 0.3$).

Temporal variation in body fat showed a pattern different from that for M_b (month: $F_{13,591} = 13.0$, $P < 0.0001$; no influence of body size, $P = 0.40$; fig. 2*B*). Indeed, from 1.90 ± 0.51 g in August, fat mass increased 246.5% to reach 6.58 ± 0.53 g in October (Aug. vs. Oct.-1: $F_{1,499} = 88.4$, $P < 0.0001$). Body fat then remained relatively stable for the remainder of winter (Oct.-1 vs. Jan.-1: $P = 0.06$) before starting to decline in January to 4.60 ± 0.48 g in March, a level

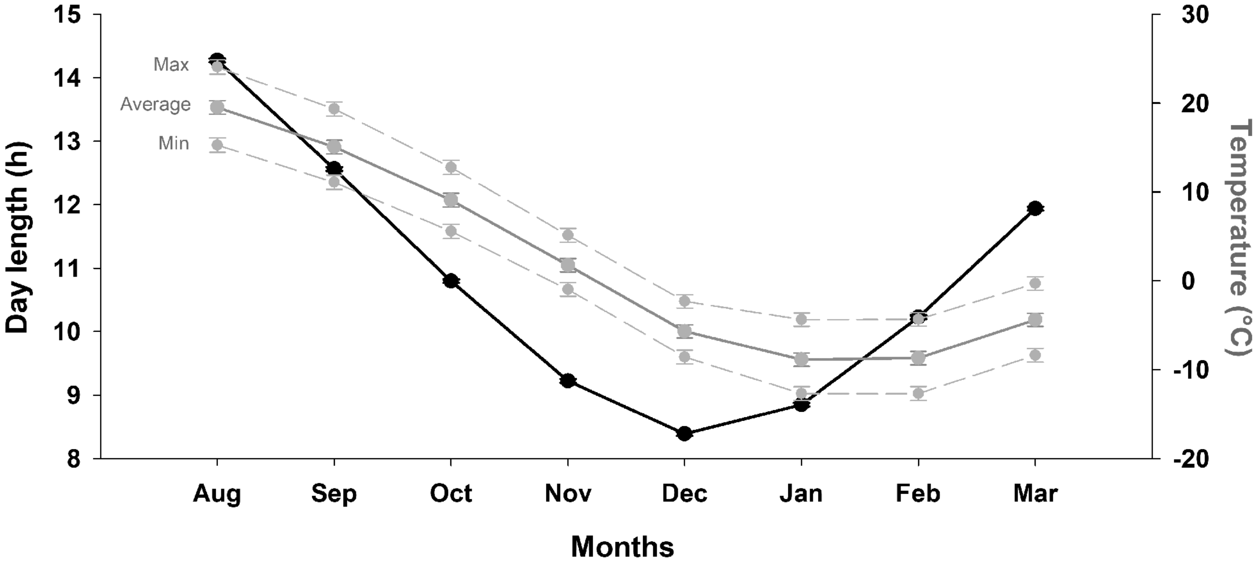

Figure 1. Variation in mean, minimum, and maximum temperatures and day length experienced by outdoor captive snow buntings in Rimouski, Quebec, from 2013 to 2018.

30.3% lower than in December (Mar. vs. Dec.-2: $F_{1,607} = 26.0$, $P < 0.0001$) but still 142.2% higher than in summer (Mar. vs. Aug.: $F_{1,441} = 34.9$, $P < 0.0001$).

Size-independent lean mass (body size: $F_{1,63} = 15.3$, $P < 0.0005$) showed a pattern opposite to that of fat (month: $F_{13,592} = 26.5$, $P < 0.0001$; fig. 2*C*). Lean mass was high in August (27.27 ± 0.31 g) and decreased to reach a value 12% lower (24.0 ± 0.32 g) in October (Aug. vs. Oct.-1: $F_{1,593} = 213.4$, $P < 0.0001$). Then lean mass gradually increased throughout winter to plateau between late December and March (Dec.-2 vs. Jan.-1 to Mar.: $P = 0.2$) at an average level (24.96 ± 0.09 g) 8.5% lower than in summer (Aug. vs. Dec.-2 to Mar.: $F_{1,587} = 184.4$, $P < 0.0001$). Lean mass in January and February was nevertheless 4.1% higher than in early October (Oct.-1 vs. Jan.-1 to Feb.-2: $F_{1,595} = 31.4$, $P < 0.0001$).

Size-independent pectoralis muscle thickness (body size: $F_{1,57} = 5.5$, $P < 0.05$) also varied over time (month: $F_{13,525} = 10.5$, $P < 0.0001$; keel height: $F_{1,522} = 1{,}325.3$, $P < 0.0001$; fig. 2*D*). Muscle thickness was 6.1 ± 0.1 mm in August and gradually increased 7.9%, reaching 6.6 ± 0.1 mm by late October (Aug. vs. Oct.-2: $F_{1,519} = 46.4$, $P < 0.0001$). Muscles then remained at that level for the rest of winter (Oct.-2 vs. Nov.-1 to Mar.: $P = 0.1$).

Hematocrit increased (month: $F_{13,560} = 38.8$, $P < 0.0001$; fig. 2*E*) from 45.2% ± 0.95% in August to 55.5% ± 0.97% in late November (Aug. vs. Nov.-2: $F_{1,553} = 251.2$, $P < 0.0001$) and remained relatively high (54.7% ± 0.2% on average) until March (Nov.-2 vs. Mar.: $P = 0.5$).

Metabolic Performance. M_{sum}, whether considered whole or mass independent, varied significantly over time (whole: month, $F_{7,248} = 10.8$, $P < 0.0001$; mass independent: month, $F_{7,160} = 13.5$, $P < 0.0001$; mass: $F_{1,190} = 20.9$, $P < 0.0001$; fig. 3*A*). It increased gradually to reach its maximal level in February (M_{sum}: +26.6% Sept. to Feb., contrast: $F_{1,189} = 33.9$, $P < 0.0001$) or January to February (mass-independent M_{sum}: +33.1% Aug. to Jan. to Feb., contrast: $F_{1,177} = 59.7$, $P < 0.0001$; no difference between Jan. and Feb., contrast: $P = 0.95$). The model using lean mass as a covariate explained more of the variation in M_{sum} (model: $r^2 = 0.68$) than the one without (model: $r^2 = 0.48$).

Time to M_{sum} varied over time (month: $F_{6,171} = 21.5$, $P < 0.0001$; mass: $P = 0.3$; fig. 3*B*). It increased progressively to stabilize between January and March (Aug. vs. Jan.: $F_{1,165} = 33.9$, $P < 0.0001$; no difference between Jan. and Mar., $P = 0.42$). As cold hardiness improved, birds took twice as long to reach their M_{sum} (1.8 ± 0.05 h) in January through March compared with in August (0.3 ± 0.3 h). T_a at M_{sum} also varied over the winter (month: $F_{7,1,425} = 17.8$, $P < 0.0001$; mass: $P = 0.06$). Between January and March (Jan. vs. Mar.: $P = 0.80$), birds reached their M_{sum} at $-22.5° \pm 0.01°C$ in a helox environment compared with $-8.3° \pm 0.9°C$ in August to September (Aug. to Sept. vs. Jan. to Mar.: $F_{1,137} = 108$, $P < 0.0001$).

As for M_{sum}, BMR considered whole or mass independent also varied over time (whole: month, $F_{7,287} = 3.3$, $P < 0.005$; mass independent: month, $F_{7,154} = 2.3$, $P < 0.05$; mass: $F_{1,204} = 17.6$, $P < 0.0001$; fig. 3*C*). However, the pattern was unclear, as BMR remained largely stable throughout winter with lower values detectable in September and February, although these differences were significant only for whole BMR (contrast between Aug. [0.53 ± 0.02 W] and Sept. [0.48 ± 0.02 W]: $F_{1,260} = 10.1$, $P < 0.005$; contrast between Aug. and Feb. [0.48 ± 0.02 W]: $F_{1,271} = 14.2$, $P < 0.0005$). For lean mass–independent BMR, the only significant difference was that

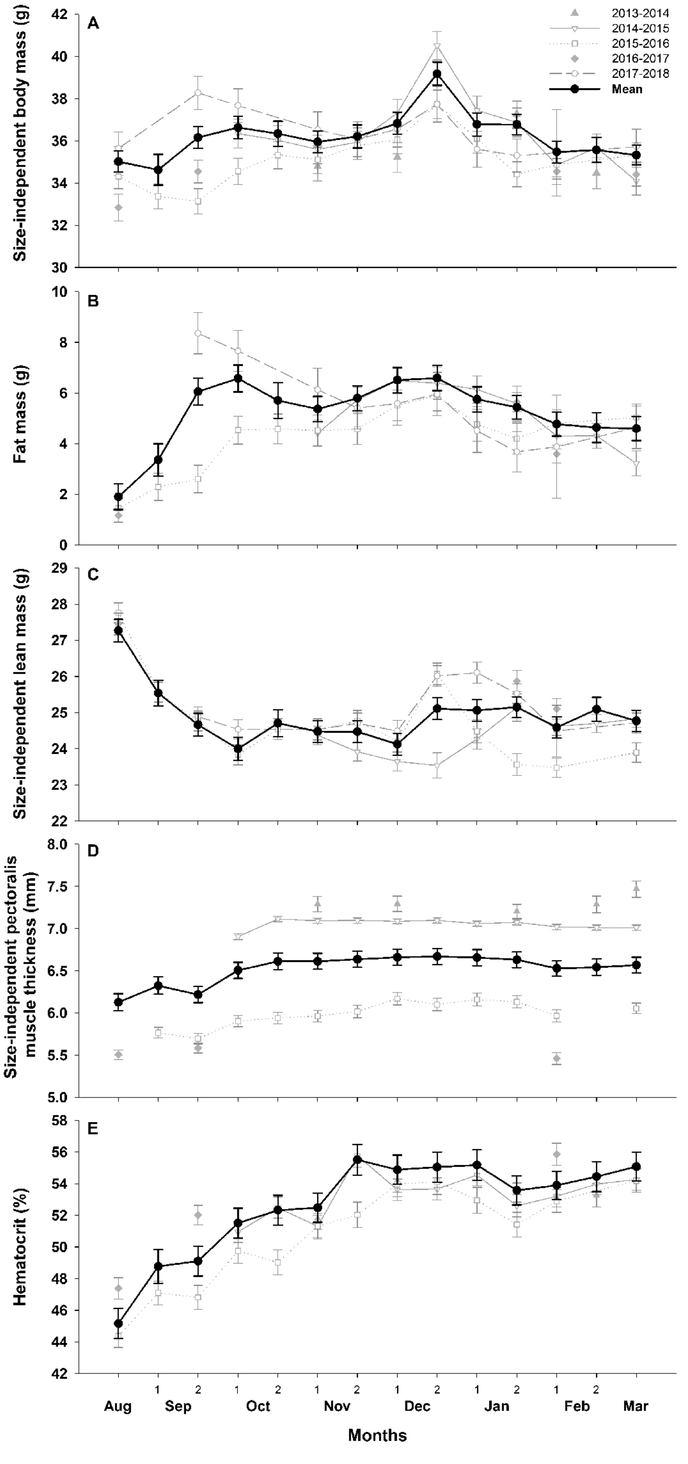

Figure 2. Intraseasonal variation in body composition and hematocrit over 5 yr in outdoor captive snow buntings. Data are least squares means (± SEM) by month for body mass (*A*), fat mass (*B*), lean mass (*C*), pectoralis muscle thickness (*D*), and hematocrit (*E*). Least squares means were obtained from a mixed general linear model (GLM) controlling for month, with year and bird ID as random parameters. Least squares means also control for structural body size (not significant for fat mass) in *A*, *C*, and *D* and for keel length in *D*. Least squares means per year (gray) were obtained from a mixed GLM controlling for the same parameters except year.

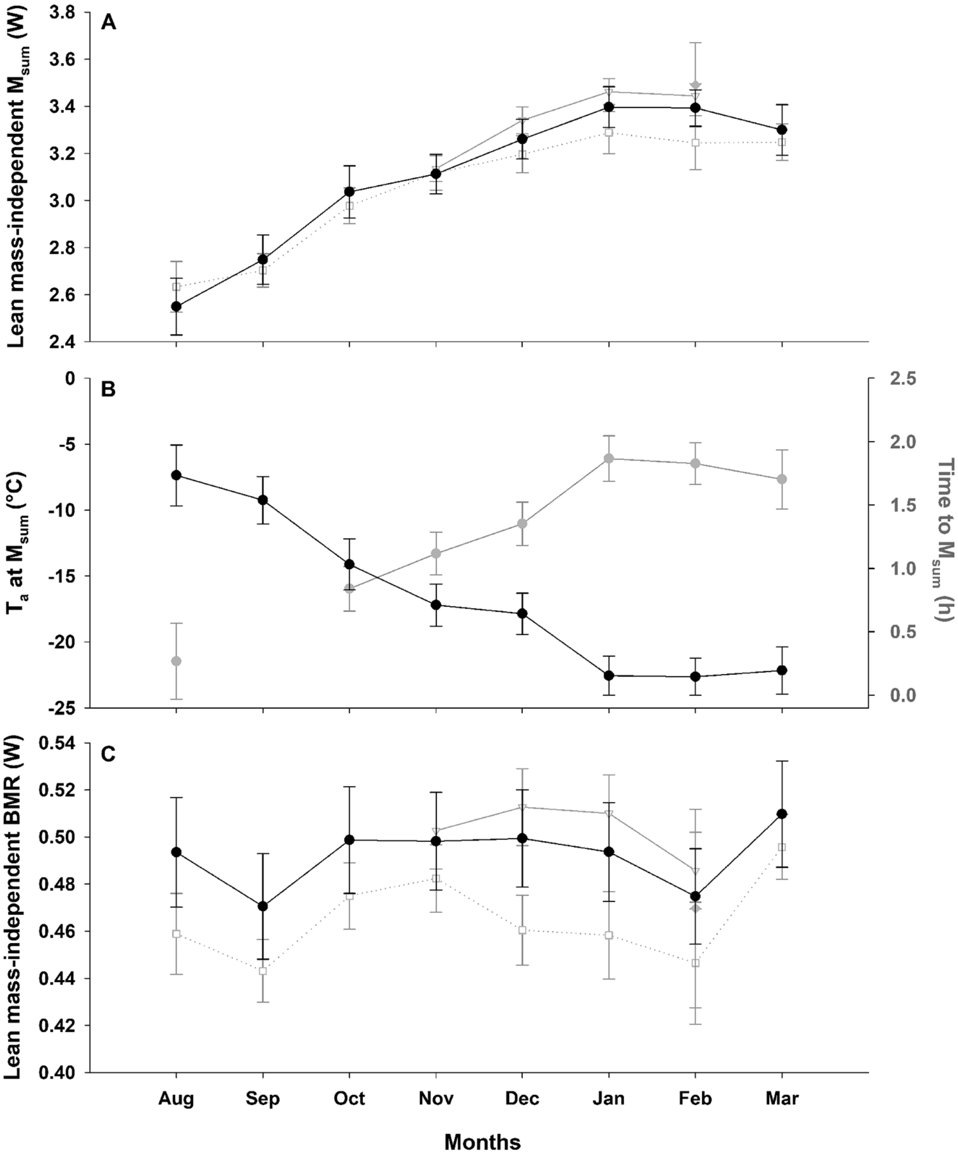

Figure 3. Intraseasonal variation in metabolic performance over 5 yr in outdoor captive snow buntings. Data are least squares means (±SEM) by month for summit metabolic rate (M_{sum}; *A*), time to M_{sum} and air temperature (T_a) at M_{sum} (*B*), and basal metabolic rates (BMR; *C*). Least squares means were obtained from a mixed general linear model (GLM) controlling for month and lean body mass for all parameters (lean mass not significant for T_a at M_{sum} and time to M_{sum}), with year and bird ID as random parameters. Least squares means per year (gray) were obtained from a mixed GLM controlling for the same parameters except year.

observed between February and March ($F_{1,182} = 5.3$, $P < 0.05$). Here again, the model including lean mass as a covariate explained more of the variation in BMR (model: $r^2 = 0.67$) than the model without (model: $r^2 = 0.49$).

Relationships between Phenotypic Traits

Comparing monthly average values in regression analyses revealed that most of the seasonal variation in total M_b was explained by variation in body fat ($n = 14$, $r^2 = 0.57$, $P < 0.005$; fig. 4*A*; no significant relationship between total mass and lean mass, $P = 0.3$). Interestingly, our results also showed a relatively strong negative relationship between lean and fat components of M_b over the season ($n = 14$, $r^2 = 0.66$, $P < 0.0005$; fig. 4*B*), where birds carried less lean mass when they were at their fattest (see also fig. 2*B*, 2*C*). Our results further indicated that in months when birds were at their fattest, pectoralis muscles were large ($n = 14$, $r^2 = 0.42$, $P < 0.05$; fig. 4*C*), and large muscles were associated with higher oxygen carrying capacity ($n = 14$, $r^2 = 0.85$, $P < 0.0001$; fig. 4*D*). Average monthly variation in hematocrit was also strongly positively associated with monthly variation in lean mass–independent M_{sum} ($n = 8$, $r^2 = 0.96$, $P < 0.0001$; fig. 4*E*). Our analyses further revealed a significant relationship

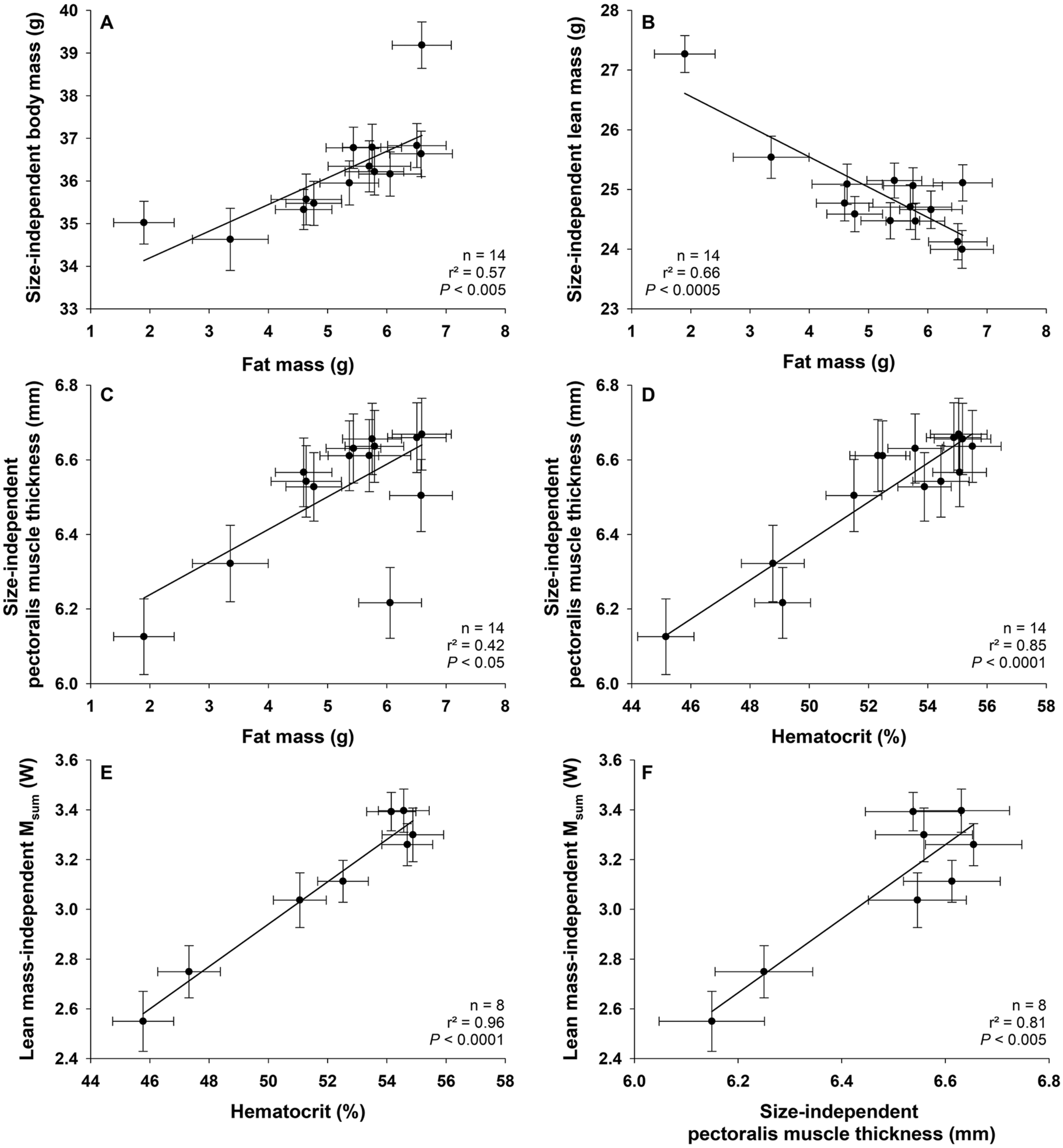

Figure 4. Relationships between phenotypic traits in outdoor captive snow buntings. Presented are results of regression analyses testing for relationships between body mass and fat mass (*A*), lean mass and fat mass (*B*), pectoralis muscle thickness and fat mass (*C*), pectoralis muscle thickness and hematocrit (*D*), summit metabolic rate (M_{sum}) and hematocrit (*E*), and M_{sum} and pectoralis muscle thickness (*F*). Values are monthly least squares means (±SEM) generated by our mixed effects models and are based on the period from August to March.

between size-independent pectoralis muscle thickness and lean mass–independent M_{sum} ($n = 8$, $r^2 = 0.81$, $P < 0.005$; fig. 4*F*). There were no correlations between lean mass–independent BMR and lean mass–independent M_{sum} ($P = 0.6$) and between whole BMR and lean mass ($P = 0.5$).

Discussion

This is the first longitudinal multiyear study to present phenotypic adjustments in a northern cold-specialized species exposed to seasonal temperature variation typical of temperate zone climates. Snow buntings showed flexibility in functions underlying thermogenic capacity and cold endurance comparable to that observed in temperate resident passerines (Dawson and Marsh 1986; Swanson and Olmstead 1999; Petit et al. 2013). Specifically, M_b, fat mass, pectoralis muscle thickness, and hematocrit increased from summer to winter. Maximal thermogenic capacity (M_{sum}) and cold endurance (time to M_{sum}) were also at their highest during the coldest months, when birds reached their M_{sum} at the coldest temperatures. However, in contrast with previous studies on resident species at this latitude (Petit et al. 2013, 2014), lean mass declined as the birds gained mass, and BMR showed no clear pattern, remaining relatively stable throughout winter.

Changes in Body Composition through Time

Our data show that snow buntings maintained heavier M_b in winter than in summer, a finding also common in passerines wintering at both temperate and northern latitudes (e.g., Brooks 1968; White and West 1977; Carey et al. 1978; Liknes and Swanson 1996; Zheng et al. 2008; Petit et al. 2014). The birds increased their M_b 13.2% from early September to late December, and this mass gain, primarily driven by body fat (fig. 4*A*), falls in the range of 9%–22% reported for northern resident species (Barnett 1970; Pohl 1971; Pohl and West 1973; Sharbaugh 2001). Interestingly, M_b was highest in late December. Although this month is not the coldest of winter, it is the month with the shortest days (fig. 1). This finding corroborates a recent report by Laplante et al. (2019), who observed a negative relationship between M_b and day length in wild wintering snow buntings, suggesting that these birds maintain larger fat reserves as an energy buffer when feeding time is limited.

Small wintering birds accumulate fat to sustain longer and colder nighttime fasting periods and to provide emergency reserves during low food availability (Blem 1976; Lehikoinen 1987; Marsh and Dawson 1989; O'Connor 1995; Gosler 1996). Snow buntings exhibited a rapid increase (nearly 250%) in fat mass between August and October and then remained relatively stable until late December, after which fat mass gradually declined. This pattern contrasts with other species, where fat increases gradually and peaks during the coldest periods of winter (Brooks 1968; Evans 1969; White and West 1977; Dawson and Marsh 1986). However, it is important to note that our findings appear largely driven by an early increase in fat mass in one of two years. During 2015–2016, birds exhibited the expected gain in fat, whereas in 2017–2018, birds already had large reserves in late September. The reasons for this difference are unknown and cannot simply be explained by differences in T_a because adding mean T_a for the days of measure in the models showed no significant influence of T_a and did not change the findings. Declining levels of fat during the peak of cold in January and February have also been seen in other resident species (Brooks 1968; Evans 1969; White and West 1977; Carey et al. 1978) and are consistent with the lower fat scores reported by Laplante et al. (2019) during the coldest days of winter in free-living snow buntings. As suggested by Mandin and Vézina (2012), this could result from thermoregulatory requirements forcing birds to consume a greater part of their daily energy intake, potentially limiting their capacity to accumulate or maintain fat reserves.

Avian cold acclimatization or acclimation is typically associated with size increases in several internal organs, including cardiopulmonary organs, skeletal muscles, and nutritional organs (e.g., Zheng et al. 2008; Liknes and Swanson 2011; Petit et al. 2014; Barceló et al. 2017). Although we could not study body composition at the organ level, we did monitor individual changes in lean mass. Counter to predictions, lean mass declined rapidly between August and October before leveling and then increasing in late December to reach a plateau for the rest of winter. These data partially conform to the pattern reported in other passerines where lean mass increases in winter (Chaplin 1974; Dawson and Marsh 1986; Petit et al. 2014), potentially as a result of higher food intake and, consequently, larger digestive and excretory organs (e.g., Zheng et al. 2008; Liknes and Swanson 2011; Petit et al. 2014; Barceló et al. 2017). However, the causes for the rapid loss of lean mass in the fall are not known.

We found that snow buntings increased the size of their pectoralis muscles from August to October and maintained it for the rest of winter. Although interseasonal variation in muscle size has never been studied at comparable temporal scales in passerines, several studies have reported increases in flight muscle mass between summer and winter (e.g., Swanson 1991*a*; Cooper 2002; Liknes and Swanson 2011; Swanson and Merkord 2013; Petit and Vézina 2014). This is often interpreted as an adjustment to improve shivering heat production (Swanson 1991*b*; Liknes and Swanson 2011; Petit et al. 2014) because M_{sum} is typically correlated with muscle mass or size (Swanson 1991*b*; O'Connor 1995; Cooper 2002; Petit et al. 2014; but see Milbergue et al. 2018), including in buntings (Dubois 2016; this study). However, in the current case, pectoralis muscle size stabilized much earlier than peak M_{sum} (figs. 2*D*, 3*A*). Given the relationship between monthly averaged muscle thickness and body fat (fig. 4*C*), the early fall increase in flight muscle size could also reflect compensation for increasing M_b to maintain flight capacity (Pennycuick 1975; Lindström et al. 2000; Dietz et al. 2007; Petit and Vézina 2014; McKechnie et al. 2015). Alternatively, because lipids are the main fuel supporting shivering thermogenesis (Vaillancourt et al. 2005), thicker muscles in wintering buntings could also partly result from larger cellular fat storage within the muscle tissue itself (Ramenofsky et al. 2017; Carter et al. 2019). Consistent with this latter argument is the obvious difference in muscle thickness between years, wherein birds carried 5% more fat and had thicker muscles in 2014–2015 compared with 2015–2016 (fig. 2). This could also explain why muscle thickness (which should mainly consist of lean tissue) increased, while total lean mass decreased (compare fig. 2*D* with fig. 2*C*).

Changes in Metabolic Performance through Time

Summit Metabolic Rate and Temperature at Summit Metabolic Rate. The observed gradual increase in M_{sum} and the 27%–33% higher values measured during the coldest time of the year

(Feb.) are very similar in pattern and values (29%–34%) to that reported for resident black-capped chickadees wintering less than 22 km away from our captive population (Petit et al. 2013, 2014). It also compares with observations in other resident species living at temperate (McKechnie et al. 2015) and Arctic (Saarela et al. 1995) latitudes. As suggested by Petit et al. (2013), the observed gradual adjustments in thermogenic capacity beginning very early in the fall (Sept.) and culminating at the peak of winter could reflect a continuous response to increasingly colder temperatures. Adjusting M_{sum} early and well ahead of peak winter conditions could be necessary if this trait responds slowly to changes in temperature (Petit and Vézina 2014; Dubois et al. 2016).

The adjustments in M_{sum} were paralleled by a gradual increase in time to M_{sum} and by a decline in T_{a} at M_{sum}. Together these results indicate that snow buntings were able to generate more heat at the peak of winter, thereby increasing their cold tolerance (Swanson 2010). In January, the mean helox T_{a} eliciting maximal heat production in these birds was $-22.2° \pm 1.1°$C. This is similar to that reported by Vézina et al. (2006) in cold-acclimated red knots (*Calidris canutus islandica*), a shorebird species more than three times heavier. This is also 8° and 11°C colder than the temperature at cold limit (helox T_{a} marking the beginning of decline in $\dot{V}\mathrm{O}_2$ following M_{sum}) reported by Swanson and Liknes (2006) in horned larks (*Eremophila alpestris*; ≈35.4 ± 2.7 g) and northern cardinals (*Cardinalis cardinalis*; ≈48.3 ± 3.6 g), respectively. Although M_{sum} is measured under maximum $\dot{V}\mathrm{O}_2$ conditions and birds likely do not operate at this level in the wild, these results clearly demonstrate that snow buntings are extreme cold specialists. Indeed, using mean conductance values from outdoor captive snow buntings measured in atmospheric air during winter (J. Drolet and F. Vézina, unpublished data), we estimated that the T_{a} required to elicit the February level of M_{sum} in snow buntings would be $-93.6° \pm 6.4°$C.

Oxygen Carrying Capacity and Summit Metabolic Rate. Higher hematocrit levels in winter appear to be a common phenomenon in birds (e.g., DeGraw et al. 1979; Piersma et al. 2000; Pap et al. 2010; Buehler et al. 2012), and at the individual level, oxygen carrying capacity has been shown to correlate positively with M_{sum} (Swanson 1990*b*; Petit and Vézina 2014; Petit et al. 2017). Accordingly, monthly average analyses revealed a close and positive association between hematocrit and lean mass–independent M_{sum} (fig. 4*E*), suggesting that buntings meet the increased oxygen requirements associated with high thermogenic capacity, in part, through an increase in hematocrit. Interestingly, as correcting M_{sum} for lean mass should also partly control for the influence of skeletal muscle mass on M_{sum} (Vézina et al. 2011), this finding further suggests a link between oxygen delivery and metabolic intensity at the cellular level during active shivering.

Basal Metabolic Rate. Small-bodied species experiencing marked seasonal weather changes typically exhibit an increase in BMR during the cold season (Pohl and West 1973; Cooper and Swanson 1994; Liknes and Swanson 1996; Zheng et al. 2008), and this can reach considerable levels (e.g., 64% in house sparrows; Arens and Cooper 2005). In our study, BMR showed no clear seasonal patterns, although some monthly variation could be detected in whole-animal values. This suggests that maintenance costs in wintering buntings remain seasonally stable. Similar patterns have also been observed in northern resident species (e.g., willow tits [Reinertsen and Haftorn 1983]; siskins [*Spinus spinus*; Saarela et al. 1995]). Sharbaugh (2001), for instance, reported no seasonal variation in resting metabolic rate measured at 25°C in Alaskan (64°N) black-capped chickadees.

King and Farner (1961), and more recently Swanson et al. (2017*b*), argued that there is no adaptive advantage for animals to increase BMR. Instead, a high BMR, as seen in species wintering in cold conditions, could result from upregulated functions required to maintain thermogenic capacities and balanced energy budgets under constraining environments. In wintering birds coping with high thermoregulatory requirements, energy conservation is paramount to minimize environmental effects on energy budgets (e.g., Reinertsen 1996). One way to reduce maintenance costs during the winter would be to decrease or minimize the amount of metabolically active lean mass (e.g., the rapid decline observed in the fall in our buntings). However, this may not be the only solution for buntings because whole-animal BMR and lean mass were not correlated when comparing monthly average patterns. Alternatively, changes in metabolic intensity (e.g., downregulation) at the cellular level could also contribute to energy economy, and this could uncouple relationships between maintenance costs and lean mass (Milbergue et al. 2018). Given the notable increase in M_{sum} observed in this study, it appears that snow buntings may be particularly efficient at minimizing the effect of winter on their maintenance costs.

Conclusions. In this investigation, we have shown that the snow bunting, a circumpolar passerine species living in the cold most of its life, exhibits considerable changes in several phenotypic traits when exposed to seasonal temperate zone temperature variation. Our results therefore suggest that flexibility in traits associated with thermal acclimatization in cold-adapted northern species is comparable to that of temperate resident species living at lower latitudes and is therefore not constrained by life in near-constant cold.

Our data also show that buntings have remarkable capacities to endure cold environments (e.g., M_{sum} reached at T_{a} equivalent to −94°C during the coldest months) while potentially minimizing the impact of life in these environments on maintenance costs (no seasonal increase in BMR). These phenotypic adaptations are consistent with snow buntings being extreme cold specialists capable of maximizing performance across multiple life-history stages under consistently cold conditions. More research is needed however to uncover the physiological mechanisms that allow buntings to managing the “worst of both worlds” across migration, breeding, and wintering. Importantly, determining these underlying mechanisms will aid in predicting whether this cold specialist can cope with the increasing T_{a} forecast in its Arctic home.

Acknowledgments

We thank Claire Bottini, Polan-Devi Darboven, Justine Drolet, Alexandre Paiement, Kim Régimbald Bélanger, and Charles Richard for their help in catching birds in the wild. We also thank Yann Bouchez, Sandrine Gingras, Sarah Piedalue, Jolanie Roy, Gabrielle Roy, and Cécile Vansteenberghe for their punctual help in measuring body composition monthly and Claire Bottini for participating in ultrasound measurements. We are also grateful to Jonathan Coudé for technical support, Alain Caron for statistical advice, and Josianne Ruest for help with bird maintenance. This research benefited from a generous donation from the Kenneth M. Molson Foundation. It was also supported by Natural Sciences and Engineering Research Council Discovery grants to F.V. and O.P.L., Canada Foundation for Innovation awards to F.V. and O.P.L., and Canada Research Chair funding to O.P.L.

APPENDIX

Table A1: Measurement sequence and sample size across years for metabolic performance (summit metabolic rate [M_{sum}] and basal metabolic rate [BMR]), hematocrit (HCT), pectoralis muscle thickness, and body composition (total, fat, and lean body mass)

	2013–2014			2014–2015			2015–2016			2016–2017			2017–2018		
Measurement sequence (month)	Meta perf	HCT and muscle	Body comp	Meta perf	HCT and muscle	Body comp	Meta perf	HCT and muscle	Body comp	Meta perf	HCT and muscle	Body comp	Meta perf	HCT and muscle	Body comp
Aug.						27	(17) 19	19	19		(24) 32	32			24
Sept.-1								(17) 19	19						
Sept.-2							16	(16) 17	17	(0) 26	31	31			23
Oct.-1				(25) 23	(25) 22	25		16	16						22
Oct.-2					(25) 20	24	(13) 14	14	14						
Nov.-1	16	19	19	22	(21) 22	22		13	13						19
Nov.-2					(18) 22	22	13	13	13						19
Dec.-1	16	17	17	(21) 24	25	25		13	13						18
Dec.-2					22	22	13	13	13						18
Jan.-1				22	23	23		12	12						18
Jan.-2	(20) 21	23	23		22	22	(9) 8	(14) 19	19			19			25
Feb.-1					24	24	8	20	20	(22) 20	23	23			
Feb.-2	(13) 15	(14) 15	15	(29) 25	25	25									
Mar.	(0) 16	16	16		(24) 25	25	(19) 18	19	19						

Note. Numbers in parentheses in the “Meta perf” columns are sample sizes for M_{sum} when different from samples sizes for BMR. Numbers in parentheses in the “HCT and muscle” columns are sample sizes for HCT when different from sample sizes for muscle thickness. Fat and lean measurements by quantitative magnetic resonance began in November 2014. As we were interested in tracking intraindividual phenotypic variation, we repeatedly measured each bird from the end of August to early March over 5 yr (metabolic performance: 4.9 ± 0.6 times, range: 1–18; body composition: 9.8 ± 1.04 times, range: 1–35; total body mass: 9.3 ± 1.04 times, range: 1–47). Measurement frequency varied from three measurement periods in 2016–2017 to 11 and 12 measurement periods in 2014–2015 and 2015–2016, respectively, when we could obtain data on each bird every other week for a full yearly cycle. Meta perf = metabolic performance; body comp = body composition.

Literature Cited

Arens J.R. and S.J. Cooper. 2005. Metabolic and ventilatory acclimatization to cold stress in house sparrows (*Passer domesticus*). Physiol Biochem Zool 78:579–589.

Banks K.W., H. Clark, I.R.K. Mackay, S.G. Mackay, and R.M. Sellers. 1991. Origins, population structure and movements of snow buntings *Plectrophenax nivalis* wintering in highland region, Scotland. Bird Study 38:10–19.

Barceló G., O.P. Love, and F. Vézina. 2017. Uncoupling basal and summit metabolic rates in white-throated sparrows: digestive demand drives maintenance costs, but changes in muscle mass are not needed to improve thermogenic capacity. Physiol Biochem Zool 90:153–165.

Barnett L.B. 1970. Seasonal changes in temperature acclimatization of the house sparrow, *Passer domesticus*. Comp Biochem Physiol 33:559–578.

Barrio A.M., S. Lamichhaney, G. Fan, N. Rafati, M. Pettersson, H. Zhang, J. Dainat, et al. 2016. The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. eLife 5:1–32.

Bartholomew G.A., D. Vleck, and C.M. Vleck. 1981. Instantaneous measurements of oxygen consumption during preflight warm-up and post-flight cooling in sphingid and saturniid moths. J Exp Biol 90:17–32.

Bengston S.E., R.A. Dahan, Z. Donaldson, S.M. Phelps, K. Van Oers, A. Sih, and A.M. Bell. 2018. Genomic tools for behavioural ecologists to understand repeatable individual differences in behaviour. Nat Ecol Evol 2:944–955.

Bennett S., C.M. Duarte, N. Marbà, and T. Wernberg. 2019. Integrating within-species variation in thermal physiology into climate change ecology. Philos Trans R Soc B 374: 20180550.

BirdLife International. 2020. Species factsheet: *Plectrophenax nivalis*. http://datazone.birdlife.org/species/factsheet/snow-bunting-plectrophenax-nivalis.

Blem C.R. 1976. Patterns of lipid storage and utilization in birds. Am Zool 16:671–684.

Brooks W.S. 1968. Comparative adaptations of the Alaskan redpolls to the Arctic environment. Wilson Bull 80:253–280.

Buehler D.M., F. Vézina, W. Goymann, I. Schwabl, M.A. Versteegh, B.I. Tieleman, and T. Piersma. 2012. Independence among physiological traits suggests flexibility in the face of ecological demands on phenotypes. J Evol Biol 25: 1600–1613.

Carey C., W.R. Dawson, L.C. Maxwell, and J.A. Faulkner. 1978. Seasonal acclimatization to temperature in cardueline finches. J Comp Physiol 125:101–113.

Carey C. and M.L. Morton. 1976. Aspects of circulatory physiology of montane and lowland birds. Comp Biochem Physiol A 54:61–74.

Carter W.A., J.P. Whiteman, C. Cooper-Mullin, S.D. Newsome, and S.R. McWilliams. 2019. Dynamics of individual fatty acids in muscle fat stores and membranes of a songbird and its functional and ecological importance. Physiol Biochem Zool 92:239–251.

Chaplin S.B. 1974. Daily energetics of the black-capped chickadee, *Parus atricapillus*, in winter. J Comp Physiol 89:321–330.

Cooper S.J. 2002. Seasonal metabolic acclimatization in mountain chickadees and juniper titmice. Physiol Biochem Zool 75: 386–395.

———. 2007. Daily and seasonal variation in body mass and visible fat in mountain chickadees and juniper titmice. Wilson J Ornithol 119:720–724.

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

Cramp S. and C.M. Perrins. 1994. Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic. Vol. 9. Buntings and New World warblers. Oxford University Press, Oxford.

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

DeGraw W.A., M.D. Kern, and J.R. King. 1979. Seasonal changes in the blood composition of captive and free-living white-crowned sparrows. J Comp Physiol B 129:151–162.

Dietz M.W., T. Piersma, A. Hedenström, and M. Brugge. 2007. Intraspecific variation in avian pectoral muscle mass: constraints on maintaining manoeuvrability with increasing body mass. Funct Ecol 21:317–326.

Dubois K., F. Hallot, and F. Vézina. 2016. Basal and maximal metabolic rates differ in their response to rapid temperature change among avian species. J Comp Physiol B 186:919–935.

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. Physiol Zool 69:1232–1254.

Evans J.K., K.L. Buchanan, S.C. Griffith, K.C. Klasing, and B.A. Addison. 2017. Ecoimmunology and microbial ecology: contributions to avian behavior, physiology, and life history. Horm Behav 88:112–121.

Evans P.R. 1969. Winter fat deposition and overnight survival of yellow buntings (*Emberiza citrinella* L.). J Anim Ecol 38: 415–423.

Gessaman J.A. and K.A. Nagy. 1988. Energy metabolism: errors in gas-exchange conversion factors. Physiol Zool 61:507–513.

Gosler A.G. 1996. Environmental and social determinants of winter fat storage in the great tit *Parus major*. J Anim Ecol 65: 1–17.

Grant P.R. and B.R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. Science 296:707–711.

Guglielmo C.G., L.P. McGuire, A.R. Gerson, and C.L. Seewagen. 2011. Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. J Ornithol 152(suppl.):75–85.

Hussell D.J.T. 1972. Factors affecting clutch size in Arctic passerines. Ecol Monogr 42:317–364.

Johnson S.R. and D.R. Herter. 1990. Bird migration in the Arctic: a review. Pp. 22–43 in E. Gwinner, ed. Bird migration. Springer, Berlin.

Karasov W.H. and C. Martinez del Rio. 2007. Physiological ecology how animals process energy, nutrients, and toxins. Princeton University Press, Princeton, NJ.

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.

Laplante M.-P., E.A. McKinnon, O.P. Love, and F. Vézina. 2019. Flexible response to short-term weather in a cold-adapted songbird. J Avian Biol 50:1–10.

Latimer C.E., S.J. Cooper, W.H. Karasov, and B. Zuckerberg. 2018. Does habitat fragmentation promote climate-resilient phenotypes? Oikos 127:1069–1080.

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

Le Pogam A., R.S. O'Connor, O.P. Love, M. Petit, L. Régimbald, and F. Vézina. 2020. Coping with the worst of both worlds: phenotypic adjustments for cold acclimatization benefit northward migration and arrival in the cold in an Arctic breeding songbird. Funct Ecol (forthcoming).

Lighton J.R.B. 2019. Measuring metabolic rates: a manual for scientists. 2nd ed. Oxford University Press, Oxford.

Liknes E.T. and D.L. Swanson. 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.

———. 2011. Phenotypic flexibility of body composition associated with seasonal acclimatization in passerine birds. J Therm Biol 36:363–370.

Lindström Å., A. Kvist, T. Piersma, A. Dekinga, and M.W. Dietz. 2000. Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. J Exp Biol 203:913–919.

Macdonald C.A., K.C. Fraser, H.G. Gilchrist, T.K. Kyser, J.W. Fox, and O.P. Love. 2012. Strong migratory connectivity in a declining arctic passerine. Anim Migr 1:23–30.

Macdonald C.A., E.A. McKinnon, H.G. Gilchrist, and O.P. Love. 2016. Cold tolerance, and not earlier arrival on breeding grounds, explains why males winter further north in an Arctic-breeding songbird. J Avian Biol 47:7–15.

Mandin C. and F. Vézina. 2012. Daily variations in markers of nutritional condition in wintering black-capped chickadee *Poecile atricapillus*. Ibis 154:791–802.

Marsh R.L. and W.R. Dawson. 1989. Avian adjustments to cold. Pp. 205–253 in L.C.H. Wang, ed. Advances in comparative and environmental physiology. Vol. 4. Animal adaptation to cold. Springer, Berlin.

Martin K. and K.L. Wiebe. 2004. Coping mechanisms of alpine and Arctic breeding birds: extreme weather and limitations to reproductive resilience. Integr Comp Biol 44:177–185.

McGuill M.W. and A.N. Rowan. 1989. Biological effects of blood loss: implications for sampling volumes and techniques. ILAR J 31:5–20.

McKechnie A.E. 2008. Phenotypic flexibility in basal metabolic rate and the changing view of avian physiological diversity: a review. J Comp Physiol B 178:235–247.

McKechnie A.E., M.J. Noakes, and B. Smit. 2015. Global patterns of seasonal acclimatization in avian resting metabolic rates. J Ornithol 156:367–376.

McKechnie A.E. and D.L. Swanson. 2010. Sources and significance of variation in basal, summit and maximal metabolic rates in birds. Curr Zool 56:741–758.

McKechnie A.E. and B.O. Wolf. 2019. The physiology of heat tolerance in small endotherms. Physiology 34:302–313.

McKinnon E.A., M.-P. Laplante, O.P. Love, K.C. Fraser, S. Mackenzie, and F. Vézina. 2019. Tracking landscape-scale movements of snow buntings and weather-driven changes in flock composition during the temperate winter. Front Ecol Evol 7:1–11.

McKinnon E.A., C.A. Macdonald, H.G. Gilchrist, and O.P. Love. 2016. Spring and fall migration phenology of an Arctic-breeding passerine. J Ornithol 157:681–693.

Meltofte H. 1983. Arrival and pre-nesting period of the snow bunting *Plectrophenax nivalis* in east Greenland. Polar Res 1:185–198.

Milbergue M.S., P.U. Blier, and F. Vézina. 2018. Large muscles are beneficial but not required for improving thermogenic capacity in small birds. Sci Rep 8:14009.

Montgomerie R. and B. Lyon. 2011. Snow bunting (*Plectrophenax nivalis*), version 2.0. In A.F. Pool, ed. Birds of North America. Cornell Lab of Ornithology, Ithaca, NY.

O'Connor T.P. 1995. Metabolic characteristics and body composition in house finches: effects of seasonal acclimitatization. J Comp Physiol B 165:298–305.

———. 1996. Geographic variation in metabolic seasonal acclimatization in house finches. Condor 98:371–381.

Pap P.L., C.I. Vágási, J. Tökölyi, G.Á. Czirják, and Z. Barta. 2010. Variation in haematological indices and immune function during the annual cycle in the great tit *Parus major*. Ardea 98: 105–113.

Peig J. and A.J. Green. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118:1883–1891.

Pennycuick C.J. 1975. Mechanics of flight. Avian Biol 5:1–73.

Petit M., S. Clavijo-Baquet, and F. Vézina. 2017. Increasing winter maximal metabolic rate improves intrawinter survival in small birds. Physiol Biochem Zool 90:166–177.

Petit M., A. Lewden, and F. Vézina. 2013. Intra-seasonal flexibility in avian metabolic performance highlights the uncoupling of basal metabolic rate and thermogenic capacity. PLoS ONE 8:e68292.

———. 2014. How does flexibility in body composition relate to seasonal changes in metabolic performance in a small passerine wintering at northern latitude? Physiol Biochem Zool 87:539–549.

Petit M. and F. Vézina. 2014. Phenotype manipulations confirm the role of pectoral muscles and haematocrit in avian maximal thermogenic capacity. J Exp Biol 217:824–830.

Piersma T., L. Bruinzeel, R. Drent, M. Kersten, J. Van der Meer, and P. Wiersma. 1996. 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., J.A. Gessaman, A. Dekinga, and G.H. Visser. 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.

Piersma T., A. Koolhaas, A. Dekinga, and E. Gwinner. 2000. Red blood cell and white blood cell counts in sandpipers (*Philomachus pugnax*, *Calidris canutus*): effects of captivity, season, nutritional status, and frequent bleedings. Can J Zool 78:1349–1355.

Piersma T. and J.A. van Gils. 2011. The flexible phenotype: a body-centred integration of ecology, physiology, and behaviour. Oxford University Press, Oxford.

Pigliucci M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends Ecol Evol 20:481–486.

Pohl H. 1971. Seasonal variations in metabolic functions of bramblings. Ibis 113:185–193.

Pohl H. and G.C. West. 1973. Daily and seasonal variation in metabolic response to cold during rest and exercise in the common redpoll. Comp Biochem Physiol 45:851–867.

Ramenofsky M., C. Priester, H. Koopman, D. Gay, and R. Dillaman. 2017. Biochemical and ultrastructural adaptations of avian flight muscle for long distance migration and arrival on the breeding grounds. Integr Comp Biol 57:e.382.

Reinertsen R.E. 1996. Physiological and ecological aspects of hypothermia. Pp. 125–157 in C. Carey, ed. Avian energetics and nutritional ecology. Springer, Boston.

Reinertsen R.E. and S. Haftorn. 1983. Nocturnal hypothermia and metabolism in the willow tit *Parus montanus* at 63°N. J Comp Physiol 151:109–118.

Rising J.D. and K.M. Somers. 1989. The measurement of overall body size in birds. Auk 106:666–674.

Rosenmann M. and P. Morrison. 1974. Maximum oxygen consumption and heat loss facilitation in small homeotherms by He-O_2. Am J Physiol 226:490–495.

Royer-Boutin P., P.A. Cortés, M.S. Milbergue, M. Petit, and F. Vézina. 2015. Estimation of muscle mass by ultrasonography differs between observers and life states of models in small birds. Physiol Biochem Zool 88:336–344.

Saarela S., B. Klapper, and G. Heldmaier. 1995. Daily rhythm of oxygen consumption and thermoregulatory responses in some European winter- or summer-acclimatized finches at different ambient temperatures. J Comp Physiol B 165:366–376.

Scholander P.F., R. Hock, V. Walters, and L. Irving. 1950*a*. Adaptation to cold in Arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. Biol Bull 99:259–271.

Scholander P.F., V. Walters, R. Hock, and L. Irving. 1950*b*. Body insulation of some Arctic and tropical mammals and birds. Biol Bull 99:225–236.

Serreze M.C. and R.G. Barry. 2005. The Arctic climate system. Cambridge University Press, New York.

Sharbaugh S.M. 2001. Seasonal acclimatization to extreme climatic conditions by black-capped chickadees (*Poecile atricapilla*) in interior Alaska (64°N). Physiol Biochem Zool 74:568–575.

Smit B. and A.E. McKechnie. 2010. Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. Funct Ecol 24:330–339.

Snell K.R.S., B.G. Stokke, A. Moksnes, K. Thorup, and F. Fossoy. 2018. From Svalbard to Siberia: passerines breeding in the High Arctic also endure the extreme cold of the Western Steppe. PLoS ONE 13:e0202114.

Swanson D.L. 1990*a*. Seasonal variation in cold hardiness and peak rates of cold-induced thermogenesis in the dark-eyed junco (*Junco hyemalis*). Auk 107:561–566.

———. 1990*b*. Seasonal variation of vascular oxygen transport in the dark-eyed junco. Condor 92:62–66.

———. 1991*a*. Seasonal adjustments in metabolism and insulation in the dark-eyed junco. Condor 93:538–545.

———. 1991*b*. Substrate metabolism under cold stress in seasonally acclimatized dark-eyed juncos. Physiol Zool 64: 1578–1592.

———. 2010. Seasonal metabolic variation in birds: functional and mechanistic correlates. Curr Ornithol 17:75–129.

Swanson D.L., M.W. Drymalski, and J.R. Brown. 1996. Sliding vs static cold exposure and the measurement of summit metabolism in birds. J Therm Biol 21:221–226.

Swanson D.L., M.O. King, W.I. Culver, and Y. Zhang. 2017*a*. Within-winter flexibility in muscle masses, myostatin, and cellular aerobic metabolic intensity in passerine birds. Physiol Biochem Zool 90:210–222.

Swanson D.L., M.O. King, and E. Harmon. 2014. Seasonal variation in pectoralis muscle and heart myostatin and tolloid-like proteinases in small birds: a regulatory role for seasonal phenotypic flexibility? J Comp Physiol B 184:249–258.

Swanson D.L. and E.T. Liknes. 2006. A comparative analysis of thermogenic capacity and cold tolerance in small birds. J Exp Biol 209:466–474.

Swanson D.L., A.E. McKechnie, and F. Vézina. 2017*b*. How low can you go? an adaptive energetic framework for interpreting basal metabolic rate variation in endotherms. J Comp Physiol B 187:1039–1056.

Swanson D.L. and C. Merkord. 2013. Seasonal phenotypic flexibility of flight muscle size in small birds: a comparison of ultrasonography and tissue mass measurements. J Ornithol 154:119–127.

Swanson D.L. and K.L. Olmstead. 1999. Evidence for a proximate influence of winter temperature on metabolism in passerine birds. Physiol Biochem Zool 72:566–575.

Swanson D.L. and F. Vézina. 2015. Environmental, ecological and mechanistic drivers of avian seasonal metabolic flexibility in response to cold winters. J Ornithol 156:377–388.

Vaillancourt E., S. Prud'Homme, F. Haman, C.G. Guglielmo, and J.-M. Weber. 2005. Energetics of a long-distance migrant shorebird (*Philomachus pugnax*) during cold exposure and running. J Exp Biol 208:317–325.

Vézina F., A. Dekinga, and T. Piersma. 2011. Shorebirds' seasonal adjustments in thermogenic capacity are reflected by changes in body mass: how preprogrammed and instantaneous acclimation work together. Integr Comp Biol 51:394–408.

Vézina F., K.M. Jalvingh, A. Dekinga, and T. Piersma. 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.

Vincent J. and J. Bédard. 1976. Fat reserves in snow buntings. Can J Zool 54:1051–1063.

White C.M. and G.C. West. 1977. The annual lipid cycle and feeding behavior of Alaskan redpolls. Oecologia 27:227–238.

White C.R. and M.R. Kearney. 2013. Determinants of interspecific variation in basal metabolic rate. J Comp Physiol B 183:1–26.

Yesou P. 1998. Afflux de Bruant des neiges Plectrophenax nivalis en France (sept. 1996–mai 1997). Ornithos 5:180–187.

Zheng W.-H., M. Li, J.-S. Liu, and S.-L. Shao. 2008. Seasonal acclimatization of metabolism in Eurasian tree sparrows (*Passer montanus*). Comp Biochem Physiol A 151:519–525.