

# Basal and maximal metabolic rates differ in their response to rapid temperature change among avian species

Karine Dubois<sup>1,2</sup> · Fanny Hallot<sup>1,2</sup> · François Vézina<sup>1,2</sup>

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**Abstract** In birds, acclimation and acclimatization to temperature are associated with changes in basal (BMR), summit ( $M_{\text{sum}}$ ) and maximal (MMR) metabolic rates but little is known about the rate at which species adjust their phenotype to short-term temperature variations. Our aims were (1) to determine the pattern of metabolic adjustments following a rapid temperature change, (2) to determine whether performance varies at similar rates during exposure to warm or cold environments, and (3) to determine if BMR,  $M_{\text{sum}}$  and MMR change at comparable rates during thermal acclimation. We measured these parameters in white-throated sparrows (*Zonotrichia albicollis*), black-capped chickadees (*Poecile atricapillus*), and snow buntings (*Plectrophenax nivalis*) after acclimation to 10 °C (day 0) and on the 4th and 8th days of acclimation to either −5 or 28 °C. Birds changed their metabolic phenotype within 8 days with patterns differing among species. Sparrows expressed the expected metabolic increases in the cold and decreases at thermoneutrality while performance in chickadees and buntings was not influenced by temperature but changed over time with inverse patterns. Our results suggest that BMR varies at comparable rates in warm and cold environments but changes faster than  $M_{\text{sum}}$  and MMR, likely due to limitations in the rate of change in organ size and function. They also suggest that maximal

metabolic capacity is lost faster in a warm environment than it is gained in a cold environment. With the expected increase in temperature stochasticity at northern latitudes, a loss of thermogenic capacity during warm winter days could, therefore, be detrimental if birds are slow to readjust their phenotype with the return of cold days.

**Keywords** Basal metabolic rate · Body composition · Maximal metabolic rate · Phenotypic flexibility · Summit metabolic rate · Thermal acclimation

## Introduction

In small avian species, thermal acclimation and acclimatization are typically associated with flexible adjustments of two components of metabolic performance (Swanson 2010). Basal metabolic rate (BMR) represents the minimal amount of energy used to maintain vital functions in a resting animal at thermoneutrality (McKechnie 2008) while summit metabolic rate ( $M_{\text{sum}}$ ) is a measure of maximal shivering heat production during cold exposure. As  $M_{\text{sum}}$  is positively correlated with cold tolerance (Swanson et al. 1996), it is commonly interpreted as an indicator of a bird's ability to endure cold environments. Both BMR and  $M_{\text{sum}}$  are known to increase in response to experimental and natural cold exposure (Broggi et al. 2007; Cooper and Swanson 1994; Klaassen et al. 2004; Liknes et al. 2002; Maldonado et al. 2008; Petit et al. 2013; van de Ven et al. 2013), and evidence suggests that this variation partly results from underlying changes in size and/or metabolic intensity of internal organs (Vézina et al. 2006; Williams and Tieleman 2000; Zheng et al. 2008). For instance, cold-induced increases in BMR are commonly associated with the enlargement of organs responsible for energy

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✉ François Vézina  
francois\_vezina@uqar.ca

<sup>1</sup> Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski, Québec, Canada

<sup>2</sup> Groupe de recherche sur les environnements nordiques BOREAS, Centre d'études nordiques, Centre de la science de la biodiversité du Québec, Rimouski, Québec, Canada

acquisition, such as the intestines, gizzard, liver or kidneys (Liu and Li 2006; Sabat et al. 2009; Williams and Tieleman 2000; Zheng et al. 2008 but see Petit et al. 2014) while larger muscles, particularly those used for flight, have been shown to result in higher thermogenic capacity (Petit and Vézina 2013; Swanson and Vézina 2015; Swanson et al. 2013, but see Swanson et al. 2014a).

Birds preparing for migration also show increases in  $M_{\text{sum}}$  (Swanson and Dean 1999; Vézina et al. 2007). This results from the development of pectoral muscles typically seen during the period preceding departure (Swanson and Dean 1999; Vézina et al. 2007). Since both shivering and locomotion use the same muscles, larger flight muscles developed for migration bring the added benefit of increasing cold endurance (Swanson and Dean 1999; Vézina et al. 2007). Similarly, cold acclimatization comes with a side benefit, where larger skeletal muscles and heart (Cooper 2002; O'Connor 1995; Saarela and Hohtola 2003; Vézina et al. 2006; Zhang et al. 2015a), as well as the associated increase in oxygen carrying capacity (Petit and Vézina 2013), lead to an improvement of maximal locomotor performance (Zhang et al. 2015a). The maximal amount of energy spent during intense exercise is called maximal metabolic rate (MMR). Like  $M_{\text{sum}}$ , MMR is thought to reflect physical endurance (McKechnie and Swanson 2010; Piersma 2011) and varies with pectoral muscle size (Wiersma et al. 2007).

Studies on flexible adjustment of metabolic performance during thermal acclimation typically report final amplitudes of response. Birds are generally separated into two temperature treatments and parameters are measured after an acclimation period varying between a few weeks (Bush et al. 2008; Maldonado et al. 2008; McKechnie et al. 2007; Williams and Tieleman 2000) to several months (Klaassen et al. 2004). The amplitude of response is then determined as the difference between final phenotypes in cold and warm treatments. However, although the amplitude of metabolic flexibility informs on the response of a species, it does not allow for predicting the rate at which that species can change its phenotype. For instance, two species could have similar amplitudes of response, which would make them considered equally flexible, while they may not be able to reach their new phenotype at the same rate. This is important in the context of ongoing climate change, since acute weather events as well as rapid daily temperature fluctuations are expected to increase in frequency and amplitude (Easterling et al. 2000; IPCC 2013; Katz et al. 2005). Adapting a phenotype to a new constraining environment is admittedly beneficial but adjusting it too late or too slowly could potentially have negative fitness consequences.

As far as we know, only one study investigated the rate of change in metabolic performance in response to

temperature variations in free-living birds. Swanson and Olmstead (1999) found that dark-eyed juncos (*Junco hyemalis*) and black-capped chickadees (*Peocile atricapillus*), which are generally associated with forest habitats (Foote et al. 2010; Nolan et al. 2002), tended to adjust their BMR and  $M_{\text{sum}}$  within 14–30 days following changes in average ambient temperature while American tree sparrows (*Spizella arborea*), a species associated with open areas (Naugler 2014), responded much more rapidly, within 5 days after a temperature change. Therefore, species living in contrasted environments could show different rates of adjustment to temperature variations. Experimental evidence also suggests that the rate of change in metabolic parameters can differ depending on whether the environment is warming up or cooling down. Indeed, Barcelo et al. (2009) found that BMR of rufous-collared sparrows (*Zonotrichia capensis*) changed more rapidly when temperature went from 22 to 15 °C (cold treatment), increasing by 29 % over a 4-week period compared to birds showing only a 13 % decline in BMR when temperature changed from 22 to 30 °C (warm treatment) over the same period (see also McKechnie et al. 2007 for a similar study). Whether a similar pattern also exists for  $M_{\text{sum}}$  and MMR remains, however, to be determined.

Recent evidence also suggests that BMR,  $M_{\text{sum}}$  and MMR could differ in the rate at which they respond to a given change in thermal environment. For example, in black-capped chickadees undergoing seasonal cold acclimatization in eastern Canada, both BMR and  $M_{\text{sum}}$  were found to peak in February (Petit et al. 2013). However, the increase in  $M_{\text{sum}}$  began more than a month before changes could be detected in BMR (Petit et al. 2013), possibly starting as early as at the end of August (Petit and Vézina 2014), while BMR began its seasonal increase only between November and December (Petit et al. 2013). Although this could reflect variables responding to different constraints (Petit et al. 2013; Petit and Vézina 2014), the pattern also suggests that  $M_{\text{sum}}$  may be slower to change than BMR. Bauchinger and McWilliams (2009, 2010) demonstrated that the rate of change in avian organ mass is determined by tissue-specific protein turnover rates. They showed that, among body components, the intestines, liver and kidneys, which are large and often found to influence BMR in cold acclimated birds (Liknes and Swanson 2011a; Liu and Li 2006; Petit et al. 2014; Williams and Tieleman 2000; Zheng et al. 2008), have the highest rate of turnover while flight and leg muscles, which are associated with maximal metabolic performance (Marsh and Dawson 1989; Petit et al. 2013; Swanson et al. 2013, 2014b; Vézina et al. 2006, 2007; Wiersma et al. 2007), have the lowest rate of tissue turnover. Therefore, assuming that changes in basal and maximal metabolic rates during thermal acclimation primarily reflect size changes in these specific organs, one

could expect BMR to respond faster than  $M_{\text{sum}}$  and MMR to a change in temperature due to intrinsic limitations in organ transformation rate.

We are not aware of experimental studies that have precisely described patterns of avian flexibility in basal and maximal metabolic rates in response to thermal changes within a time period shorter than 3 weeks (see Rezende et al. 2004 and Zhu et al. 2010 for studies in mammals). For this experiment, our objectives were (1) to characterize the pattern of adjustment in metabolic performance following a rapid change in temperature and determine whether these patterns were consistent across species contrasted by their natural thermal environments, (2) to determine whether BMR,  $M_{\text{sum}}$  and MMR change at comparable rates during thermal acclimation and (3) to determine whether metabolic parameters vary at similar rates during exposure to warm or cold environments. We, therefore, measured these metabolic parameters, as well as components of body composition and food intake, in captive black-capped chickadees, white-throated sparrows (*Zonotrichia albicollis*), and snow buntings (*Plectrophenax nivalis*) kept for a minimum of 6 weeks at 10 °C (day 0) and then on the 4th and 8th days of thermal acclimation to constant temperature of either −5 or 28 °C.

## Materials and methods

### Experimental birds and acclimation protocol

For this study, we worked with three passerine species that live in contrasting thermal environment. Black-capped chickadees (10–14 g) are non-migratory birds mostly found in forested habitats of the United States and Canada. Chickadees must face highly seasonal environments throughout the year. For example our source population, in eastern Québec, Canada, experiences monthly mean temperatures varying between 16.8 °C ( $\pm 2.1$  °C) in July and −8.9 ( $\pm 4.1$  °C) in February (Environment Canada climate database). White-throated sparrows are associated with woodlands (Falls and Kopachena 2010) and migrate between their breeding grounds in southern Canada and their wintering habitat in southern USA. Therefore, although they can acclimate to cold ambient temperatures (Kontogiannis 1968; McWilliams and Karasov 2014; Metcalfe et al. 2013; Seibert 1949), these birds remain in relatively warm environments throughout the year [0–20 °C in winter (Odum 1949) to 5–21 °C in summer at our location (Environment Canada climate database)]. In contrast, the snow bunting is a circumpolar species that breeds in the Arctic tundra. In North America, these birds spend the cold season in open fields of southern Canada and northern USA. Therefore, except for a few weeks during the breeding season where

temperatures are above freezing (e.g., mean July temperature is  $6.0 \pm 3.7$  °C at our breeding study site at Alert, Nunavut Canada, Environment Canada climate database), this species remains in a cold and snowy environment year round (Montgomerie and Lyon 2011).

Snow buntings ( $n = 24$ ) were captured using baited walk-in traps in open fields, around Rimouski, Québec, Canada between February 24 and March 2, 2013. White-throated sparrows ( $n = 24$ ) were captured at the Forêt d'enseignement et de recherche Macpès, near Rimouski, by mist net as well as in private gardens using a walk-in trap between April 27 and May 7 2013. Black-capped chickadees were captured at the Forêt d'enseignement et de recherche Macpès using Potter traps (Mandin and Vézina 2012). A first group of 24 chickadees that was planned for this experiment was captured between December 2012 and May 2013. However, part of our captive population died before the experiment due to a bacterial infection and only 9 healthy birds could be measured. Another 16 chickadees were then captured in October and November 2013 for a second round of measures. The potential difference between groups was considered in statistical analyses but no significant differences were found. Therefore, data from all individuals were combined and are presented here ( $n = 25$ ).

All birds used in this experiment were housed at the avian facility of the Université du Québec à Rimouski, where the experiment took place. After capture, birds were kept at room temperature and constant photoperiod (12 L: 12 D) in individual cages (31 cm D  $\times$  40 cm H  $\times$  40 cm W) where they had access to water and food ad libitum. The experiment began by first maintaining the birds at 10 °C ( $\pm 2$  °C) for a minimum of 6 weeks, which gives time, based on previous work, for stabilizing the metabolic phenotype (Barcelo et al. 2009). Then each species experienced the same procedure. All birds were randomly assigned to either of two groups of 12 individuals, one to be acclimated to a “thermoneutral treatment” and the other to be acclimated to a “cold treatment” (final  $n$  for chickadees: 12 in the thermoneutral treatment and 13 in the cold treatment).

A day prior to the change in temperature (day 0), a first series of measurement (body mass, food intake, muscle thickness and fat reserves, BMR,  $M_{\text{sum}}$  and MMR) was obtained (see below). The next morning (at around 8:00), birds were transferred from the 10 °C room to a second identical room where temperature was set to either 28 °C ( $\pm 4$  °C) for the thermoneutral group (within the thermoneutral zone for all species Cooper and Swanson 1994; Kontogiannis 1968; Scholander et al. 1950) or −5 °C ( $\pm 2$  °C) for the cold group. The transfer took place in less than 20 min and birds were kept in this new thermal environment until the end of the experiment. We then proceeded

to repeat all measurements on the 4th and 8th days after the change in temperature for all birds. Our respirometry procedure limited the number of individuals that could be measured simultaneously to three birds per day. To ensure that all birds were measured at the same time after the change in temperature, we used an experimental sequence where three individuals of a single species were transferred to the experimental room every day until 12 birds had been moved (species were randomly picked each day). This allowed for monitoring each group of three birds at their 4th and 8th days of acclimation. Due to the overlapping schedule for these groups of birds, this experiment lasted 72 consecutive days.

### Food intake

We measured individual food intake on the day preceding metabolic measurements to evaluate raw energy input but also because an increase in daily food consumption could indirectly influence BMR through adjustments in gut size (Williams and Tieleman 2000). Snow buntings and white-throated sparrows (granivores) were fed with oil sunflower seeds and a commercial wild bird seed mix (Coopérative fédérée de Québec, Trois-Rivières, Canada) while black-capped chickadees (mainly insectivores) were fed with oil sunflower seeds and a commercial preparation of insect mash (Insect Patee, Orlux, Deinze, Belgium). A pre-weighted amount of food was offered in the morning (at around 8:00 h) in a 1.7 L glass receptacle placed on the cage floor to limit food spillage. The next morning, the remaining food was removed at the same hour before ad libitum access was resumed. This food was kept dry and weighed within the next day. As birds could have preferred sunflower seeds over other types of seeds (Devost et al. 2014), sunflower seeds were manually separated from the other seed types and weighed again.

### Body mass, fat scores, muscle scores and ultrasonography

On days 0, 4 and 8, we measured body mass (0.01 g) as well as the size of fat reserves and pectoral muscles of all birds. We estimated the size of fat reserves visually using standard fat scores (on a scale of 1 to 5), according to Gosler (1996). The size of pectoral muscles was then first estimated by visual examination using scores ranging from 0 to 4 according to Busse (2000). Eighty-nine percent of scores were obtained by the same observer and there were no observer effect on the measures (data not shown). Pectoral muscle thickness and cross-sectional area of thigh muscles were then determined non-invasively using ultrasonography (Dietz et al. 1999; Royer-Boutin et al. 2015; Swanson and Merkord 2012). This was done using a

LOGIQe ultrasound scanner fitted with a linear probe set at 12 MHz (GE Healthcare, Wauwatosa, WI, USA). We obtained three independent measurements of the left pectoral muscles and the right thigh muscles for each bird on each measurement day (mean repeatability of replicate measurements among species  $r = 0.825 - 0.899$ ). To avoid contaminating the birds' feathers with ultrasound transmission gel, the gel (Parker Aquasonic 100) was contained in a latex condom attached around the probe, which was dipped in lukewarm water before measurements. This arrangement created a cushion of gel on the probe tip, which covered the left pectoral muscles or the whole thigh during measurements. To expose the skin, we wetted the birds' feathers with water and pushed feathers aside. Pectoral muscle thickness was measured by placing the probe transversally on the breast at an angle of  $90^\circ$  to the keel, 1–2 mm from the upper keel tip. This formed a cross-sectional image on which the length between the base of the keel and the muscle surface could be measured with a  $45^\circ$  angle relative to the keel. The size of thigh muscles was obtained by fitting an ellipse measuring tool on images and by measuring the length of horizontal and vertical axes to calculate thigh cross-sectional area. Using an average of the two closest measures (data not shown) rather than the mean of the three replicates did not change our results and therefore means of the three measures are presented here. Ultrasound examinations lasted 8–12 min per bird.

### Respirometry

For each respirometry trial, we followed the same sequence. MMR was measured first in the morning starting at around 10:30 (1 h after ultrasound measurement), followed by a minimum of 2 h of rest before  $M_{\text{sum}}$  measurement. BMR was started at the end of the day (between 18:00 and 19:00) after a rest period ranging from 1 to 5.5 h (depending on duration of  $M_{\text{sum}}$ , see below).

#### Maximal Metabolic Rate

We measured MMR using a handmade PVC hop-flutter wheel (Chappell et al. 1999) with an effective volume of 2.97 L. Flow rates (averaging at  $2864 \text{ ml min}^{-1}$ ) were controlled by mass flow valves (Sierra Instruments, Side-Trak<sup>®</sup>, Monterey, CA, USA) previously calibrated for air with a bubble-O-meter (Dublin, OH, USA). Before a trial, the wheel was first flushed for at least 5 min with dry,  $\text{CO}_2$ -free air (Servomex gas purity analyzer, model 4100, Boston, MA, USA, set to 21.95 % oxygen). Birds were then weighed ( $\pm 0.01 \text{ g}$ ) and introduced into the wheel, which was covered with a sheet, where they were allowed to calm down for 10 min. The sheet was then removed and wheel rotation was initiated and accelerated every 5 min. During



MMR trials, all individuals were active, engaging in short jumps and fluttering. When birds showed signs of fatigue (reluctance to move, resting on the wheel floor) and/or  $O_2$  levels were stable for several minutes even if the bird kept exercising, the wheel was stopped. Trials lasted around 10–15 min. Birds were then given a few minutes of rest in the chamber and were weighed a second time. Reported body mass was calculated as the average between values measured before and after MMR measurement. During trials with snow buntings, four ping-pong balls were added into the chamber to stimulate the birds to move. This technique was not needed for the other two species.

#### *Summit metabolic rate*

$M_{\text{sum}}$  was measured using a sliding cold exposure protocol (Swanson et al. 1996), measuring three birds simultaneously. Birds were first weighed ( $\pm 0.01$  g) and placed in airtight stainless steel metabolic chambers (1.5 L for black-capped chickadees and 2.1 L for white-throated sparrows and snow buntings) fitted with perches and copper-constantan thermocouples connected to a Sable Systems TC-2000 thermocouple reader (Sable Systems, Las Vegas, NV, USA) for chamber temperature measurements. The chambers were placed inside a homemade temperature cabinet controlled with the EXPEDATA software (Sable Systems, Las Vegas, NV, USA). Birds were then exposed to air for a 10-min habituation period. Average flow rates and temperatures during this period were  $450 \text{ mL min}^{-1}$  and  $3 \text{ }^\circ\text{C}$  for black-capped chickadees,  $550 \text{ mL min}^{-1}$  and  $-6 \text{ }^\circ\text{C}$  for white-throated sparrows, and  $650 \text{ mL min}^{-1}$  and  $-15 \text{ }^\circ\text{C}$  for snow buntings. We then switched gas influx to helox (79 % helium and 21 % oxygen) and used respective flow rates of  $900 \text{ mL min}^{-1}$ , for chickadees and sparrows and  $1200 \text{ mL min}^{-1}$  for snow buntings (mass flow valves calibrated for helox with a bubble-O-meter). We measured a helox baseline value for approximately 3–5 min before recording the birds' oxygen consumption every 5 s until the end of trial. During this time, temperature was lowered by  $3 \text{ }^\circ\text{C}$  every 20 min until the birds entered hypothermia (i.e., oxygen consumption steadily declining for several minutes), or until birds reached the end of their species-specific program (145 min for chickadees, 125 min for sparrows, and 155 min for buntings). Birds were then immediately removed from chambers and, to confirm hypothermia, their body temperature was measured with a thermocouple reader (Omega model HH-25KC, NIST-traceable, Omega, Montréal, QC, Canada) using a copper-constantan thermocouple inserted into the cloacae approximately 10 mm deep. Following this procedure, we found that two chickadees, 6 sparrows and 13 buntings were not hypothermic at the end of the trial. However, hypothermia is not a necessary prerequisite to validate  $M_{\text{sum}}$ , as the maximal oxygen

consumption occurs earlier than the decline indicating hypothermia during cold exposure (Dutenhoffer and Swanson 1996). Removing non-hypothermic measures from analyses had no influence on final findings. These data were therefore kept in our datasets. As for MMR, birds were weighed again at the end of trials and average body mass was used for  $M_{\text{sum}}$  analyses.

#### *Basal metabolic rate*

Birds were placed into metabolic chambers for BMR measurements between 18:00 and 19:00, and taken out the next morning between 08:00 and 08:30. Metabolic chambers were put in a PELT-5 (Sable Systems, Las Vegas, NV, USA) temperature cabinet in which temperature was set at  $25 \text{ }^\circ\text{C}$  [within the thermoneutral zone for all species (Cooper and Swanson 1994; Kontogiannis 1968; Scholander et al. 1950)]. Birds received constant dry,  $\text{CO}_2$ -free air ( $450 \text{ mL min}^{-1}$ ,  $550 \text{ mL min}^{-1}$  and  $650 \text{ mL min}^{-1}$  for chickadees, sparrows and buntings, respectively) throughout the night. During recordings, the air sent to the analyzer alternated between reference air (10 min) and chambers' air (40 min) using a multiplexer (Sables Systems MUX, Sable Systems, Las Vegas, NV, USA). Body mass was measured before and after BMR trials and average values were used in analyses.

MMR,  $M_{\text{sum}}$ , and BMR calculations were, respectively, based on the highest averaged 1 min, highest averaged 10 min, and lowest averaged 10 min of oxygen consumption using Eq. 10.1 in Lighton (2008).  $M_{\text{sum}}$  and MMR were calculated using the instantaneous measurement technique (Bartholomew et al. 1981), whereas BMR was calculated with the steady state approach. Birds were fasted for an hour before BMR trials and the duration of trials insured a post-absorptive state at time of measurement (BMR attained after  $4:45 \text{ h} \pm 12 \text{ min}$  of measurement on average). Shivering and exercise in birds are mainly supported by lipid oxidation (Vaillancourt et al. 2005). We therefore estimated energy consumption for all trials using a constant equivalent of  $19.8 \text{ kJ L}^{-1}\text{O}_2$  and converted units to Watts (Gessaman and Nagy 1988).

Factorial aerobic scopes for exercise (MMR/BMR) measured with a hop-flutter wheel typically varies around 10 X BMR in birds (McKechnie and Swanson 2010) although lower values such as 6 to 7 X BMR have been observed for tropical birds (Wiersma et al. 2007) and juvenile house sparrows (Chappell et al. 1999). In our study, MMR exceeded BMR by 4.5- to 5.6-fold, which may be considered low relative to available observations (McKechnie and Swanson 2010). However, although there are no comparable data for black-capped chickadees and snow buntings, it should be noted that Price and Guglielmo (2009) obtained MMR values ranging between 8.5 and 10.5

mL<sub>O2</sub> g<sup>-1</sup> h<sup>-1</sup> in white-throated sparrows while our values in that species averaged 13.04 mL<sub>O2</sub> g<sup>-1</sup> h<sup>-1</sup>. Given this comparable set of values, and the fact that all birds showed signs of fatigue justifying the end of measures after sustained efforts, we are confident in the values we obtained.

### Molt in snow buntings

In this experiment, all birds were exposed to constant photoperiod. This, however, did not prevent 16 snow buntings from entering into some level of feather molt during the course of the experiment. Since feather molt may influence metabolic parameters such as BMR (Lindström et al. 1993; Vézina et al. 2009) and potentially influence heat loss in a cold environment (but see Vézina et al. 2009), we measured the intensity of molt by scoring wing and tail feather growth as well as body plumage growth and included these variables in our analyses. The ten primary feathers of both wings together with the rectrix feathers were each scored on a scale of 0 (new, very small pin) to 5 (full grown feather) for a maximum score of 150 for a non-molting individual. Body plumage was scored on a scale of 0 to 2, with 0 = old plumage, 1 = few pins visible and 2 = more than half of the body surface covered with pins.

### Statistical analysis

We used linear mixed models (with REML approach) to investigate the effects of day (0, 4 or 8), thermal treatment (cold and thermoneutral), and their interaction on body mass, food intake, muscle size, fat reserves and metabolic performance (BMR,  $M_{\text{sum}}$ , and MMR). All models controlled for repeated measures by including “bird ID” as a random variable. When studying effects on metabolic performance, we also controlled for the influence of body size by including a composite measurement of structural size as a covariate in models. We used body size rather than body mass in this study because metabolic variations in response to cold are likely to result in large part from body remodeling (Liknes and Swanson 2011a; Petit et al. 2014; Swanson and Vézina 2015; Zheng et al. 2008). Therefore, statistically removing the effect of body mass would eliminate most of the variation that we were interested in. Furthermore, in individuals containing large amounts of body fat, controlling for body mass can create underestimations of metabolic rate values due to the low metabolic activity of fat tissue (Petit et al. 2010; Scott and Evans 1992), and this could in turn generate biased treatment effects in the current study. Structural size was computed using a principal component analysis combining variations in beak, head, tarsus, wing and tail length for each species (feather measurements taken at time of capture). The first component was then extracted and used as a covariate in models

including metabolic parameters. We estimated the rate of change of each metabolic parameter by calculating the percent change at day 4 and day 8 relative to day 0 (set at 100 %) in each thermal treatment. In snow buntings, we considered the potential effect of feather molt on metabolic parameters by including molt scores (body plumage and wing plus tail feathers) as covariates in models. One individual was molting heavily compared to the rest of the group (wing and tail score of 56 vs. mean score of 152) and was removed from all analyses. Differences were then analyzed using post hoc Tukey tests.

We used two approaches to investigate relationships between variations in food intake and BMR and between muscle size and  $M_{\text{sum}}$  and MMR. Firstly, we included individual food intake variables as covariates in each species’ BMR models (one set of analyses per food intake variable). Similarly, we tested for effects of muscle size on MMR and  $M_{\text{sum}}$  by including ultrasound measures of either pectoral or thigh muscles as covariates in corresponding models. Secondly, we calculated the changes in food intake, muscle size, BMR,  $M_{\text{sum}}$  and MMR between day 0 and day 8. We then used least square linear regressions to test for relationships, across treatments, between the change in food intake and BMR as well as between the change in muscle size and  $M_{\text{sum}}$  and MMR.

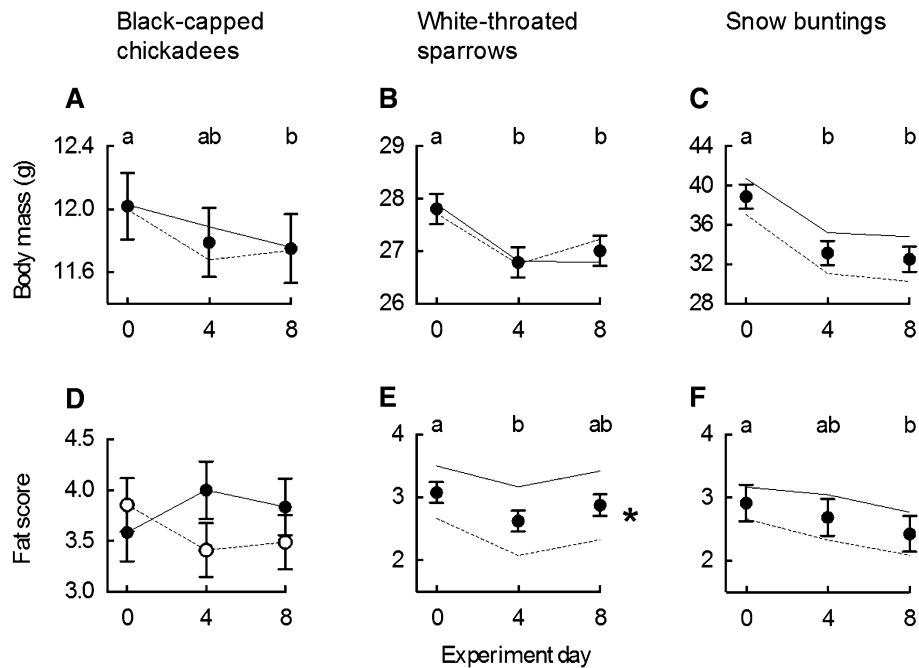
We visually confirmed normality of residuals for all models. All statistical analyses were performed with JMP v.10.0 and data are presented as mean  $\pm$  s.e.m.

## Results

### Body mass, muscle size and fat reserves

All species showed the same pattern of body mass variation. Body mass did not differ between thermal treatments (treatment:  $p > 0.8$  in sparrows and chickadees), although snow buntings tended to be 11.1 % lighter in the cold ( $p = 0.09$ ). However, body mass decreased significantly with time in all species (black-capped chickadees:  $F_{2,46} = 4.5$ ,  $p = 0.016$ ; white-throated sparrows:  $F_{2,44} = 9.6$ ,  $p < 0.0005$ ; snow buntings  $F_{2,38} = 34.9$ ,  $p < 0.0001$ ) (Fig. 1). The loss of mass in chickadees appeared relatively gradual, being significant only when comparing day 0 and day 8 (–2.2 %) (Fig. 1a). In the other two species, body mass declined within the first 4 days (white-throated sparrows: –2.9 %, snow buntings: –16.3 %) and remained stable afterwards, as it did not differ between day 4 and day 8 (Fig. 1b, c).

In black-capped chickadees, fat reserves changed over time in a way that differed between treatments (time  $\times$  treatment:  $F_{2,46} = 5.01$ ,  $p = 0.011$ ). Birds exposed to the cold lost body fat within the first 4 days while those kept at thermoneutrality appeared to increase the size of



**Fig. 1** Changes in body mass and fat reserves during thermal acclimation. Data are presented as least square means of body mass (a–c) and fat score (d–f) in black-capped chickadees, white-throated sparrows, and snow buntings measured on day 0 (before temperature change) and on days 4 and 8 of thermal acclimation. Temporal changes within temperature treatments are represented by dashed (cold,  $-5\text{ }^{\circ}\text{C}$ ) and solid (thermoneutral,  $28\text{ }^{\circ}\text{C}$ ) lines. In cases of sig-

nificant interaction treatment  $\times$  time, open circles represent birds from the cold treatment and filled circles represent birds from the thermoneutral treatment. Different letters above graphs indicate significant difference among days (filled circles) and a star on the side indicates a significant effect of temperature treatments. The interaction in (d) was significant but differences between means could not be detected by a Tukey test. See text for details

their fat reserves. However, the interacting effects of time and treatment were not strong enough to reach significance in the post hoc analysis (Fig. 1d). In sparrows, fat scores were higher in the thermoneutral group (mean score:  $3.36 \pm 0.21$ ) than in the cold group (mean score:  $2.36 \pm 0.21$ , treatment:  $F_{1,22} = 11.44$ ,  $p < 0.005$ ). However, in both groups there was a loss of fat reserves in the first 4 days, which was partly recovered by day 8 (time:  $F_{2,44} = 6.03$ ,  $p < 0.005$ , Fig. 1e). In snow buntings, fat reserves were not affected by thermal treatments (treatment:  $p = 0.3$ ) but declined over the 8 days of the experiment (time:  $F_{2,38} = 3.25$ ,  $p = 0.0498$ , Fig. 1f).

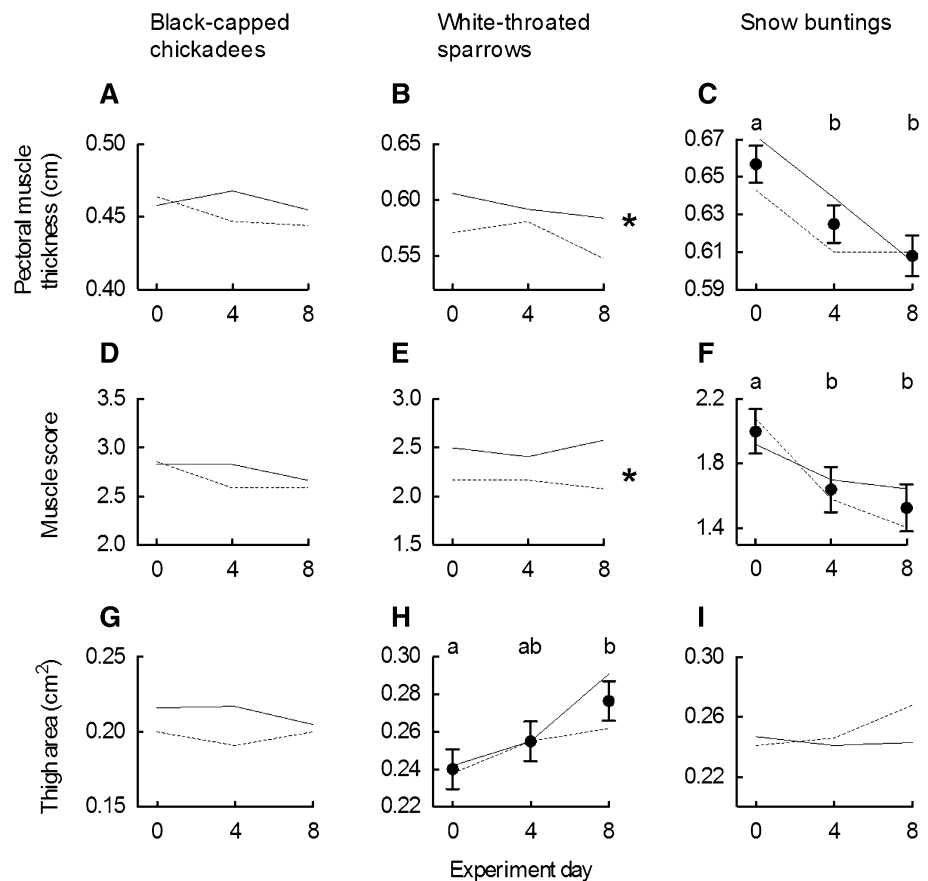
We found no time and treatment effects on muscle scores or on pectoral and thigh muscle size measured by ultrasound in black-capped chickadees ( $p > 0.09$  in all cases). In white-throated sparrows, birds from the thermoneutral group had 4.7 % thicker pectoral muscles on average ( $5.94 \pm 0.08\text{ mm}$ ) than those forming the cold group ( $5.67 \pm 0.08\text{ mm}$ , treatment:  $F_{1,22} = 5.66$ ,  $p = 0.027$ , Fig. 2b), a finding also apparent in muscle scores (treatment:  $F_{1,22} = 7.07$ ,  $p = 0.014$ , cold:  $2.14 \pm 0.1$ , thermoneutral:  $2.5 \pm 0.1$ , Fig. 2e). However, pectoral muscles did not change over time (time:  $p > 0.1$  in both cases) and the interaction was not significant (treatment  $\times$  time:  $p = 0.5$

in both cases). In contrast, sparrows of both thermal treatments grew their thigh muscles by 16.7 % over the 8 days of the experiment (time:  $F_{2,44} = 15.32$ ,  $p < 0.0001$ ; treatment:  $p = 0.6$ ). This effect appeared to differ between thermal treatments, with the greatest growth registered in birds kept at  $28\text{ }^{\circ}\text{C}$ , but the interaction was not significant (treatment  $\times$  time:  $p = 0.08$ , Fig. 2h). Snow buntings lost 7.6 % of their pectoral muscles over the 8 days as measured by ultrasound (time:  $F_{2,42} = 6.73$ ,  $p = 0.003$ , treatment:  $p = 0.2$ ), although this effect was significant only between day 0 and day 4 (6.0 % loss, Fig. 2c), and the same pattern was found for muscle scores (time:  $F_{2,38} = 8.46$ ,  $p < 0.001$ ; treatment:  $p = 0.8$ , Fig. 2f). However, we could not detect any changes in thigh muscle size in this species (time:  $p = 0.4$ ; treatment:  $p = 0.5$ ).

**Food intake**

Total food intake in chickadees was influenced by both time ( $F_{2,47} = 11.08$ ,  $p < 0.0005$ ) and treatment, being higher in the cold ( $141.2 \pm 6.1\text{ mg h}^{-1}$ ) than at thermoneutrality ( $91.3 \pm 6.6\text{ mg h}^{-1}$ ,  $F_{1,24} = 30.81$ ,  $p < 0.0001$ ). However, this was driven by the birds kept in the cold treatment. Indeed, within the first 4 days of being exposed to the

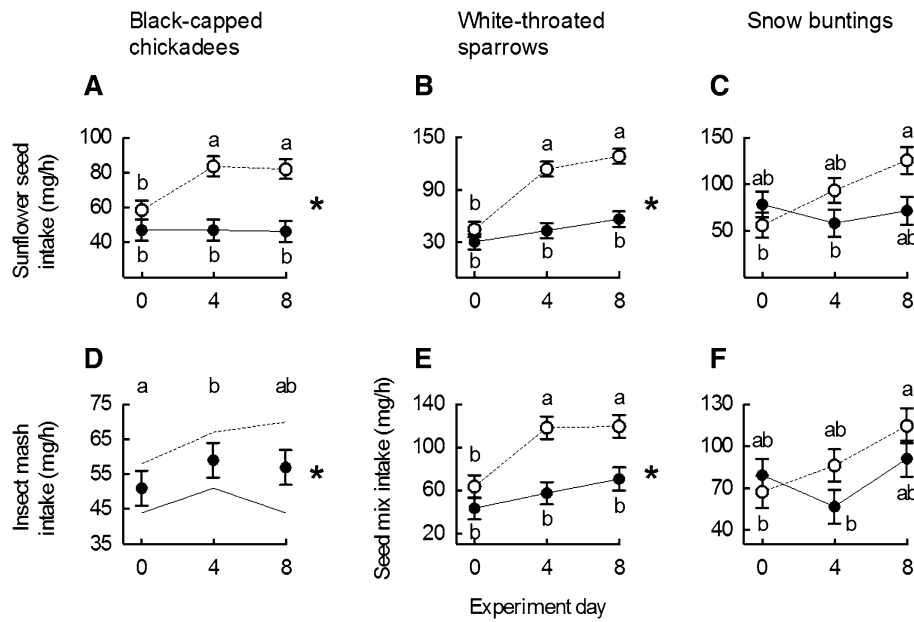
**Fig. 2** Changes in muscle size during thermal acclimation. Data are least square means of pectoral muscle thickness, pectoral muscle score and thigh muscle cross-sectional area in black-capped chickadees, white-throated sparrows and snow buntings. Data are presented for day 0 (before temperature change) and for day 4 and 8 of thermal acclimation. Temporal changes within temperature treatments are represented by dashed (cold,  $-5\text{ }^{\circ}\text{C}$ ) and solid (thermoneutral,  $28\text{ }^{\circ}\text{C}$ ) lines. Different letters above graphs indicate significant difference among days (filled circles shown only for significant effect) and a star on the side indicates a significant effect of temperature treatments



cold, these individuals increased their total food intake by 31.8 % while no significant changes were observed in those kept at thermoneutrality (treatment  $\times$  time:  $F_{2,47} = 8.97$ ,  $p < 0.001$ ). Sunflower seeds consumption followed the same pattern. Birds ate 42.6 % more sunflower seeds after 4 days when exposed to the cold while no changes were observed at thermoneutrality (Fig. 3a, treatment  $\times$  time:  $F_{2,43} = 5.06$ ,  $p = 0.011$ , time:  $F_{2,43} = 4.75$ ,  $p = 0.014$ , treatment:  $F_{1,21} = 18.83$ ,  $p < 0.005$ ,  $-5\text{ }^{\circ}\text{C}$ :  $74.8 \pm 4.4\text{ mg h}^{-1}$ ,  $28\text{ }^{\circ}\text{C}$ :  $46.9 \pm 4.7\text{ mg h}^{-1}$ ). Chickadees acclimating to both treatments also increased their consumption of insect mash in the first 4 days but showed a slight decrease by day 8 (time:  $F_{2,46} = 4.22$ ,  $p = 0.021$ , Fig. 3d). This, however, seemed mainly driven by birds kept at  $28\text{ }^{\circ}\text{C}$ , although the interaction was not quite significant ( $F_{2,46} = 2.69$ ,  $p = 0.079$ , Fig. 3d). On average, birds living in the cold also ate 41.3 % more insect mash ( $65.1 \pm 5.8\text{ mg h}^{-1}$ ) than those kept at thermoneutrality ( $46.2 \pm 6.2\text{ mg h}^{-1}$ , treatment:  $F_{1,24} = 4.96$ ,  $p = 0.036$ , Fig. 3d). White-throated sparrows showed a similar pattern. Birds exposed to the cold increased their total food consumption by 114.7 % within the first 4 days while those at thermoneutrality showed no significant changes, despite an increasing trend (treatment  $\times$  time:  $F_{2,44} = 9.69$ ,  $p < 0.0005$ , time:  $F_{2,44} = 30.47$ ,  $p < 0.0001$ , treatment:  $F_{1,22} = 29.67$ ,  $p < 0.0001$ ,  $-5\text{ }^{\circ}\text{C}$ :

$197.8 \pm 12.9\text{ mg h}^{-1}$ ,  $28\text{ }^{\circ}\text{C}$ :  $98.1 \pm 12.9\text{ mg h}^{-1}$ ). Likewise, sunflower seed consumption increased by 153.3 % between day 0 and day 4 in the cold group while the thermoneutral group tended to increase its consumption, albeit not significantly (Fig. 3b treatment  $\times$  time:  $F_{2,44} = 12.02$ ,  $p < 0.0001$ , time:  $F_{2,44} = 36.43$ ,  $p < 0.0001$ , treatment:  $F_{1,22} = 28.80$ ,  $p < 0.0001$ ,  $-5\text{ }^{\circ}\text{C}$ :  $96.0 \pm 6.9\text{ mg h}^{-1}$ ,  $28\text{ }^{\circ}\text{C}$ :  $43.9 \pm 6.9\text{ mg h}^{-1}$ ). Although the interaction did not reach significance (treatment  $\times$  time:  $F_{2,44} = 3.14$ ,  $p = 0.053$ ), the trend was similar for the mixed seeds intake. Post hoc Tukey test nevertheless showed that sparrows from the cold treatment increased their intake of mixed seeds by 84.4 % to reach a plateau in the first 4 days while birds at thermoneutrality showed a non-significant increase in seed consumption (Fig. 3e, time:  $F_{2,44} = 14.48$ ,  $p < 0.0001$ , treatment:  $F_{1,22} = 13.95$ ,  $p = 0.001$ ,  $-5\text{ }^{\circ}\text{C}$ :  $100.5 \pm 8.2\text{ mg h}^{-1}$ ,  $28\text{ }^{\circ}\text{C}$ :  $57.3 \pm 8.2\text{ mg h}^{-1}$ ). Snow buntings kept in the cold increased their total food intake throughout the experiment with no apparent plateau. On the 8th day, the birds were consuming 99.1 % more food than on day 0. This, however, was not apparent in birds kept at thermoneutrality (treatment  $\times$  time:  $F_{2,39} = 8.90$ ,  $p < 0.001$ , time:  $F_{2,39} = 7.78$ ,  $p = 0.001$ , treatment:  $p = 0.1$ ). The same pattern was visible for sunflower seed consumption, with birds kept in the cold eating 130.7 %





**Fig. 3** Changes in food intake during thermal acclimation. Data are least square means of sunflower seed intake (a–c), insect mash intake (d) and mixed seed intake (e and f) for black-capped chickadees, white-throated sparrows, and snow buntings. Data are presented for day 0 (before temperature change) and for day 4 and 8 of thermal acclimation. Temporal changes within temperature treatments are represented by dashed (cold, -5 °C) and solid (thermoneutral, 28 °C)

lines. In cases of significant interaction treatment × time, open circles represent birds from the cold treatment and filled circles represent birds from the thermoneutral treatment. Different letters above graphs indicate significant difference among days (filled circles) and a star on the side indicates a significant effect of temperature treatments. Different letters within graphs indicate significant differences among days and temperature treatments

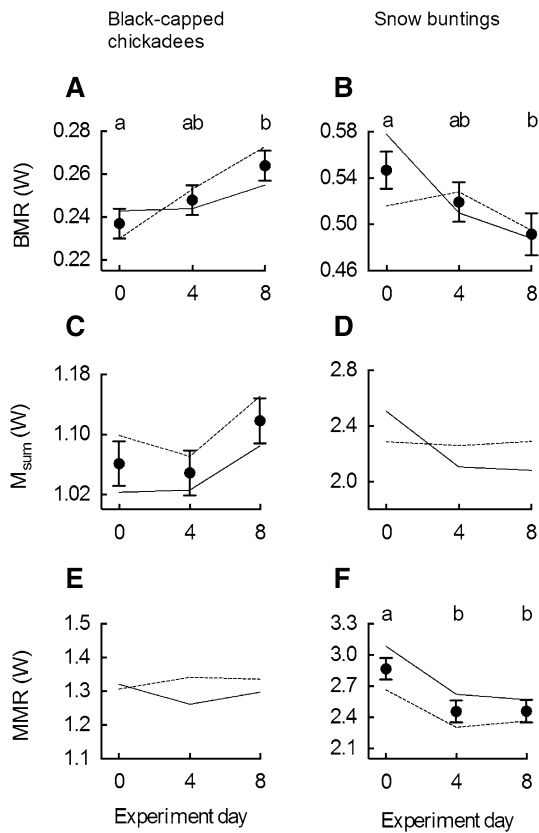
more on average on the 8th day compared to day 0 (Fig. 3c, treatment × time:  $F_{2,39} = 8.26, p = 0.001$ , time:  $F_{2,39} = 5.04, p = 0.011$ , treatment:  $p = 0.2$ ). This phenomenon was also found for consumption of mixed seeds. Although the interaction treatment × time was marginally significant ( $F_{2,40} = 3.14, p = 0.052$ ), post hoc Tukey test nevertheless revealed a clear increase (+69.8 %) in food consumption in birds acclimating to the cold between day 0 and day 8 while birds maintained at thermoneutrality showed no significant differences among days (Fig. 3f).

**Metabolic parameters**

Within the 8 days of the experiment, all species showed changes in parameters of metabolic performance but the patterns differed among species. In black-capped chickadees, body size was not related to any metabolic parameter (>0.6 in all cases). BMR was not affected by thermal treatments ( $p = 0.7$ ) but increased over time, reaching a level on day 8 that was 11.4 % higher than on day 0 (time:  $F_{2,44} = 5.97, p < 0.005$ , Fig. 4a).  $M_{sum}$  also appeared to change with time and independently from thermal treatment but the effect was not quite significant (time:  $F_{2,43} = 2.66, p = 0.08$ ; treatment  $p = 0.2$ ). However, taking the interaction term ( $p = 0.8$ ) and body size ( $p = 0.7$ ) out of the model showed a significant influence of

time on  $M_{sum}$  ( $F_{2,47} = 3.47, p = 0.04$ ). In this model,  $M_{sum}$  increased by 7.1 % between day 4 and day 8 but this effect was not strong enough for a post hoc Tukey test to find significant differences among days (Fig. 4c). MMR remained independent from thermal treatments and did not change significantly over time (time:  $p = 0.9$ ; treatment:  $p = 0.6$ ).

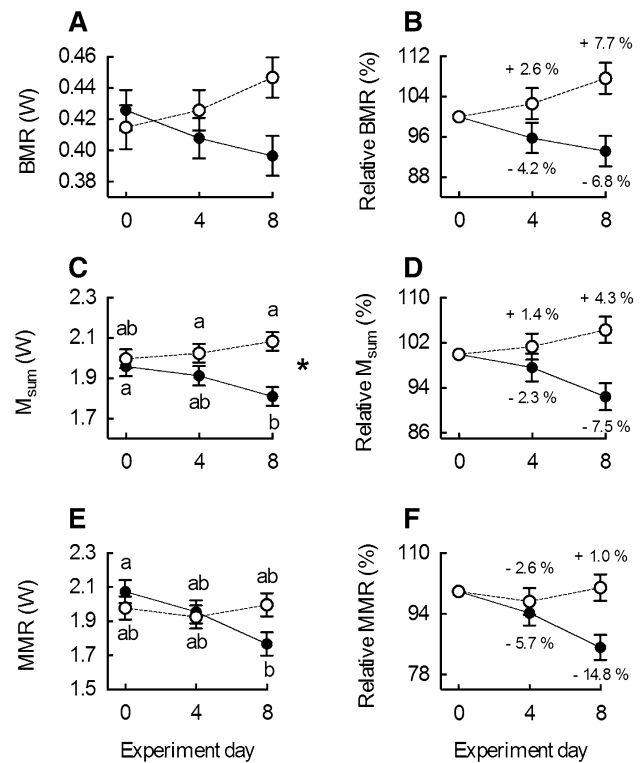
BMR decreased by 10.9 % in snow buntings between day 0 and day 8 (time:  $F_{2,32} = 4.06, p = 0.027$ , Fig. 4b) and this effect was independent from thermal treatments ( $p = 0.8$ ), body size ( $p = 0.6$ ), and molt (wing and tail:  $p = 0.7$ ; body:  $p = 0.8$ ). Part of this decline appeared stronger in birds acclimating to thermoneutrality (Fig. 4b) but the interaction term was not significant (treatment × time:  $F_{2,32} = 2.77, p = 0.077$ ). Time and thermal treatment did not influence size-corrected  $M_{sum}$  in buntings (size:  $F_{1,13} = 10.68, p = 0.006$ ; time:  $p = 0.1$ ; treatment:  $p = 0.6$ ), which was also not influenced by molt (wing and tail:  $p = 0.9$ ; body:  $p = 0.2$ ). In contrast, MMR decreased by 14.3 % in the first 4 days in birds from both treatments (time:  $F_{2,38} = 14.02, p < 0.0001$ ; treatment:  $p = 0.1$ ; size:  $p = 0.4$ , Fig. 4f). Molt also influenced MMR, with birds either not molting or molting only partially their wing and tail feathers showing higher MMR (wing and tail:  $F_{1,34} = 5.23, p = 0.029$ ; body:  $p = 0.4$ ), presumably because molting individuals were less active in the hop-flutter wheel than non-molting birds. Removing two individuals showing high molting activity



**Fig. 4** Changes in BMR,  $M_{\text{sum}}$  and MMR in black-capped chickadees and snow buntings during thermal acclimation. Data are least square means presented for day 0 (before the temperature change) and for day 4 and 8 of thermal acclimation. Temporal changes within temperature treatments are represented by dashed (cold,  $-5\text{ }^{\circ}\text{C}$ ) and solid (thermoneutral,  $28\text{ }^{\circ}\text{C}$ ) lines. Different letters above graphs indicate significant difference among days (filled circles shown only for significant effect). The time effect in (c) was significant but differences between means could not be detected by a Tukey test. See text for details

from the dataset made wing and tail molt non-significant ( $p = 0.06$ ) but did not change the effect of time on MMR ( $F_{2,37} = 13.17$ ,  $p < 0.0001$ ).

Body size did not influence metabolic parameters in sparrows ( $>0.1$  in all cases) but metabolic performance changed over time with patterns that depended on temperature. BMR varied in interaction with time and treatment ( $F_{2,41} = 7.24$ ,  $p < 0.001$ , Fig. 5a), increasing in the cold by 7.7 % over 8 days and decreasing by 6.8 % over the same period at thermoneutrality (Fig. 5b, time  $p = 0.8$ , treatment  $p = 0.3$ ). However, this effect was not strong enough for a post hoc Tukey test to detect significant differences between days and treatments.  $M_{\text{sum}}$  followed a similar pattern (treatment  $\times$  time:  $F_{2,42} = 8.18$ ,  $p = 0.001$ , Fig. 5c). Birds kept at thermoneutrality showed a significant 7.5 % decline in  $M_{\text{sum}}$  between day 0 and day 8 while individuals maintained at  $-5\text{ }^{\circ}\text{C}$  increased their thermogenic capacity



**Fig. 5** Changes in BMR,  $M_{\text{sum}}$ , and MMR in white-throated sparrows during thermal acclimation. Left panel show least square means of BMR,  $M_{\text{sum}}$  and MMR on day 0 (before the temperature change) and on day 4 and 8 of thermal acclimation. Right panels show the same data expressed as percent change relative to values measured on day 0 (100 %). Temporal changes are represented by dashed lines and open circles in cold ( $-5\text{ }^{\circ}\text{C}$ ) treatment and solid line and filled circles in thermoneutral ( $28\text{ }^{\circ}\text{C}$ ) treatment. Different letters indicate significant differences among days and temperature treatments. The interaction in (a) was significant but differences between means could not be detected by a Tukey test. See text for details

by 4.3 % over the same period (Fig. 5d), although this latter change was not significant (Fig. 5c). Thermal treatment on its own also influenced  $M_{\text{sum}}$  ( $F_{1,21} = 6.07$ ,  $p = 0.023$ ), with birds kept in the cold expressing a  $M_{\text{sum}}$  7.4 % higher ( $2.03 \pm 0.02\text{ W}$ ) on average than those experiencing thermoneutrality ( $1.89 \pm 0.04\text{ W}$ , time alone  $p = 0.6$ ). White-throated sparrows also showed a general decline in MMR over time (time:  $F_{2,44} = 3.50$ ,  $p = 0.039$ ) but this effect was due to birds from the thermoneutral treatment since the time effect depended on the treatment (treatment  $\times$  time:  $F_{2,44} = 4.95$ ,  $p = 0.012$ , treatment alone  $p = 0.7$ ). Indeed, birds maintained at  $28\text{ }^{\circ}\text{C}$  showed a 14.8 % decline in MMR between day 0 and day 8 while no significant changes were observed in birds kept at  $-5\text{ }^{\circ}\text{C}$  (1.0 % increase, Fig. 5e, f).

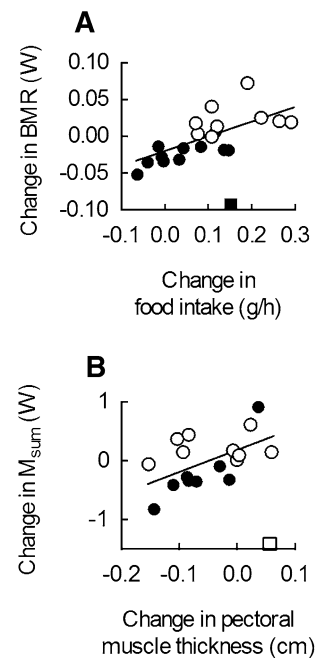
Neither total, sunflower seed, insect mash or seed mix intakes were related to BMR in black-capped chickadees and white-throated sparrows when considered as covariates ( $p > 0.3$  in all cases for both species). In snow buntings,

when considering the significant effect of time, sunflower seed intake was significantly and positively related to BMR ( $F_{1,53} = 4.28$ ,  $p = 0.04$ , time:  $F_{2,34} = 6.19$ ,  $p < 0.005$ ; total food intake  $p = 0.2$ , seed mix intake  $p = 0.9$ ). Pectoral and thigh muscle size were not significantly related to either  $M_{\text{sum}}$  or MMR in sparrows and buntings ( $p > 0.4$  in all cases) but the size of thigh muscles significantly and positively varied with  $M_{\text{sum}}$  and MMR in black-capped chickadees ( $M_{\text{sum}}$ :  $F_{1,44} = 6.21$ ,  $p = 0.02$ , MMR:  $F_{1,43} = 5.41$ ,  $p = 0.02$ ). MMR also tended to vary positively with pectoral muscle thickness ( $F_{1,61} = 2.92$ ,  $p = 0.092$ ), and this effect was significant when removing the effect of structural body size ( $p = 0.9$ ) from the model ( $F_{1,65} = 4.12$ ,  $p = 0.046$ ), but this trend was not found for  $M_{\text{sum}}$  ( $p = 0.3$ ).

Using least square regressions to investigate relationships between changes in parameters from day 0 to day 8, food intake and BMR were not related in black-capped chickadees and snow buntings ( $p > 0.1$  in all cases) but white-throated sparrows showed a positive relationship between the change in all food intake variables and the change in BMR (total food intake:  $r^2 = 0.27$ ,  $n = 21$ ,  $p = 0.017$ ; sunflower seed intake:  $r^2 = 0.22$ ,  $n = 21$ ,  $p = 0.033$ ; seed mix intake:  $r^2 = 0.22$ ,  $n = 21$ ,  $p = 0.031$ ). One bird from the thermoneutral treatment appeared as an outlier, showing a relatively large increase in food intake associated with a decrease in BMR. Removing this individual from analyses substantially increased the fit of all relationships (total food intake:  $r^2 = 0.51$ ,  $n = 20$ ,  $p = 0.004$ ; sunflower seed intake:  $r^2 = 0.43$ ,  $n = 20$ ,  $p = 0.002$ ; seed mix intake:  $r^2 = 0.46$ ,  $n = 20$ ,  $p = 0.001$ , Fig. 6a). Investigating relationships between changes in muscle size and maximal metabolic rates, regressions were not significant for any species when considering thigh muscles ( $p > 0.2$  in all cases). When considering pectoral muscles, we also found no relationships ( $p > 0.1$  in all cases) but, here again, one individual snow bunting appeared as an outlier, expressing one of the largest increases in muscle size and the largest decline in  $M_{\text{sum}}$ . Removing this individual from the analysis revealed a positive relationship between the change in pectoral muscles and the change in  $M_{\text{sum}}$  ( $r^2 = 0.29$ ,  $n = 17$ ,  $p = 0.026$ , Fig. 6b).

## Discussion

Using three passerine species differing in their natural thermal environments, our aims in this study were (1) to determine the pattern of variation in metabolic performance after a rapid change in ambient temperature, (2) to investigate whether components of metabolic performance vary at comparable rates during thermal acclimation, and (3) to determine whether rates of change in metabolic performance differ during exposure to warm and cold



**Fig. 6** Influence of food consumption and muscle size on parameters of metabolic performance during thermal acclimation. The relationship between the change in total food intake and the change in BMR in white-throated sparrows is shown in (a) and the relationship between the change in pectoral muscle thickness and the change  $M_{\text{sum}}$  in snow buntings is shown in (b). Changes are calculated as the differences between values measured on day 8 of thermal acclimation and on day 0, before the temperature change. *Open circles* represent birds from the cold treatment ( $-5\text{ }^{\circ}\text{C}$ ) and *filled circles* represent birds from the thermoneutral treatment ( $28\text{ }^{\circ}\text{C}$ ). *Open and filled squares* show outliers accordingly. Regression lines represent analyses excluding outliers

environments. Our results showed significant phenotypic adjustments in the first 8 days following a  $15\text{--}18\text{ }^{\circ}\text{C}$  temperature change in all species. They also showed that these adjustments differed among species.

## Energy balance

Regardless of thermal treatment, all species lost body mass in the first 4 days of the experiment to reach stable mass between day 4 and day 8. This was paralleled by a decline in fat score in the first 4 days in cold acclimating chickadees and in sparrows of both treatments, while all buntings lost fat reserves throughout the experiment. We could not detect significant changes through time in muscle size of chickadees measured by muscle scores or by ultrasound (but see Royer-Boutin et al. (2015) for a discussion on the limit of precision in measurement by ultrasound in this species). Similarly, white-throated sparrows kept the size of their pectoral muscles relatively constant throughout the experiment. In contrast, snow buntings acclimating to both thermoneutrality and cold showed a continuous decline in

pectoral muscle score and thickness. Therefore, despite a 32–115 % increase in total food consumption in birds acclimating to cold, it appears that individuals from all species, whether they were facing cold or thermoneutral temperatures, were in negative energy balance in the first half of the experiment.

The loss of mass and fat reserves in birds acclimating to cold is not surprising given the added thermoregulation cost. Working with white-throated sparrows, McWilliams and Karasov (2014) showed that birds undergo negative energy balance when a decline in ambient temperature requires an increase in energy intake above the immediate spare capacity provided by the size of their digestive organs (McWilliams and Karasov 2014). Therefore, although all cold acclimating birds increased their food consumption, energy expenses likely outweighed the income in the first half of the experiment, leading to a loss of mass, fat reserves and, in snow buntings, a loss of muscles. However, the finding of a negative energy balance in birds being alleviated from thermoregulatory requirements came as a surprise given that these individuals did not change their daily food consumption. Birds wintering in cold environments typically maintain a heavier body mass, larger fat reserves and pectoral muscles than during summer (Blem 1976; Liknes and Swanson 2011a; Petit et al. 2014). It is, therefore, expected that individuals undergoing a reduction of thermostatic costs would decrease the size of these body components to reduce locomotion costs and maintain maneuverability (Dietz et al. 2007; Krams 2002; Kullberg et al. 1996; Lind et al. 1999). The mechanism by which this change may have happened in captive birds maintaining constant energy intake is not clear, however, and will require further investigation.

White-throated sparrows were the only species showing significant positive changes in body components, increasing the size of their thigh muscle in both cold and thermoneutral treatments. We believe that this unexpected muscle growth could result from a training effect caused by the MMR trials. We noticed that sparrows hopped to a greater extent than the other species during trials (pers. obs.). Chickadees tended to spend more time fluttering in the center of the wheel while snow buntings were much less active and needed a motivation measure (ping-pong balls) to stimulate locomotion. Therefore, it is possible that the increase in thigh muscle size recorded in sparrows has been provoked by the MMR trials, a finding consistent with the recently reported positive effect of 45 min daily flight training on pectoral muscles thickness in house sparrows (*Passer domesticus*) (Zhang et al. 2015a).

### Patterns of variation in metabolic performance across species

Because seasonal acclimatization to winter at northern latitudes typically leads to elevated metabolic performance (Marsh and Dawson 1989; Swanson 2010; Swanson and Vézina 2015), we expected an increase in BMR,  $M_{\text{sum}}$  and MMR in birds exposed to the cold and a reverse pattern in birds acclimating to thermoneutrality. Although white-throated sparrows did show the expected pattern, black-capped chickadees and snow buntings, not only showed variations in metabolic rate that were inconsistent with our predictions, but they also showed completely opposite patterns (Fig. 4). Therefore, our observations suggest that species differing in life histories and natural thermal environments may show very different responses to a given change in ambient temperature.

Chickadees acclimating to both cold and thermoneutrality showed the same response, a 12 % increase in BMR over 8 days, a moderate, but non-significant, 7 % increase in  $M_{\text{sum}}$  between day 4 and day 8 and no changes in MMR over time. The causes for these changes in chickadees are not obvious. In birds, BMR variations are expected to reflect changes in size and activity of internal organs (Barcelo et al. 2009; Cavieres and Sabat 2008; Kersten and Piersma 1986; Maldonado et al. 2008; McKechnie 2008; Petit et al. 2014; Piersma et al. 1996) and, in birds acclimating to cold, organs associated with energy acquisition are thought to play an important role in BMR changes (Liu and Li 2006; Sabat et al. 2009; Tieleman et al. 2003; Williams and Tieleman 2000; Zheng et al. 2008). However, the lack of relationship between daily food intake and BMR in chickadees suggests that the adjustments in digestive organs that may have occurred over the 8 days of acclimation had little influence on maintenance energy expenditure. Using body composition data from dissections, Petit et al. (2014) also found little support for the influence of digestive organs on BMR in this species. The reasons for the increase in BMR observed here therefore remain unexplained. Variation in  $M_{\text{sum}}$  and MMR is expected to relate positively with muscle size (Chappell et al. 1999; Petit and Vézina 2013; Swanson et al. 2013; Swanson and Vézina 2015; Hammond et al. 2000) but we could not detect changes over time in pectoral or thigh muscle size in chickadees using either muscles scores or ultrasonography. However, our finding of a significant influence of thigh muscles on  $M_{\text{sum}}$  and MMR and nearly significant effect of pectoral muscles on MMR does bring some support to the role of muscles size in driving maximal metabolic rates (Petit et al. 2014; Swanson et al. 2013, 2014b; Swanson and Vézina 2015).

In snow buntings, irrespective of thermal treatment, BMR declined by 11 % and MMR decreased by 14 % between day 0 and day 8. Of the three species, buntings experienced the greatest relative loss of mass (16 %). They also lost 8 % of their pectoral muscles based on thickness measurements. Assuming that the loss of muscles reflects an overall decrease in lean mass, negative changes in lean body component presumably drove the observed decline in BMR (Scott and Evans 1992). However, in contrast with observations in chickadees, we found that food consumption was positively correlated with BMR in this species. Therefore, data for snow buntings are consistent with the expected influence of digestive organs on BMR in cold acclimated birds (Liu and Li 2006; Sabat et al. 2009; Williams and Tieleman 2000; Zheng et al. 2008) and the contrast with results in chickadees suggests that this relationship may not be common to all species. Given that both  $M_{\text{sum}}$  and MMR reflect active aerobic metabolism of skeletal muscles (Chappell et al. 1999; Hammond et al. 2000; Petit and Vézina 2013; Swanson et al. 2013), the observed decrease in muscle size could have driven the changes in maximal metabolic performance. We could not detect an effect of time on  $M_{\text{sum}}$  per se, but the positive relationship across treatments between the change in  $M_{\text{sum}}$  and the change in pectoral muscle thickness between day 0 and day 8 nevertheless supports this hypothesis.

Of the three species, white-throated sparrows were the only birds showing the expected pattern of variation in metabolic parameters. BMR was 8 % higher after 8 days of cold acclimation while the opposite was found at thermoneutrality, with birds exhibiting a 7 % loss.  $M_{\text{sum}}$  and MMR also changed in these birds. However, the only clear effect in this case was observed at thermoneutrality, where birds lost up to 15 % of their maximal metabolic capacity over the course of the experiment. Therefore, although changes over 8 days were moderate relative to seasonal variations observed in other species (e.g., 5–50 % for BMR and  $M_{\text{sum}}$ , Cooper and Swanson 1994; Liknes and Swanson 1996; Liknes et al. 2002; Petit et al. 2013), these findings demonstrate that metabolic parameters associated with thermal acclimation can respond within days to changes in ambient temperature.

As for snow buntings, we also found evidence that daily food consumption, and presumably physiological adjustments for digestion (Liu and Li 2006; Sabat et al. 2009; McWilliams and Karasov 2014; Williams and Tieleman 2000; Zheng et al. 2008), had an influence on BMR in sparrows (Fig. 6a). However, although  $M_{\text{sum}}$  seemingly began to increase in birds acclimating to cold (non-significant 4 % increase), muscle thickness did not vary significantly over time. In fact, birds from the cold group had smaller pectoral muscles on average than individuals from the thermoneutral group (Fig. 2b, e). Furthermore, in contrast with observations in snow buntings, birds kept at 28 °C showed a significant decrease of both  $M_{\text{sum}}$  and MMR despite

the lack of change in muscle size. These findings therefore suggest that variations in  $M_{\text{sum}}$  and MMR were not the sole result of muscle size variations in white-throated sparrows. In accordance with this reasoning, Barcelo et al. (unpublished) found that this species can increase its  $M_{\text{sum}}$  by as much as 18 % when acclimated to a temperature of –8 °C for 30 days without changing the size of its pectoral muscle. Studies have demonstrated that cellular metabolic activity can parallel winter increments of thermogenic capacity and be directly correlated to  $M_{\text{sum}}$  (Marsh and Dawson 1989; Swanson 2010), although that can differ among species (Liknes and Swanson 2011b; Pena-Villalobos et al. 2014; Swanson et al. 2013, 2014a, b; Zhang et al. 2015b). Consequently, if an increase of skeletal muscle mass may improve maximal metabolic rate in birds (Swanson and Vézina 2015), physiological systems underlying changes in  $M_{\text{sum}}$  and MMR could vary among species. In white-throated sparrows, a loss of metabolic capacity at the cell level in response to thermoneutral conditions could perhaps result in a decrease in whole animal performance before one can detect notable changes in muscle size.

### Rate of change in BMR, $M_{\text{sum}}$ and MMR

Considering percent changes relative to day 0, our data for white-throated sparrows acclimating to cold support the prediction that BMR responds faster to a change in ambient temperature than maximal metabolic rate measured as  $M_{\text{sum}}$  or MMR. Indeed, over a period of 8 days, birds acclimating to –5 °C showed a significant 8 % increase in BMR but no change in MMR (<1 %) and a 4 % gain of  $M_{\text{sum}}$  that was not significant when considered within treatment (Fig. 5). Although metabolic performance in chickadees did not respond to thermal treatment, our data are nevertheless also consistent with the prediction. Indeed, over the course of the experiment, we observed a significant 12 % increase in BMR, a non-significant elevation (7 %) of  $M_{\text{sum}}$  and no changes in MMR. In fact, the increase in BMR found in chickadees was greater than the interseasonal mass-corrected variation observed in our source population (6 %) (Petit et al. 2013). It was also comparable to the seasonal variation observed by others in whole BMR in chickadees (13–18 % in South Dakota, Cooper and Swanson 1994). Therefore, independent of temperature, chickadees showed as much amplitude of variation in their BMR within a week than within a year, while short-term variation in maximal metabolic rates was much more limited (7 % increase in  $M_{\text{sum}}$  relative to 32 % in our source population, Petit et al. 2013). Taken together, these results support the hypothesis that BMR can change quite rapidly and faster than maximal metabolic rate, possibly as a result of intrinsic limitations in the rate of change



in organ size and function (Bauchinger and McWilliams 2009, 2010; Petit et al. 2013; Swanson et al. 2012; Vézina et al. 2011). Consequently, it appears that 8 days may not be sufficient for some species facing a sudden cold spell to upregulate their maximal metabolic performance, a finding consistent with the 24–30 days required for an  $M_{\text{sum}}$  response to temperature in free-living black-capped chickadees and dark-eyed juncos (Swanson and Olmstead 1999). Birds could instead have to rely on developing thermogenic capacity ahead of the peak of winter cold (Petit et al. 2013) and on maintaining a spare capacity in prevision of cold spells (McWilliams and Karasov 2014; Petit and Vézina 2014).

Tissue turnover rate is assumed to explain anabolic and catabolic processes involved in organ remodeling, meaning that fast growing organs are also expected to be fast regressing organs (Bauchinger and McWilliams 2009). Alone, the rate of change in organ size therefore does not easily explain why, in buntings and sparrows maintained at thermoneutrality,  $M_{\text{sum}}$  and MMR, which are thought to reflect organs changing slowly (Bauchinger and McWilliams 2009, 2010; Petit and Vézina 2013; Swanson and Vézina 2015; Swanson et al. 2013), declined at rates comparable to or faster than that of BMR, which should reflect fast changing organs (Bauchinger and McWilliams 2009, 2010; Liu and Li 2006; Sabat et al. 2009; Williams and Tieleman 2000; Zheng et al. 2008). However, we found evidence that BMR variation might have been influenced by digestive functions in both these species. Individuals kept at thermoneutrality also tended to maintain their food consumption constant over time. It is therefore possible that body components may not all have changed at the same rate or in the same direction in response to thermoneutrality. As BMR variations should be influenced by total lean body mass and internal organs, while  $M_{\text{sum}}$  and MMR should vary with muscle size (Marsh and Dawson 1989; Petit and Vézina 2013; Swanson et al. 2013, 2014b; Vézina et al. 2006, 2007; Williams and Tieleman 2000), at least in snow buntings, in absence of the need for active thermoregulation, muscle maximal performance could thus have declined faster than the overall resting energy consumption. An alternative but non-exclusive hypothesis is that maximal metabolic rate could also be influenced to a greater extent by variations in cellular metabolic pathways than basal metabolic rate. For example, a down-regulation in the activity of specific enzymes, either by changes in protein levels or allosteric regulation, would be expected to have a greater effect on  $M_{\text{sum}}$  and MMR, where enzymes are operating closer to their maximal capacities, than on BMR, where enzymes should operate at much lower levels of activity (Swanson 2010; Vézina and Williams 2005).

### Rate of change in metabolic parameters in warming and cooling environments

Barcelo et al. (2009) studied BMR variations in captive rufous-collared sparrows during acclimation to either cold or warm temperatures. They found that, compared to initial values measured at 22 °C, birds acclimating to 15 °C had increased their BMR by 29 % over 4 weeks, while those experiencing a temperature of 30 °C had decreased their BMR by only 13 % over the same time period. Similarly, using three acclimation temperatures (10, 22, and 35 °C), McKechnie et al. (2007) found in laughing doves (*Streptopelia senegalensis*), not only the expected negative relationship between BMR and air temperature, but also that for a given amount of time spent at these temperatures (21 days), the magnitude of change in BMR was greater when the birds faced colder than warmer temperatures (see Fig. 5 in McKechnie et al. 2007). These studies therefore suggest that birds adjust their BMR faster when experiencing a cooling rather than a warming environment.

The only species for which we could compare the rate of change in metabolic parameters in relation to acclimation temperature is the white-throated sparrow. In contrast with findings by Barcelo et al. (2009) and McKechnie et al. (2007), BMR varied at comparable rates between treatments (+8 % in the cold and −7 % at thermoneutrality over 8 days, Fig. 5b). One important difference between our study and those cited above, however, is the time span between measurements. Barcelo et al. (2009) and McKechnie et al. (2007) acclimated birds for periods ranging from 21 days to 4 weeks before re-measuring individual BMR, while our measures were spaced in time by 4 days. It is not possible to determine whether BMR had already attained stability at the time of second measure in these previous studies. However, considering the rates of change documented here, it is reasonable to assume that BMR could have progressed at comparable rates in both thermal treatments, therefore reaching stability over a longer time period in birds exposed to cold temperatures relative to birds exposed to a warm environment. Additional research is required to determine whether this pattern is generalizable to other species.

As far as we know, this is the first study to document the rate of change in  $M_{\text{sum}}$  and MMR over less than 8 days in response to temperature in birds. Maximal metabolic capacity was lost by as much as 15 % over 8 days in white-throated sparrows kept at thermoneutrality, while the cold treatment induced, at best, a moderate and non-significant increase in metabolic performance (1–4 %). Therefore, it appears that maximal metabolic capacity is easier to lose than to build in this species. A possible explanation for this phenomenon could be that acclimating birds to 10 °C prior to the change in temperature

already provided enough thermogenic capacity for sparrows to live at  $-10\text{ }^{\circ}\text{C}$ , thus requiring little change in  $M_{\text{sum}}$  in the cold treatment. However, this is clearly not the case since another study on this species conducted in the same facilities showed that  $M_{\text{sum}}$  had stabilized at  $2.56 \pm 0.06\text{ W}$  after 30 days of acclimation to  $-8\text{ }^{\circ}\text{C}$  (Barcelo et al. unpublished). This temperature is warmer than the cold treatment used here but the  $M_{\text{sum}}$  value obtained by Barcelo et al. is nevertheless 23 % higher than what we recorded in the current study after 8 days in the cold ( $2.08 \pm 0.05\text{ W}$ ). The exact causes for the different rates of change in cold and warm conditions are therefore not obvious and will require more investigations, especially if changes happen independently from muscle mass variations (Barcelo et al. unpublished). In a context where temperature stochasticity is expected to increase at northern latitudes (Easterling et al. 2000; IPCC 2013; Katz et al. 2005), a rapid decline of thermogenic capacity during warm winter days could be detrimental if birds are slow to readjust their phenotype with the return of cold. This therefore emphasizes the need for more data on physiological responses to short-term temperature variations in birds.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All bird manipulations were approved by the Université du Québec à Rimouski animal care committee (CPA-52-13-113) and have been conducted under scientific (SC-47) and banding (10704D) permits from Environment Canada - Canadian Wildlife Service.

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