Title page

Thermal acclimation in a non-migratory songbird occurs via changes to thermogenic capacity, but not conductance

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Abstract

Thermoregulatory performance can be modified through changes in various subordinate traits, but the rate and magnitude of change in these traits is poorly understood. We investigated flexibility in traits that affect thermal balance between black-capped chickadees (*Poecile* *atricapillus*) acclimated for six weeks to cold (-5℃) or control (23℃) environments (n = 7 per treatment). We made repeated measurements of basal and summit metabolic rates via flow-through respirometry and of body composition using quantitative magnetic resonance of live birds. At the end of the acclimation, we measured thermal conductance of the combined feathers and skins. Cold-acclimated birds had a higher summit metabolic rate, reflecting a greater capacity for endogenous heat generation, and an increased lean mass. However, birds did not alter their thermal conductance. These results suggest that chickadees respond to cold stress by increasing their capacity for heat production rather than increasing heat retention, an energetically expensive strategy.

Introduction

During winter, reductions in ambient temperature present energetic challenges to many animals. Animals exposed to seasonal cold temperatures may migrate to avoid them but if they do not, they must stay and acclimatize. For the latter, animals must endure decreased temperatures that often coincide with less daylight for foraging. For small non-hibernating endotherms, thermoregulatory performance can be a primary predictor of survival in cold environments (Fontanillas et al., 2005; Hayes and O'Connor, 1999; Lustick and Adams, 1977; Petit et al., 2017).

For the most part, birds that overwinter in cold environments do not hibernate. Seasonal acclimatization allows individuals to adjust to changes in temperature through physiological modifications that alter heat generation and/or retention. Mechanisms for increasing thermogenesis include muscle hypertrophy, greater density of mitochondria, and/or changes to efficiency/intensity of mitochondrial activity (reviewed in Swanson, 2010). Additionally, birds can change thermal conductance primarily through feather growth and, to a lesser extent, through changing conductive properties of the skin to diminish heat loss (Wolf and Walsberg, 2000). The relative importance of these two thermoregulatory strategies (heat generation vs. retention) is not well understood, but is key to understanding how non-migratory birds survive harsh winters.

While a large body of work has advanced our understanding of how birds acclimatize to increase summit metabolic rate (M_{sum}, representing total thermogenic capacity and measured as a cold-induced VO_{2max}), and the subordinate traits that support it (e.g., Barceló et al., 2017; Dubois et al., 2016; Petit et al., 2014; Swanson, 1991; Swanson, 1993; Swanson and Liknes, 2006), fewer studies have focused on the relative contributions of heat generation versus heat conservation mechanisms in that process. Recent research has demonstrated that dark-eyed juncos (*Junco hyemalis*) adjust their ability to thermoregulate over the course of a 9-week cold exposure via both increased metabolic heat production and decreased thermal conductance, but these modifications occur over different timescales (Stager et al., 2020). This result suggests that juncos may meet specific thermal stresses of their environment by combining multiple responses, but the generality of this finding is unknown.

We investigated patterns of cold acclimation in the black-capped chickadee (*Poecile atricapillus*), a small (9-14 g) passerine that is a year-round resident in temperate North America. In free-living black-capped chickadees, elevated thermogenic capacities are associated with enhanced over-winter survival probabilities (Petit et al., 2017). A previous acclimation experiment of black-capped chickadees from Quebec, Canada, demonstrated that cold acclimation increased basal metabolic rate (BMR), M_{sum}, and aerobic capacity of pectoralis muscle and liver tissues over a period of 28 days (4 weeks) (Milbergue et al., 2022). It remains unknown, whether other traits that can influence thermoregulatory ability are affected, and what the time series is of acclimation in those and metabolic traits. While increases in capacity for heat generation are well-established, they represent only part of the strategies available for maintaining thermal balance. We hypothesized that birds acclimated to cold temperatures would demonstrate heightened thermoregulatory abilities via higher M_{sum}, higher heat retention, and increased oxygen-carrying capacity as measured through hematocrit, hemoglobin, and heart mass.

Materials and Methods

Collection and care of chickadees

We live-trapped 16 individuals from Boulder, CO, USA (40.0150° N, 105.2705° W; Colorado Parks and Wildlife Permit 1709964528, USFWS Permit MB06336A-1), using basket traps at feeders, on August 16, 2020, then drove them to Missoula, MT, USA, over 13 hours. All individuals were kept individually housed for both transport and the duration of the experiment. Upon initial arrival to Missoula, we inspected each bird for disease, assigned a sample identifier, and transferred it to a cage (76 cm x 45 cm x 45 cm). Birds had *ad libitum* access to food and water. Every day, individuals received a mix of sunflower seeds, ~5-10 live mealworms, and ~5-10 pine nuts, and every other day, ~1 tsp of insect pattee (Orlux, Belgium) and ~1 tsp of ground high-protein dog food. We supplemented water with vitamin drops (Wild Harvest, Blacksburg, VA, USA). For three weeks after arrival (August 17 to September 9, 2020), birds adjusted to captive conditions (23^{o} C) and we aligned birds to a 12H:12H day:night schedule (05:00 on, 17:00 off) incrementally over that period. Birds were not observed to have undergone a molt while in captivity. All birds were maintained at this 12H:12H light:dark schedule for the remainder of the experiment. Two birds died during the first week of captivity. Sex was determined by identification of gonads after euthanasia (8 females, 6 males). All animal care was approved by the University of Montana IACUC (AUP 043-19), and experimental animals were regularly inspected by ourselves and veterinary staff.

Acclimation trials and metabolic assays

After the initial period of adjustment to captive conditions, we randomly assigned individuals to one of two 6-week acclimation treatments of either control (23℃; n=7 birds) or cold (-5℃; n=7 birds) temperatures. We measured birds at week 0 (prior to placement in experimental treatment), week 3, and week 6 of the acclimation, as follows. To measure basal metabolic rate (BMR), we removed food from cages at least 2 hours prior (between the hours of 14:00 and 15:00 h) to ensure birds were postabsorptive prior to measurement (as in Olson et al., 2010), and used a Sable Systems flow-through respirometry system. BMR trials began at approximately 17:00 h. Immediately prior to the BMR measurement, we weighed each bird and placed it in a 1L volume Nalgene bottle chamber outfitted with input and output connection for air flow into and out of the chamber, and the bird was placed inside a dark temperature-controlled chamber (Sable Systems Pelt Cabinet with Pelt-5 Temperature Controller, North Las Vegas, NV, USA) held at ~32℃. This temperature is within the thermoneutral zone of chickadees (Olson et al., 2010; Rising and Hudson, 1974). We dried ambient air using Drierite, then used an FB8 mass flow meter (Sable Systems), to control air flow into each chamber at ~500 mL/min. For each animal or baseline chamber, we manually subsampled output airflow at 100 - 150 ml/min using barrel syringes, dried air with Drierite, scrubbed CO_2 using Ascarite, then re-dried air with Drierite prior to measuring O_2 using a FoxBox (Sable Systems). We let the birds adjust and acclimate to the respirometry chambers for ~15 minutes, then we started measuring with an empty baseline chamber of 15 min, followed by measuring the bird chamber for 15 min. Bird and baseline chambers were all 1L Nalgene containers. Three individually-housed birds were measured concurrently by cycling through each bird three times, with a 15 min baseline between each measurement, for a total measurement period of 45 min per bird over 3 hours. Each day, before the respirometry experiments, we spanned the FoxBox using ambient (undried) air (20.95% O_2). At the end of the BMR trials, birds were returned to their acclimation treatment and were provided food.

The morning after each BMR trial, we measured M_{sum}, or the cold-induced maximum metabolic rate (VO_2max) on unfasted birds. Starting at approximately 9:00 h, we weighed each bird and placed it in a chamber within a temperature cabinet kept at ~-5℃. Incurrent air to the chamber was a mix of 21% oxygen and 79% helium, with flow rates and mixing regulated using a mass flow controller (Alicat Scientific, Tucson, AZ, USA) with a flow rate of ~750 ml/min. Given the higher conductance of heliox over that of ambient air, the use of heliox during measurement of M_{sum} enables eliciting a response to cold stress at higher ambient temperatures than without heliox, resulting in less potential for damage to the organism (Rosenmann and Morrison, 1974; Swanson, 2010). After a 5-minute baseline measurement, we measured oxygen consumption for a maximum 30 minute period or until birds became hypothermic (visible by a peak of oxygen consumption followed by a sharp decline). Before and after each trial, we measured body temperature using a thermocouple (Omega HH802U, 0.05% reading accuracy) inserted into the cloaca; birds with a body temperature at or below 37℃ were considered hypothermic. At week 3 and week 6, control birds had their M_{sum} measured in a temperature cabinet at -5℃, as at week 0, while birds that were in the cold treatment had their M_{sum} measured in a temperature cabinet (Accucold VLT65, Bronx, NY, USA) maintained at -20℃. We reasoned that if birds in the control group are hypothermic at the end of the -5℃ trial, they likely would have been as cold challenged as the cold acclimated birds at -20℃, given that hypothermia suggests the inability to regulate body temperature. We chose a static cold exposure over a step-down exposure because both approaches yield similar $VO2_{max}$ estimates (Swanson et al. 1996), and a static exposure reduces the time need for each trial. These conditions were similar to those implemented previously (Stager et al., 2020). At week 3, 12 of 14 birds had a body temperature ≤37℃ (indicating hypothermia; Stager et al., 2020; Swanson et al., 2014). Two birds did not have reliable body temperature estimates, but their oxygen traces indicated M_{sum} was reached so they were included in analysis. At week 6, all birds had a body temperature ≤37°C after the M_{sum} trial. A single researcher (R.M.S.) performed all metabolic assays.

Following Stager et al. (2020) and the custom R scripts therein (https://github.com/Mstager/batch_processing_Expedata_files), we corrected for baseline drift using a linear correction, then, following (Lighton, 2008), calculated BMR as the lowest oxygen consumption rate (ml O_2/min) averaged over a 10-min period. After baseline corrections, we calculated M_{sum} as the highest oxygen consumption (ml O_2/min) over a 5-min period.

Physiological phenotyping

At week 0, week 3, and week 6 of the acclimation experiments, we measured body fat and lean mass using an EchoMRI quantitative magnetic resonance (QMR) system (E26-281-BHlab, Houston, TX, USA) immediately after M_{sum} trials. We measured three standards at the beginning and end of each set of QMR measurements, then calculated a standard curve and adjusted the sample values accordingly using linear regression in R to account for measurement drift. After week 6 of the acclimation experiment, we euthanized birds via thoracic compression, and dissected and weighed the heart, determined sex via inspection of the gonads. Given the influence of hemoglobin on blood O_2 carrying capacity and aerobic performance, we measured hemoglobin concentration using a HemoCue Hb 201 (Brea, CA, USA) and, after spinning down blood-filled capillary tubes for 5 min at 12,000 RPM in a hematocrit centrifuge (Unico C-MH30), measured hematocrit as the relative proportion of packed red blood cells using digital calipers. We filled each body cavity with a damp paper towel, then froze the skins at -20℃ until the conductance assays were performed.

Conductance assays

We measured the thermal conductance of skin and plumage as the power input required to maintain a constant internal temperature of 39 □ (a typical body temperature of free living black-capped chickadees; Lewden et al. 2017), following Stager et al. (2020). Each skin was thawed and any remaining adipose or muscle tissue was removed from the body cavity, and feathers were brushed using corn meal to remove any excess oils. We then created an epoxy mold (fit to the internal cavity of a ~9 g chickadee) composed of a thermocouple and nichrome wire contained in PC-Marine Epoxy Putty (Allentown, PA, USA). We then stitched the skins closed around the mold within the thoracic and abdominal cavity. Skins were then placed in an upright position hanging by threads attached to external nares with wings tucked to sides. We monitored temperature of the mold with a thermocouple, and measured the power input (milliwatts, mW) necessary to maintain the epoxy mold at 39℃ using a voltage logger (Omega OM-CP-Quadvolt, Norwalk, CT, USA), an amperage logger (Omega OM-CP-Process 101A-3A) and a temperature controller (Omega CNI1622-C24-DC). A 12 V DC battery was used to power the board as well as to heat the epoxy mold.

We performed conductance trials in a small room with limited airflow and a relatively constant ambient room temperature (mean±s.d. = 23.7 ±0.7°C). Epoxy molds were heated to 39℃ and power was required to increase temperature of the thermocouple in the mold when it fell below a lower threshold of 37℃. Throughout a 20-minute period both amperage and Voltage were recorded at every second. We calculated average power input by taking the mean of Volts*amps over the 20-minute interval for a sample size of n=14. Trials were conducted by a single person (A.R.) blind to each bird's treatment. We did not find a significant effect of variation in room temperature (p=0.872) or sampling date (p=0.086) on average power (p=0.872) so neither were included in models testing for treatment effects.

Statistical analyses

We performed all statistical analyses in R, and the code and R notebook are available on Github (https://github.com/renaschweizer/chickadee_thermal_acclimation_paper). We quantified the effects of acclimation on phenotypic traits with linear mixed-effects models using the Lme4 package in R (Bates et al., 2015). For BMR and M_{sum}, we tested for an effect of a treatment*timepoint interaction, including body mass (M_b) as a covariate and bird ID as a random effect (e.g., phenotype ~ mass + treatment*timepoint + (1| bird ID)). We used the corresponding M_b measured just before BMR or just before M_{sum}. We performed the same tests for QMR measures but did not include body mass as a covariate. Models testing an effect including sex (e.g., ~ M_b + Sex + (1|ID)) were not the best fit for BMR and M_{sum} (see below), so we excluded sex from those final models. For M_b, we included the treatment*timepoint interaction with bird ID as a random effect. We used ANOVA to assess significance, and compared models using AICc in MuMIn (Barton, 2010). To test for treatment effects on conductance, we used a standard linear regression model (Lm function in R). We used post-hoc tests to find pairwise differences of treatment*timepoint on phenotypes with significant treatment or timepoint effects using the emmeans package in R and a Benjamini-Hochberg correction for multiple testing. We also used linear models to test treatment and timepoint effects on hemoglobin, percent red blood cells (hematocrit), and heart mass, given their critical roles in oxygen carrying capacity and aerobic performance. Finally, we tested for significant pairwise associations between phenotypes using Pearson correlation (cor.test in base R).

Results and Discussion

Cold acclimation increases thermogenic capacity

After adjusting to captivity, but prior to experimental acclimation, chickadees did not differ by treatment in BMR ($F_{1,11}$=0.1912, p=0.6704) nor M_{sum} ($F_{1, 11}$=0.0482, p=0.8303) (Table S1; Table S2). Birds gained body mass over the experimental acclimation (Figure S1A), with timepoint and a treatment*timepoint interaction having significant effects (Table 1).

BMR was significantly affected and best explained by M_b (F_1=4.547, p-value: 0.044; BMR ~ M_b + (1|ID)); in a more complex model (BMR ~ M_b + Treatment*Timepoint + (1|ID)), we found no significant effect of M_b, treatment, timepoint, or interaction on BMR (Figure 1A; Table 1; Figure S1B). However, when we examined the week 6 BMR data separately, we did find a significant effect of treatment ($F_{1,11}$=4.923,p=0.048) and mass ($F_{1,11}$=6.369, p=0.028), with a higher BMR in cold birds (Table S2).

The best fit model for M_{sum} included M_b, treatment*timepoint, and bird ID, but not sex (Table 1). In this model, we found significant effects on M_{sum} of treatment (F_1=11.583, p=0.046) and time point (F_2= 15.782, p=3.607e-05) (Figure 1B), but no effect of M_b (F_1= 4.0519, 0.062)(Table 1; Figure S1C). Cold treatment birds increased their M_{sum} from T_{0wk} to T_{3wk} (df=30, p=0.001) and from T_{0wk} to T_{6wk} (df= 32.6, p=0.001), but not from T_{3wk} to T_{6wk} (df= 24.3, p=0.986)(Table S2). Control birds also increased their M_{sum} from T_{0wk} to T_{6wk} (df= 25.5 , p=0.026, but not from T_{3wk} to T_{6wk} (df= 24.1, p=0.275) nor from T_{0wk} to T_{3wk} (df= 24.4 , p= 0.1812; Table S2). At both T_{3wk} and T_{6wk}, the mean M_{sum} was significantly different between treatment groups (p=0.003 and p=0.022, respectively), with cold-acclimated birds having an M_{sum} elevated by ~14-18%.

Contrary to some previous findings (Dubois et al., 2016; Fristoe et al., 2015; Liknes et al., 2002; Milbergue et al., 2022; Nilsson and Nilsson, 2016 but see Smit et al. 2010), cold acclimation did not affect BMR (Figure 1A). Cold-acclimated birds did demonstrate a higher mean BMR after 6 weeks than control birds (cold: 0.745 mL O_2/min; control: 0.675 O_2/min), even after accounting for mass differences between the two groups (cold: 0.059 mL O_2/min/g; control: 0.058 O_2/min/g). The cause of this lack of an effect is not known, but a similar study of black-capped chickadees from Quebec, Canada, also found that BMR did not change seasonally despite seasonal increases in M_{sum} (Petit et al. 2014). Nonetheless, other studies of black-capped chickadees have identified significant effects cold acclimatization (Cooper and Swanson, 1994; Swanson and Liknes, 2006) and acclimation (Milbergue et al., 2018; Milbergue et al., 2022), suggesting that the effects of cold acclimation on BMR may be population specific.

We did not measure the masses of digestive organs or food intake so we are unable to assess whether cold-acclimation affected these traits. Although we measured heart size and blood oxygen capacity, we did not find any significant effects of treatment (Table S2). If changes in BMR are uncoupled from M_{sum}, they may be tied to fitness in unmeasured ways, or linked to other behavioral (e.g., energy expenditure) or metabolic traits. For example, Milbergue et al. (2022) found that, in cold-acclimated black-capped chickadees, heightened BMR and M_{sum} were correlated with divergent patterns of mitochondrial metabolism in different tissues (i.e., with complex I activity in the liver and with mitochondrial proton leak in the muscle, respectively).

Our M_{sum} results are consistent with previous studies demonstrating an increased ability to tolerate cold in winter-acclimatized birds relative to those sampled in the summer (Cooper and Swanson, 1994) and in cold-acclimated birds relative to control (Milbergue et al. 2022). M_{sum} is a proxy for the maximum thermogenic capacity of an endotherm and, as a result, is positively correlated with cold tolerance (i.e., endurance under cold stress; Swanson 2001; Swanson et al., 2006; Swanson et al., 2009; Barceló et al. 2017). Increases in thermogenic capacity underscore the importance of internal heat generation as chickadees acclimate to colder temperatures.

Cold acclimation increases lean mass, but not fat mass

For total fat mass, we did not find an effect of treatment ($p>0.5$) or timepoint ($p>0.5$) over the acclimation period (Figure S1D; Table 1). Lean mass significantly increased over time with cold acclimation (Figure 2A). Within cold-acclimated birds, lean mass was significantly different from T_{0wk} to T_{3wk} (df=24, $p= 3x10^{-4}$), T_{0wk} to T_{6wk} (df=24, $p<0.001$), and T_{3wk} to T_{6wk} (df=24, $p=0.001$), but there were no significant pairwise differences within the control birds (Table S2). While chickadees did not alter fat mass over the experimental period, there was a non-significant trend towards increased total fat mass in cold-acclimated birds (Figure S1D).

The lack of significant increases in fat mass is in contrast to previous studies on free-living dark-eyed juncos and black-capped chickadees that found increases in winter fat stores (Chaplin, 1974; Swanson, 1991). While one of the functions of fat is to provide fuel stores for wintering birds, studies have demonstrated that fat mass may not directly influence thermogenic capacity (Marsh and Dawson 1989). Because M_{sum} in birds is largely the result of active shivering by skeletal muscles, the flight muscles (pectoralis) are considered to be the primary thermogenic organ (Marsh and Dawson 1989; Swanson et al., 2013). Seasonal flexibility of M_{sum} should thus be rooted in seasonal flexibility of the pectoralis. An important mechanism for increasing organismal thermogenic capacity in winter is to increase the size of the pectoralis, and this has been observed in a variety of bird species (Swanson and Vézina, 2015).

We found an increase in lean mass, occurring on a similar time course as M_{sum} (Figure 2A). Stager et al. (2020) found in dark-eyed juncos that thermogenic mechanisms change over different timescales, given that temperature regulation and resistance to hypothermia continued to improve in the absence of improvements in M_{sum}. Although pectoralis muscle size is a prominent predictor of M_{sum} in birds (Swanson, 2010), other factors that influence aerobic capacity of muscle fibers could explain improvements in M_{sum} in the absence of changes to lean mass. For example, changes in mitochondrial densities, metabolic enzyme activities, or blood supply to respiring tissues have all been shown to influence metabolic capacity (Suarez, 1998).

In cold-acclimated black-capped chickadees, specifically, elevated M_{sum} has been positively correlated with mitochondrial proton leak (Milbergue et al., 2022), which has been suggested as a heat-generating mechanism. Changes in these factors could alter metabolic intensity without concurrent changes in muscle mass (Milbergue et al., 2018; Swanson, 2010). Other studies have also found increases in mass of body muscles (pectoralis, legs, supracoracoideus, skeletal) and cardiopulmonary organs as well as hematocrit levels during winter (Petit et al., 2014; Swanson, 1991; Swanson, 2010). The overall increase in lean mass (including the pectoralis muscle) are consistent contributors to the winter phenotype of small birds in cold climates (Liknes and Swanson, 2011; Petit et al., 2013; Vézina et al., 2011).

Chickadees do not modify their thermal conductance in response to cold exposure

Despite the expectation that changes in heat retention could improve thermoregulation in the cold, we found that cold acclimation did not significantly affect thermal conductance (cold birds: 385.5 W, control birds: 403.0 W, F_1= 0.264, p=0.616; Figure 2B; Table 1). This suggests that black-capped chickadees do not simultaneously alter physiological processes that improve both capacity to generate (Figure 1) and retain (Figure 2B) heat, solely in response to cold exposure at least over the timescales studied here.

While our study shows a lack of significant difference in thermal conductance between birds acclimated to -5°C and birds acclimated to 23°C, it is important to note previous studies that have found an increase in conductance for birds acclimated to summer temperatures (Swanson, 1991) as well as decreasing conductance for cold-acclimated birds (Stager et al., 2020). One possible explanation could be simply that our experimental trial lengths were not long enough to show changes. For example, Stager et al. (2020) found that cold-acclimated dark-eyed juncos showed reduced conductance only at 9 weeks (and not at weeks 1, 2, 3, or 6). An additional important factor to consider is that conductance may respond to different seasonal cues. Specifically, large changes to conductance in birds may require a molt, which is often triggered by changes in photoperiod (reviewed in Dawson et al., 2001). In a study focusing on red knots, an arctic shorebird, seasonal changes in thermal conductance were likely due at least in part to changes in insulative feathers (Piersma et al., 1995). Additionally, Swanson (1991) measured the dry mass of contour plumage in dark-eyed juncos, where the contour plumage was an index of insulation, and found the mass increased by 37% in birds captured during the winter. While temperature and photoperiod covary as seasonal cues and the full seasonal phenotype may be the outcome of response to one or the other cue, in our experiment we only modified temperature and kept photoperiod constant. Black-capped chickadees typically molt once a year in the late summer, so our individual birds were collected after molting. Such experimental separation of seasonal cues (i.e., temperature and photoperiod) provides important insight into the cues that stimulate flexible responses.

While we did not detect changes in thermal conductance here, behavioral changes can also alter heat retention and its necessity (Wolf and Walsberg, 2000). For example, birds may thermoregulate through behaviors such as clustering with conspecifics (McKechnie and Lovegrove, 2001) or posturing (Ferretti et al., 2019). Additionally, because chickadees cache food during the winter, they may be less dependent on energy saving strategies like increasing conductance.

Mechanisms of thermoregulation in black-capped chickadees

Our results suggest that, in response to cold, black-capped chickadees modify their thermoregulatory ability through changes in internal heat generation rather than changes in heat retention, with a few caveats. The 6-week acclimation period may not have been long enough to capture additional changes in thermal conductance, or the birds may have arrived in Montana after molting in the wild. Regardless, our results indicate that chickadees may be able to respond to short-term bouts of cold stress by increasing their M_{sum} rather than changing their conductance. Cold snaps might therefore require a greater energetic investment to offset if they are solely dealt with via changes to metabolism (Linek et al., 2021). To better understand the underlying mechanisms driving thermoregulation in birds, it would be beneficial to study both the behavioral and physiological aspects of the avian response to cold throughout the acclimation period. Further studies could also shed light on specific physiological changes that contribute to improvements in whole-organism performance.

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Competing interests

The authors declare that no competing interests exist.

Author contributions

Conceptualization: RMS, SAT, ZAC, AMR, MDC

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Formal analysis: RMS, AR

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Data curation: RMS, AR

Writing - original draft: RMS, AR

Writing - review & editing: BWT, SAT, AMR, GAS, ZAC, MDC

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Data Availability

Metadata compiled for each individual is available in Table S1 and provided as individual files with a R data analysis script on R.M.S.'s Github page (https://github.com/renaschweizer/chickadee_thermal_acclimation_paper). Raw respirometry, QMR, and conductance data are available upon request. Scripts for processing raw respirometry data are similar to those available on Github (https://github.com/Mstager/batch_processing_Expedata_files).

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Tables

Table 1. Results of linear models testing the effects of mass, treatment, timepoint, and the treatment x timepoint interaction (Treat:Time) on various physiological measurements. For BMR and M_{sum}, multiple models were tested and the corresponding AICc values are reported. For BMR and M_{sum}, the "mass" term corresponds to the body mass of the bird measured just prior to the BMR or M_{sum} trial, respectively, and we measured those body masses pre-acclimation as well as at 3 weeks and 6 weeks of acclimation.

Trait	Model	AICc	Factor	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Mass	~ Treatment*Timepoint + (1|ID)	--	Treatment	0.129	0.129	1	12.000	1.463	0.2497

			Timepoint	3.372	1.686	2	24.000	19.168	**0.0000**
			Treatment:Timepoint	1.739	0.869	2	24.000	9.884	**0.0007**
BMR	~ Mass + (1|ID)	**-73.162**	Mass	0.026	0.026	1	22.053	4.547	**0.0444**
	~ Mass + Sex + (1|ID)	-66.525	Mass	0.006	0.006	1	32.347	1.048	0.3135
			Sex	0.004	0.004	1	18.015	0.769	0.3921
	~ Mass + Treatment*Timepoint + (1|ID)	-42.133	Mass	0.008	0.008	1	13.924	1.496	0.2415
			Treatment	0.005	0.005	1	10.746	1.038	0.3306
			Timepoint	0.028	0.014	2	26.408	2.721	0.0842
			Treatment:Timepoint	0.009	0.004	2	25.213	0.867	0.4325
VO2max	~ Mass + (1|ID)	86.758	Mass	4.775	4.775	1	19.697	15.098	**0.0009**
	~ Mass + Sex + (1|ID)	89.965	Mass	3.326	3.326	1	29.193	10.711	**0.0027**
			Sex	0.067	0.067	1	15.190	0.215	0.6493

	~ Mass + Treatment*Timepoint + (1|ID)	**80.042**	Mass	0.644	0.644	1	14.636	4.052	0.0629
			Treatment	0.795	0.795	1	11.583	4.999	**0.0459**
			Timepoint	5.017	2.509	2	25.181	15.783	**0.0000**
			Treatment:Timepoint	0.749	0.374	2	27.843	2.356	0.1135
Fat	~ Treatment*Timepoint + (1|ID)	**24.516**	Treatment	0.029	0.029	1	12.000	1.206	0.2936
			Timepoint	0.075	0.037	2	24.000	1.535	0.2359
			Treatment:Timepoint	0.094	0.047	2	24.000	1.923	0.1680
	~ Treatment*Timepoint + Sex + (1|ID)	29.817	Sex	0.001	0.001	1	11	0.0374	0.8501
			Treatment	0.028	0.028	1	11	1.1328	0.31
			Timepoint	0.075	0.037	2	24	1.5348	0.2359
			Treatment:Timepoint	0.094	0.047	2	24	1.9233	0.168

Lean mass	~ Treatment*Timepoint + (1|ID)	29.267	Treatment	0.006	0.006	1	9.609	0.572	0.4678
			Timepoint	0.086	0.043	2	21.518	4.170	**0.0295**
			Treatment:Timepoint	0.071	0.036	2	23.063	3.463	**0.0484**
	~ Sex + Treatment*Timepoint + (1|ID)	**22.615**	Sex	0.117	0.117	1	12.977	11.844	**0.0044**
			Treatment	0.000	0.000	1	10.045	0.003	0.9608
			Timepoint	0.096	0.048	2	23.351	4.887	**0.0169**
			Treatment:Timepoint	0.089	0.045	2	24.660	4.538	**0.0210**
Average Power	~ Treatment	--	Treatment	1076.835	1076.835	1	*NA*	0.2646106	0.6163117
			Residuals	48834.089	4069.507	12		*NA*	*NA*

The table reports results of reduced linear mixed effect models. NumDF and DenDF are the numerator and denominator degrees of freedom, respectively. P-values ≤0.05 are bolded.

Figure Legends

Figure 1. Repeated measures of BMR and M_{sum} for control (23℃, n=7) and cold (-5℃, n=7) experimental treatments. A) Over the 6 week acclimation, there was no significant effect of treatment or timepoint on BMR. B) Chickadees showed significant effects of treatment ($p<0.05$) and time point ($p<0.01$) on M_{sum}, with significant changes in cold-acclimated M_{sum} from T_{0wk} to T_{3wk} ($p=0.0010$) and from T_{0wk} to T_{6wk} ($p=0.0010$), and in control-acclimated M_{sum} from T_{0wk} to T_{6wk} ($p=0.0259$).

Figure 2. Lean mass and conductance over experimental acclimation. A) Lean mass was significantly affected by mass ($p=0.0009$), timepoint ($p=0.0169$), sex ($p=0.0044$), and timepoint*treatment ($p=0.0210$), and significantly increased in cold-acclimated birds from T_{3wk} to T_{6wk} ($p=0.0211$) and from T_{0wk} to T_{6wk} ($p=0.029$). B) Average power measured between birds from cold and control treatments. Power was not significantly affected by treatment ($p>0.05$).

Figure 1

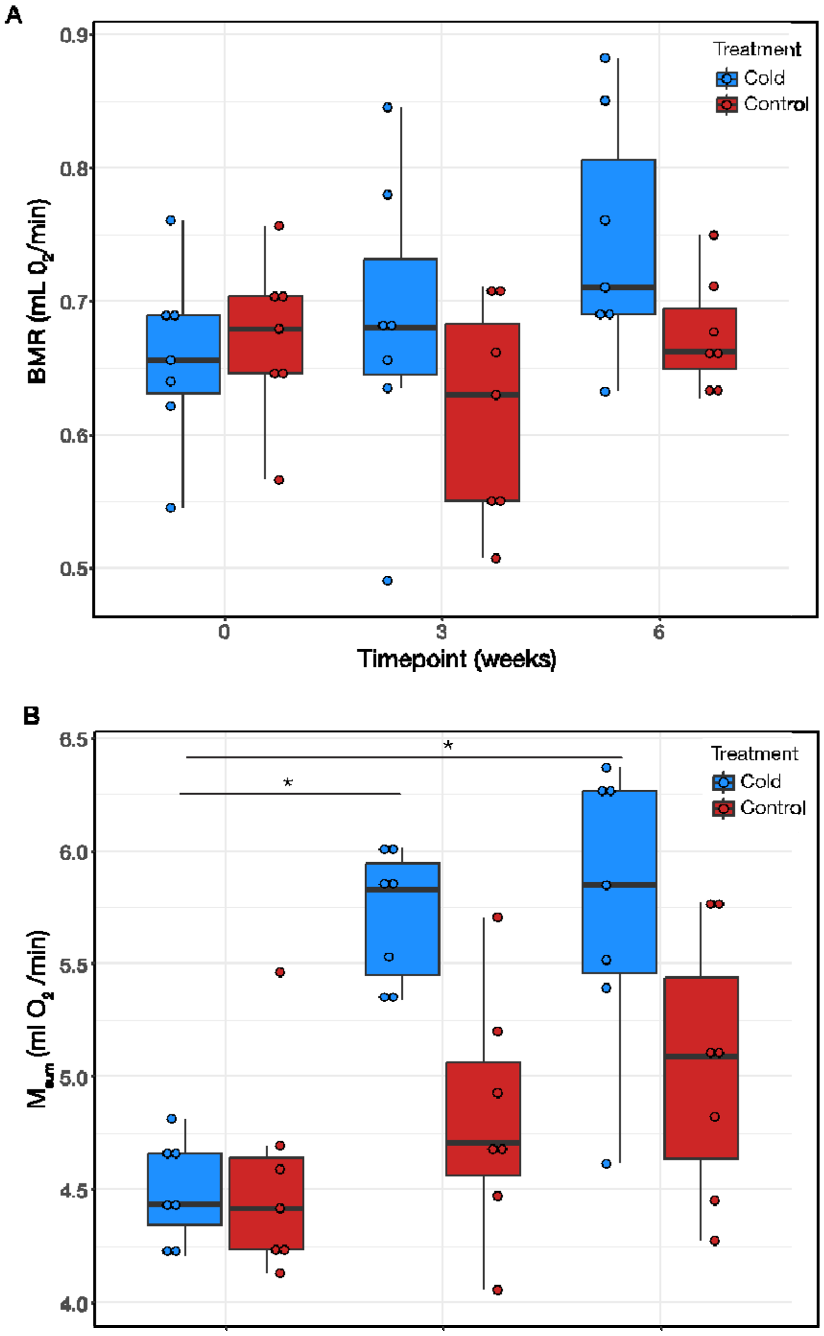
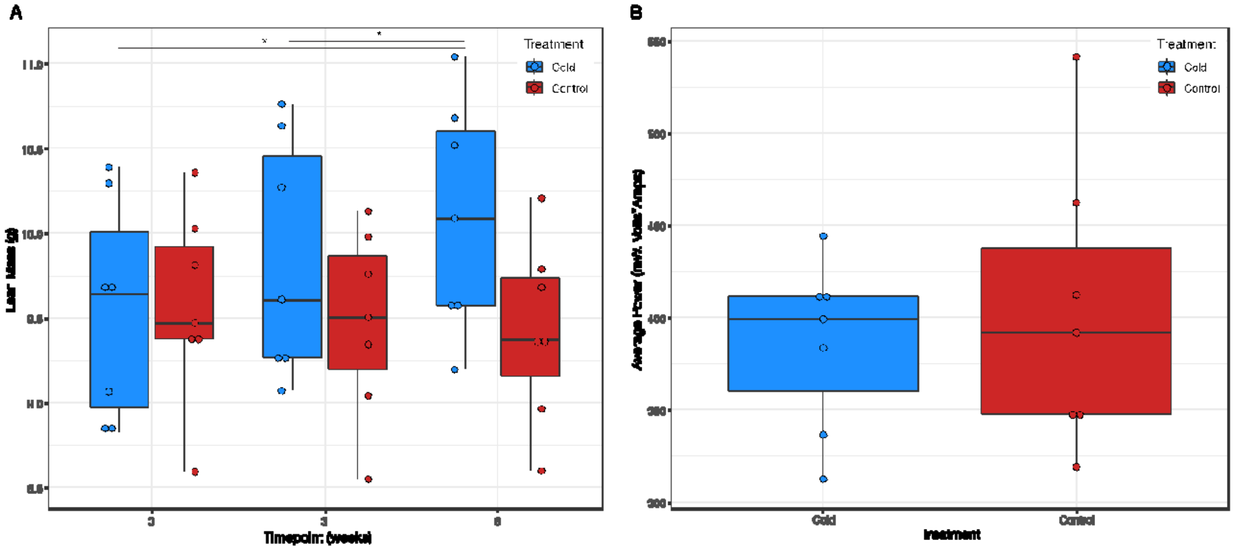
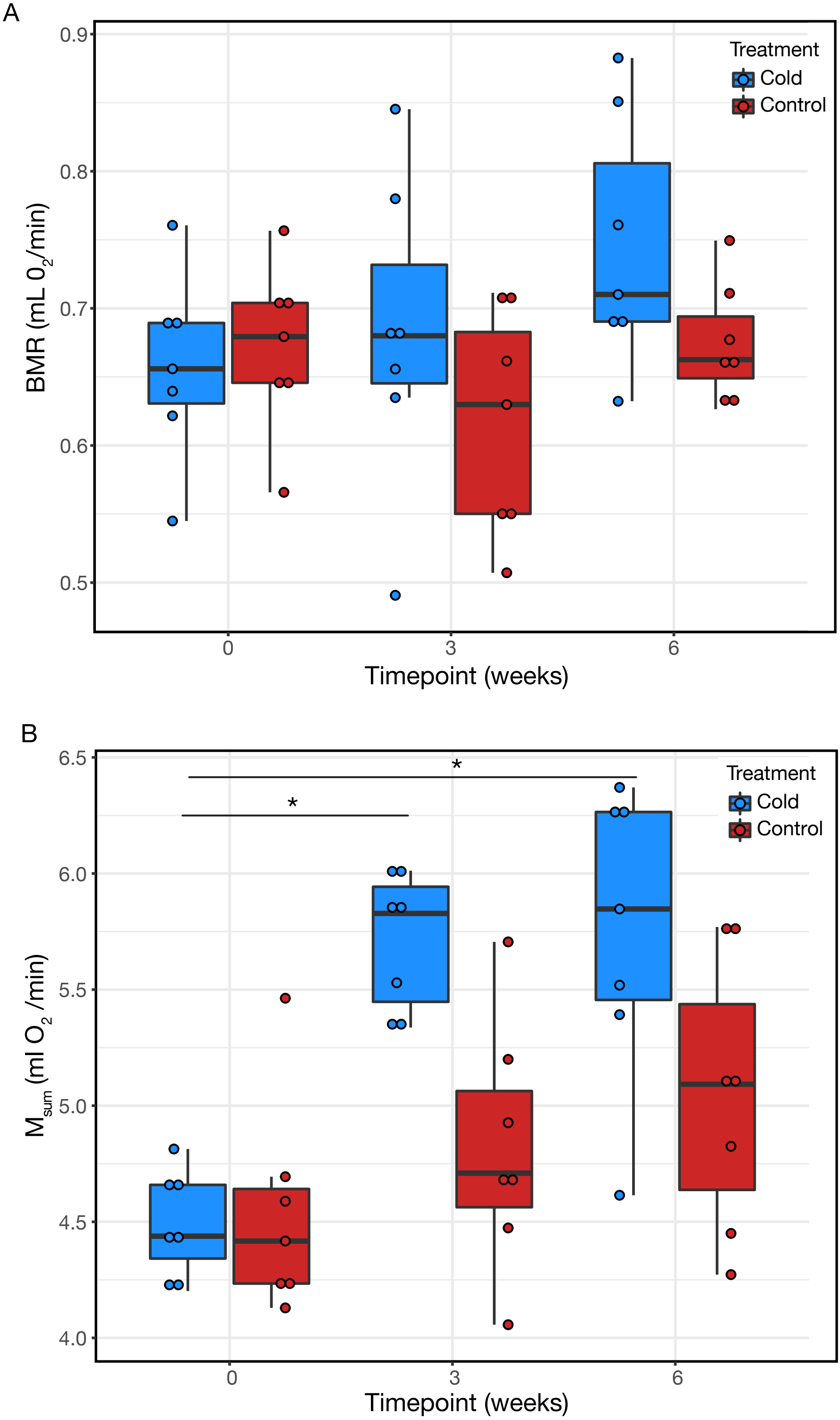
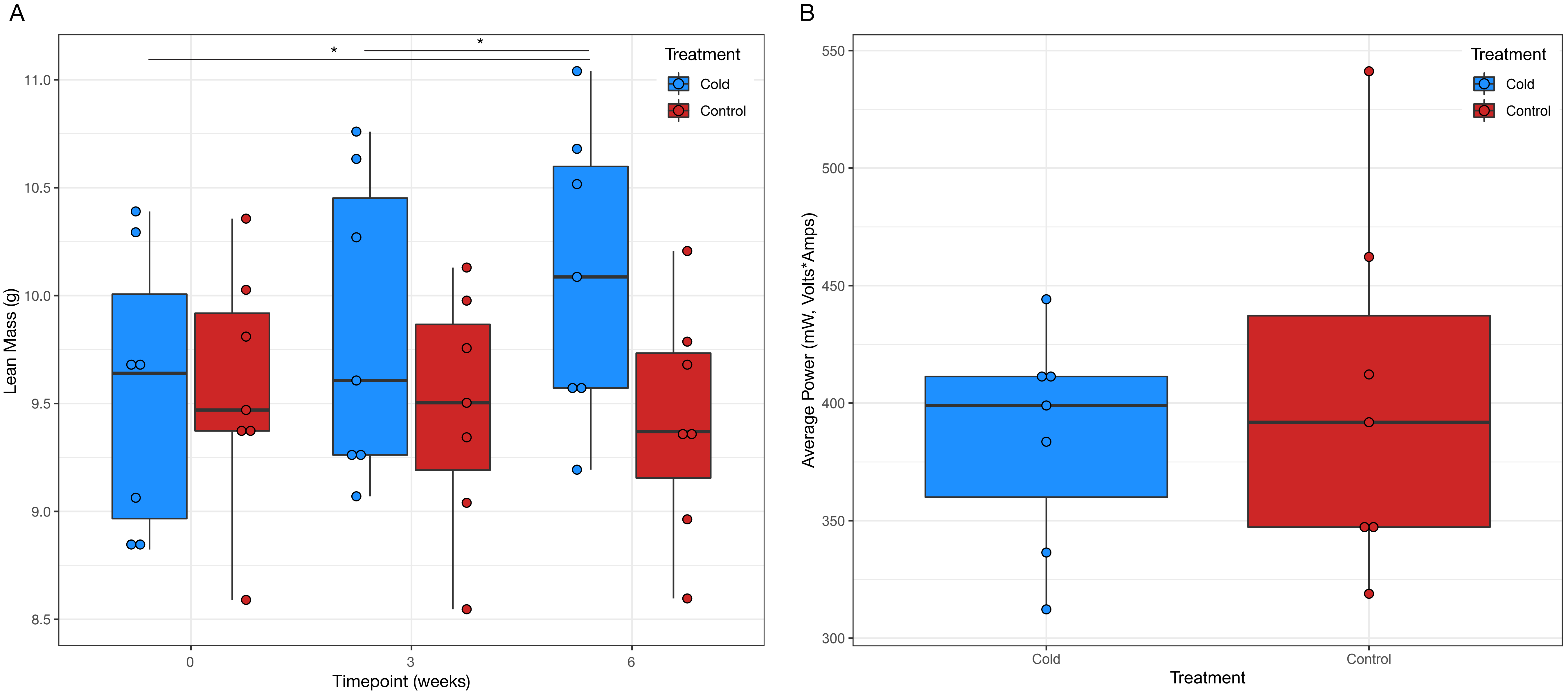

A
Treatment
Cold
Control
BMR (mL O_2/min)
0.9
0.8
0.7
0.6
0.5
0
3
6
Timepoint (weeks)
B
Treatment
Cold
Control
*
*
M_{sum} (ml O_2 /min)
6.5
6.0
5.5
5.0
4.5
4.0

Figure 2


A
Treatment
Cold
Control
*
*
11.0
10.5
10.0
9.5
9.0
8.5
Lean Mass (g)
Timepoint (weeks)
B
Treatment
Cold
Control
550
500
450
400
350
300
Average Power (mW, Volts*Amps)
Cold
Control
Treatment


A
BMR (mL 0$_2$/min)
0.9
0.8
0.7
0.6
0.5
0
3
6
Timepoint (weeks)
Treatment
Cold
Control
B
M$_{sum}$ (ml O$_2$ /min)
6.5
6.0
5.5
5.0
4.5
4.0
*
*
0
3
6
Timepoint (weeks)
Treatment
Cold
Control


A
Lean Mass (g)
11.0
10.5
10.0
9.5
9.0
8.5
*
*
Treatment
Cold
Control
0
3
6
Timepoint (weeks)
B
Average Power (mW, Volts*Amps)
550
500
450
400
350
300
Treatment
Cold
Control
Cold
Control
Treatment