Article

Differences between temperate and tropical birds in seasonal acclimatization of thermoregulatory traits

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Phenotypic flexibility can be an important determinant of fitness in variable environments. The climatic variability hypothesis (CVH) predicts that phenotypic flexibility in thermoregulatory traits will be greater in temperate species than tropical species as a means of coping with increased temperature seasonality at higher latitudes. However, support for the CVH has been mixed, and recent studies suggest that tropical birds are capable of substantial phenotypic flexibility. To test the generality of the CVH, we used flow-through respirometry to quantify seasonal acclimatization in thermoregulatory traits in suites of temperate (n = 6) and tropical (n = 41) birds. We used W/S ratios (winter/summer trait values) to quantify the direction and magnitude of seasonal change (W/S ratio of 1 means no seasonal change). Temperate species exhibited coordinated changes in thermoregulatory traits in winter, including large increases in thermoneutral zone (TNZ) breadth and reductions in heat loss below the lower limit of the TNZ. Conversely, tropical species exhibited idiosyncratic seasonal thermoregulatory responses, and mean W/S ratios were close to 1 for all traits, indicative of little seasonal change and consistent with predictions of the CVH. Nevertheless, mean W/S ratios did not differ significantly between temperate and tropical species for either M_b or BMR, demonstrating that tropical birds can also exhibit substantial thermoregulatory flexibility. Our results highlight the need for complementary acclimation experiments to determine if latitudinal differences in seasonal acclimatization are due to inherent differences in capacity for flexibility.

Keywords: basal metabolic rate, climatic variability hypothesis, latitude, seasonal acclimatization, thermoneutral zone

Introduction

Phenotypic flexibility – the ability to undergo reversible phenotypic adjustments in response to changing environmental conditions (Piersma and van Gils 2011) – is an important component of fitness for organisms that inhabit variable environments (Piersma and Drent 2003).

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Flexibility in thermoregulatory traits is likely to be especially important in seasonal environments where organisms must tolerate a broad range of temperatures throughout the year. For example, many temperate birds exhibit seasonal acclimatization in key energetic and thermoregulatory traits such as basal metabolic rate (BMR – the minimum maintenance metabolism required for homeostasis) and summit metabolic rate (M_{sum} – the maximum metabolic heat production elicited in response to cold stress and a measure of thermogenic capacity) (Kersten and Piersma 1987, Swanson 2010). Although seasonal adjustments of BMR and M_{sum} have been well-studied in temperate birds (reviewed by Swanson 2010), less is known about these adjustments in species that inhabit tropical environments.

An emerging framework for understanding geographic variation in phenotypic flexibility of thermoregulatory traits is the climatic variability hypothesis (CVH), which predicts that species from temperate latitudes will exhibit greater seasonal flexibility than those from tropical latitudes, where temperature seasonality is comparatively modest (Chown et al. 2004, Bozinovic et al. 2011, Naya et al. 2012). The CVH has received some empirical support – temperate birds exhibit greater seasonal variation in M_{sum} compared to tropical species (Swanson and Garland 2009, Swanson 2010, Wells and Schaeffer 2012, McKechnie et al. 2015, Stager et al. 2016). However, patterns of seasonal acclimatization for other thermoregulatory traits do not conform to the predictions of the CVH. For example, a recent global synthesis of seasonal adjustments in avian BMR (McKechnie et al. 2015) found that the magnitude of seasonal acclimatization of BMR was similar in temperate and tropical species. Thus, temperature seasonality does not necessarily predict patterns of seasonal acclimatization of all thermoregulatory traits in birds, and as a result, the general applicability of the CVH remains unclear.

Another important thermoregulatory trait in endotherms is the thermoneutral zone (TNZ) – the range of ambient temperatures at which an organism is at BMR and not expending energy to maintain heat balance (McNab 2002) (Fig. 1). Outside the lower (lower critical temperature – LCT) and upper (upper critical temperature – UCT) bounds of the TNZ, an endotherm must allocate energetic resources to maintaining internal temperature homeostasis (McNab 2002). Thus, the breadth of the TNZ influences the energetic costs of thermoregulation and greater temperature variability may be expected to select for increased TNZ breadth to reduce the energetic costs of thermoregulation in thermally variable environments (Khaliq et al. 2014). Indeed, TNZ breadth increases with latitude (Araújo et al. 2013, Khaliq et al. 2014, Pollock 2016), indicating that it may be important for coping with temperature seasonality. However, the few studies that have characterized seasonal variation in TNZ have found mixed results (Bush et al. 2008, Nzama et al. 2010, Zhao et al. 2014, Thompson et al. 2015, Wu et al. 2015), and it is unclear whether temperate species exhibit greater seasonal acclimatization in TNZ breadth as predicted by the CVH. To test the generality of the CVH in
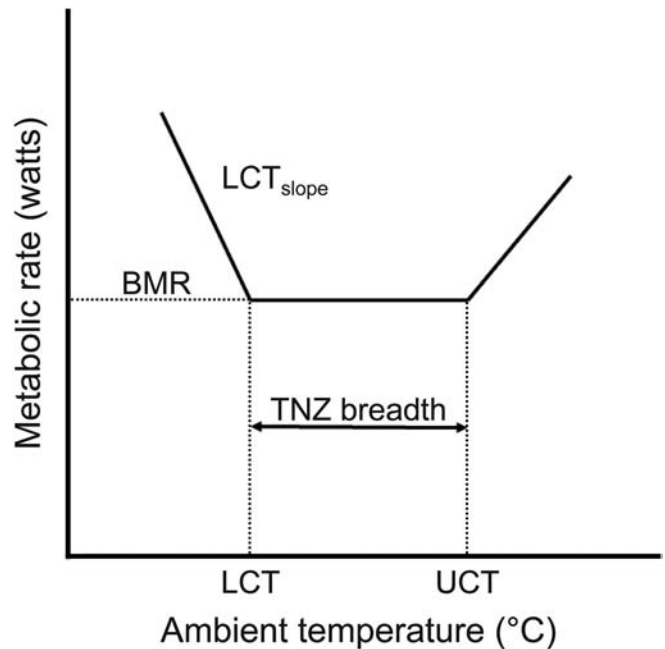

Figure 1. Schematic of the endothermic thermoneutral zone (TNZ). Within the TNZ, an endotherm is able to maintain a basal metabolic rate (BMR) and the metabolic costs of thermoregulation are minimal. However, at ambient temperatures below the lower critical temperature (LCT) or above the upper critical temperature (UCT), an endotherm must expend energetic resources on thermoregulation to maintain a stable, high body temperature, thereby increasing metabolic rate (LCT_{slope}).

birds, we quantified seasonal acclimatization in thermoregulatory traits (body mass, BMR and TNZ breadth) in suites of tropical and temperate bird species. We predicted that temperate species would exhibit seasonal increases in thermoregulatory traits, reflecting the greater temperature variation and energetic demands that they face in winter. In contrast, we predicted that the seasonal acclimatization responses of tropical species would be more variable and of a smaller magnitude due to their occurrence in a relatively stable thermal environment.

Methods

Bird capture and handling

We sampled birds over the course of two years (2014 and 2015) at one lowland tropical (Gamboa, Panama – 09°07′N, 79°42′W) and one temperate (Urbana, Illinois – 40°06′N, 88°12′W) sampling locality. We sampled temperate species in winter (December–February) and summer (May–August) and tropical species in dry season (February–April) and wet season (June–November). To facilitate seasonal comparison between the temperate and tropical sampling localities, we designated a 'summer' (temperate summer, tropical wet season) and 'winter (temperate winter, tropical dry season)

season based on seasonal temperature data obtained from each locality (Supplementary material Appendix 1 Fig. A1), although we acknowledge that there were only slight differences in 'summer' and 'winter' temperature regimes at the tropical locality (mean temperature was 0.86°C higher during the tropical summer). Diurnal and seasonal temperature variability was greater at the temperate locality (Supplementary material Appendix 1 Fig. A1). Although sampling dates were not temporally synchronized between localities, our approach allowed us to examine seasonal differences in thermal physiology across the annual spectrum of ambient temperature conditions experienced by birds at each locality.

We captured birds in mist-nets (12×2.6 m; 36-mm mesh) between 14:00 and 18:00 h and transported them back to the laboratory in cloth bags, where we banded each individual with a uniquely numbered aluminum leg-band to facilitate future identification. We then held birds at 27°C (within the TNZ of most focal species; Pollock 2016) in cloth-covered cages with water provided ad libitum until beginning temperature experiments.

Measuring metabolic responses to ambient temperature

At sunset on the day of capture, we weighed focal individuals using a digital scale (American Weigh Scales model AWS-201, 200 ± 0.01 g) and transferred them to respirometry chambers (see Respirometry system and gas measurement below) inside a PTC-1 temperature cabinet (Sable Systems) controlled by a Peltier device (Pelt-5, Sable Systems). On a given night, we then conducted either a temperature increase experiment or a temperature decrease experiment to determine the UCTs or LCTs of focal individuals (three per experiment), respectively. Throughout the temperature exposures, we precisely regulated ambient temperature in the cabinet and continuously monitored chamber temperatures using thermistor probes (model SEN-TH, Sable Systems, $\pm$ 0.2°C accuracy). We allowed birds to acclimate to metabolic chambers for at least three hours at 27°C, which ensured that focal birds had been post-absorptive for at least four hours and had reached BMR (following the criteria required for BMR as outlined in McKechnie and Wolf 2004). We used infrared cameras (model WCM-6LNV, Sabrent) to monitor bird behavior and activity levels inside the chambers. We only included data in our subsequent analysis from birds that remained calm and resting throughout the duration of the experiment. Following the acclimation period, we either increased (to determine UCT) or decreased (to determine LCT) T_a in 3°C increments and held birds at each T_a for at least one hour while measuring their O_2 consumption (see Supplementary material Appendix 1 Fig. A2 for an exemplar O_2 trace). We measured each individual twice per ambient temperature, sampling the three birds in series for 10 min at a time interspersed with a 5 min baseline period (i.e. 10:10:10:5). Following the second series of measurements at a given temperature, we measured an empty baseline chamber until the cabinet temperature had reached the next 3°C interval, which usually took between 10 and 15 min. To ensure that focal individuals had exceeded the limits of their TNZs, we estimated O_2 consumption throughout each experiment by subtracting $\%O_2$ of the animal chamber gas stream from $\%O_2$ of the previous baseline chamber gas stream (i.e the difference is a proxy for O_2 consumption). We concluded experiments when the difference between the baseline $\%O_2$ and animal chamber $\%O_2$ had increased in three successive sampling intervals, indicating that the focal individual was increasing O_2 consumption and had exceeded its TNZ. The average length of UCT experiments did not differ between tropical (6.1 ± 2.4 h) and temperate (6.4 ± 3.1 h) species (p = 0.61); however the length of LCT experiments were significantly longer (p < 0.001) in temperate birds (mean $\pm$ SD = 10.1 ± 3.9 h) than tropical birds (mean $\pm$ SD = 6.3 ± 2.8 h). At both sites, experiments were terminated at least one hour prior to sunrise, ensuring that elevations in O_2 consumption were not due to circadian rhythms in energy metabolism (Aschoff and Pohl 1970). Immediately after concluding temperature experiments, we re-weighed birds, held them in cages until sunrise, and then released them at the site of capture.

Respirometry system and gas measurement

We used push-mode flow-through respirometry (Withers 2001, Lighton and Halsey 2011) to measure gas exchange of focal bird species. During each experiment, we pumped incurrent air (PP2 pump; Sable Systems) through a column of Drierite to remove water and then pumped dried air into a mass-flow controller (Flowbar-8; Sable Systems) that divided the air stream into four channels, each plumbed through Bevaline IV tubing (Cole-Parmer) to separate Plexiglas metabolic chambers (one empty baseline chamber as a reference, three animal chambers), each equipped with a rubber gasket in the lid and sealed with binder clips (ACCO Brands Corporation) to prevent leakage. During experiments, birds rested in the chambers on perches made of wire mesh. The chamber inlet was situated on the lid of the chamber and the chamber outlet was on the side of the chamber opposite to the inlet to ensure that incurrent air flowed directly across the bird before exiting the chamber. Flow rates (300–1500 ml min^{-1}) and chamber sizes (1.97 or 4.53 l) varied depending on the size of the focal species, with higher flow rates and larger chambers used for larger species. Excurrent air from one chamber at a time was subsampled manually at 100–150 ml min^{-1} through barrel syringes, scrubbed of water vapor (Drierite) and CO_2 (Ascarite), and analyzed for $\%O_2$ (FoxBox; Sable Systems).

During each experiment, we measured flow rate, chamber T_a, and $\%O_2$ from one chamber at a time in series (i.e. using the 10:10:10:5 intervals described above) at one-second intervals using the program Expedata (Sable Systems). Following experiments, we used a Catmull-Rom spline correction during data processing to correct for drift in the O_2 analyzer.

We used customized macros within Expedata to convert %O_2 to $\dot{V}O_2$ (rate of O_2 consumption, measured in ml O_2 min^{-1}) using the following equation:

$$\dot{V}O_2 = FR \times (F_iO_2 - F_eO_2)/(1 - F_eO_2)$$

where FR was the flow rate of the animal chamber (ml min^{-1} STPD), F_iO_2 was the incurrent fractional oxygen concentration (0.2095), and F_eO_2 was the excurrent fractional oxygen concentration.

We calculated BMR as the lowest stable 5-min average $\dot{V}O_2$ measured during the experiment (Londoño et al. 2015) and used a coefficient of 20.08 J ml^{-1} O_2 (Schmidt-Nielsen 1997) to convert $\dot{V}O_2$ to metabolic rate (Watts). We calculated M_b as the mean of the pre- and post-experiment weights. Due to the well-known allometric relationship between M_b and BMR (Lasiewski and Dawson 1967, McKechnie and Wolf 2004), we log$_{10}$-transformed M_b and BMR to examine metabolic scaling relationships in our dataset. As expected, BMR scaled positively with M_b during both winter ($R^2 = 0.77$, $F_{1,45} = 154.2$, $p < 0.0001$; Supplementary material Appendix 1 Fig. A3a) and summer ($R^2 = 0.83$, $F_{1,45} = 224.1$, $p < 0.0001$; Supplementary material Appendix 1 Fig. A3b). Seasonal changes in avian BMR are driven by two primary mechanisms: 1) increases in metabolically active tissue (i.e. increases in central organ masses, such as the brain, heart, gut and kidneys; Swanson 2010) and 2) increases in cellular aerobic capacity (i.e. increases in oxygen transport capacity, elevated mitochondrial densities, increased catabolic enzymatic activity, etc.; Swanson 2010). To distinguish between these two mechanisms, we examined both seasonal changes in whole-animal BMR and mass-specific BMR. A common practice to control for calculating mass-specific BMR is to use the residuals of the M_b–BMR regression as data in subsequent analyses, yet this methodology can lead to biased parameter estimates if M_b is correlated with other variables of interest in subsequent analyses (Freckleton 2009). To avoid biased parameter estimates associated with using residuals (Freckleton 2009), we opted for an alternative approach to account for the effects of M_b on metabolic rate. Rather than using the log-transformed residuals, we empirically calculated the allometric scaling relationship between M_b and BMR (Stager et al. 2016), which is described by the equation $BMR = aM_b^b$, where a is the y-intercept and b is the scaling exponent. Typical values of b reported in the literature range from 0.65 to 0.75 for BMR (McKechnie and Swanson 2010). We obtained slightly lower values of $b = 0.49$ (winter) and $b = 0.51$ (summer), probably because our dataset was comprised almost exclusively of species from one order (passeriformes) with a narrow range of M_b (3.94–119.85 g). We then calculated mass-adjusted BMR by dividing each BMR measurement by M_b^b using the above scaling exponent for the season of interest (e.g. winter mass-specific $BMR = BMR/M_b^{0.49}$).

To estimate the limits of the TNZ (LCT or UCT) of focal individuals, we obtained 3-min averages of $\dot{V}O_2$ and corresponding T_a for each individual throughout the experiment, generating a series of paired T_a and $\dot{V}O_2$ measurements. We then identified inflection points in the relationship between T_a and $\dot{V}O_2$ using piecewise linear regression (R package 'segmented'; Muggeo 2009). As a proxy for thermal conductance, we also calculated the slope of the relationship between T_a and $\dot{V}O_2$ below the LCT (LCT_{slope}). While this method can underestimate minimal thermal conductance (McNab 1980), we still included the metric in the interest of comparing energy costs of thermoregulation below the LCT. TNZ breadth of each species was calculated as the difference between the mean UCT and mean LCT (TNZ = UCT – LCT; Khaliq et al. 2014). Due to the aforementioned relationship between M_b and BMR, we also tested for associations between M_b and a) LCT, b) UCT, and c) TNZ breadth in birds measured in both winter and summer. However, we did not detect any significant relationships between M_b and any of the three traits in either season (all $p > 0.05$; Supplementary material Appendix 1 Table A1), so we did not control for the influence of M_b on LCT, UCT or TNZ breadth in subsequent analyses.

Quantifying seasonal acclimatization in thermoregulatory traits

Phenotypic flexibility is defined as reversible, within-individual phenotypic changes across a range of environmental conditions (Piersma and Drent 2003). Due to the logistical challenges associated with recapturing individual birds across seasons, we were unable to obtain within-individual measurements across seasons for most of the individuals within our dataset. Instead, we compared differences in species-level trait values among individuals sampled across seasons.

To quantify seasonal acclimatization in thermoregulatory traits, we calculated species-specific winter/summer (W/S) ratios for M_b, BMR, TNZ breadth and LCT_{slope} (i.e. we divided the mean winter trait value by the mean summer trait value; McKechnie 2008). W/S ratios may be equal to 1 (indicates that the trait does not change seasonally), greater than 1 (indicates that the trait value increases in winter), or less than 1 (indicates that the trait value decreases in winter).

Statistical analyses

Phylogenetic generalized least squares regressions

To account for the influence of phylogeny on thermoregulatory traits, we generated a phylogeny (Supplementary material Appendix 1 Fig. A4) by pruning a maximum likelihood avian phylogenetic tree (Burleigh et al. 2015) with the 'drop.tip' function in the R package 'ape' (Paradis et al. 2004) to include all focal species. We then employed our phylogeny to conduct phylogenetic generalized least squares (PGLS) regressions. To assess phylogenetic signal in our dataset, we employed Pagel's λ (Pagel 1999) – a metric of phylogenetic signal ranging from 0 (no phylogenetic signal) to 1 (species' traits covary in direct proportion to their shared evolutionary history) (Freckleton et al. 2002). We estimated

Table 1. Results of PGLS regression models testing for differences between temperate (n = 6) and tropical (n = 41) species in winter/summer (W/S) ratios of body mass (M_b), whole-animal and mass-adjusted BMR, LCT_{slope} (a proxy for thermal conductance) and TNZ breadth. The phylogenetic signal (λ), F-value, p-value and R^2 are listed for each model. Bold font indicates a significant p-value (α = 0.05). λ fixed at 1 (maximum phylogenetic signal) for TNZ and LCT_{slope} because PGLS models did not converge.

| Model | W/S ratio ± SD | | λ | F-value (df₁, df₂) | p-value | R^2 |
	Temperate	Tropical				
M_b	1.05 ± 0.07	0.98 ± 0.06	0.46	3.30 (1.45)	0.08	0.07
BMR	1.11 ± 0.06	0.99 ± 0.06	0.71	3.62 (1.45)	0.06	0.05
$BMR_{massadj}$	1.15 ± 0.14	1.06 ± 0.12	0.73	2.16 (1.45)	0.15	0.05
LCT_{slope}	0.64 ± 0.19	1.04 ± 0.19	–	13.10 (1.13)	**0.005**	0.57
TNZ	1.35 ± 0.14	1.04 ± 0.26	–	7.13 (1.13)	**0.02**	0.35

Pagel's λ (Pagel 1999) in the residual error of each regression model while simultaneously estimating regression parameters (Revell 2010) and then scaled regression models using estimates of λ. The maximum-likelihood estimates of λ were moderate to high for most traits (Table 1), justifying our use of PGLS regressions, and we therefore report results from PGLS analyses (following Freckleton 2009).

Characterizing geographic variation in seasonal flexibility of thermoregulatory traits

To test for differences between temperate and tropical species in the direction and magnitude of seasonal flexibility of M_b, BMR, TNZ breadth and LCT_{slope}, we compared W/S ratios using PGLS regressions with sampling locality (tropical versus temperate) as the independent variable.

Relationships between seasonal flexibility in LCT, UCT and TNZ breadth

To determine the relative influence of LCT and UCT on seasonal flexibility in TNZ breadth, we performed bivariate PGLS regressions of ΔLCT and ΔUCT on ΔTNZ breadth. We then compared the two candidate models (ΔLCT ~ ΔTNZ; ΔUCT ~ ΔTNZ) using a corrected Akaike information criterion (AIC_c) for small sample sizes (Hurvich and Tsai 1989). Finally, we conducted a bivariate PGLS regression between ΔLCT and ΔUCT to test for functional linkages between the two traits.

In total, we estimated seasonal acclimatization in one or more of the thermoregulatory traits described above [body mass (M_b), whole-animal basal metabolic rate (BMR), mass-adjusted BMR, lower critical temperature (LCT), slope of BMR below the LCT (LCT_{slope}), upper critical temperature (UCT) and thermoneutral zone (TNZ) breadth] in 365 individuals of 47 species (six temperate, 41 tropical) (Supplementary material Appendix 1 Table A2). Because we sampled far fewer temperate species, we used Levene's tests (R package 'car'; Fox and Weisberg 2011) to test for heteroscedasticity in trait variances of tropical and temperate species within each season. We found no evidence of heteroscedasticity in either winter or summer in any of the thermoregulatory traits (Supplementary material Appendix 1 Table A3), indicating that the sample size disparity between temperate and tropical species was not influencing the validity of our inferences about potential differences in seasonal acclimatization. Because many of the focal species we sampled had small

sample sizes, we also re-ran all analyses for the subset of focal species with n ≥ 3 individuals (n = 18 species; Supplementary material Appendix 1 Table A4) to control for the effect of sample size on model effect sizes and associations. We found quantitatively similar results for both datasets, and so we present results for the full complement of focal species here.

Data deposition

All data are available on GitHub at <https://github.com/henRyPollock/Avian-Seasonal-Thermoregulation_JAB>.

Results

Geographic variation in seasonal acclimatization of thermoregulatory traits

We found that temperate species exhibited a greater magnitude of seasonal acclimatization in TNZ breadth than tropical species (Table 1, Fig. 2a). The direction of seasonal change in TNZ breadth and its component traits was also much more consistent in temperate species than tropical species. For example, five of six temperate species exhibited winter reductions (−6.3 ± 2.87°C) to LCT (Fig. 3a), which were coupled with more modest reductions (−1.95 ± 1.81°C) to UCT (Fig. 3b), resulting in a net increase (+4.77 ± 1.57°C) in winter TNZ breadth (Fig. 3c). Similarly, all temperate species decreased LCT_{slope} (a proxy for thermal conductance) in winter, indicative of reduced heat loss and decreased energy expenditure at lower temperatures (Fig. 3d). In contrast, the direction of seasonal acclimatization in both TNZ breadth and LCT_{slope} was more variable in tropical species (Fig. 3a–d). Although several tropical species showed large seasonal changes in TNZ breadth (e.g. *Euphonia laniirostris* – W/S ratio = 0.58; *Ramphocelus dimidiatus* – W/S ratio = 1.43), most tropical species made only small (~1–3°C) seasonal adjustments to LCT and UCT, resulting in modest mean seasonal change in TNZ breadth (mean W/S ratio = 1.04; Table 1), and the same pattern was evident in LCT_{slope} (mean W/S ratio = 1.04; Table 1).

With respect to the other thermoregulatory traits, five of six temperate species exhibited modest winter increases in M_b (mean W/S ratio = 1.15, range = 0.97–1.12) and both metrics of BMR (whole-animal BMR: mean W/S
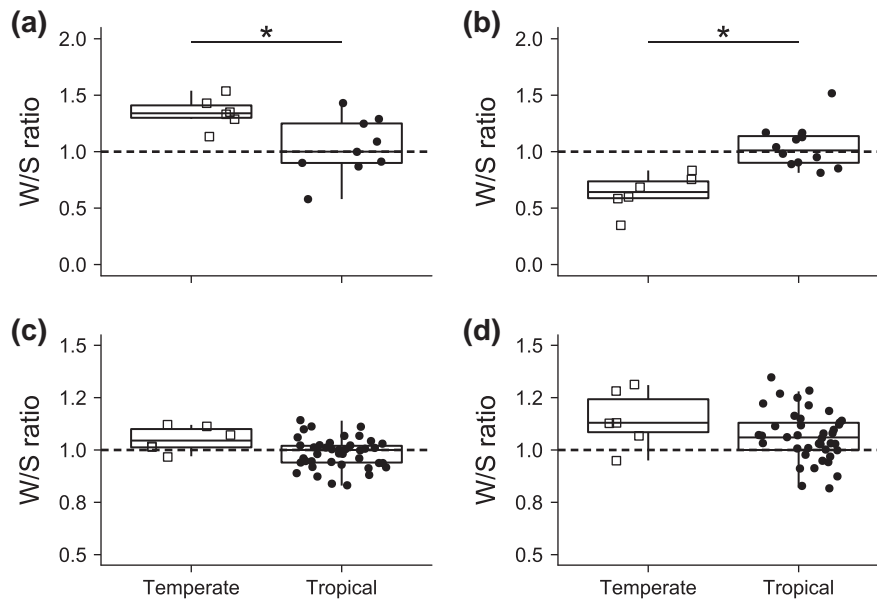
Figure 2. Box plots of winter/summer (W/S) ratios (i.e. the ratio of the winter/summer trait values – a measure of seasonal change) of (a) thermoneutral zone (TNZ) breadth, (b) LCT_{slope} (the slope of the metabolic rate below the LCT), (c) body mass (M_b) and (d) mass-adjusted basal metabolic rate (BMR) in temperate (white squares) and tropical (black circles) species. Dashed line indicates a W/S ratio of 1, i.e. no seasonal change. * $p < 0.05$.
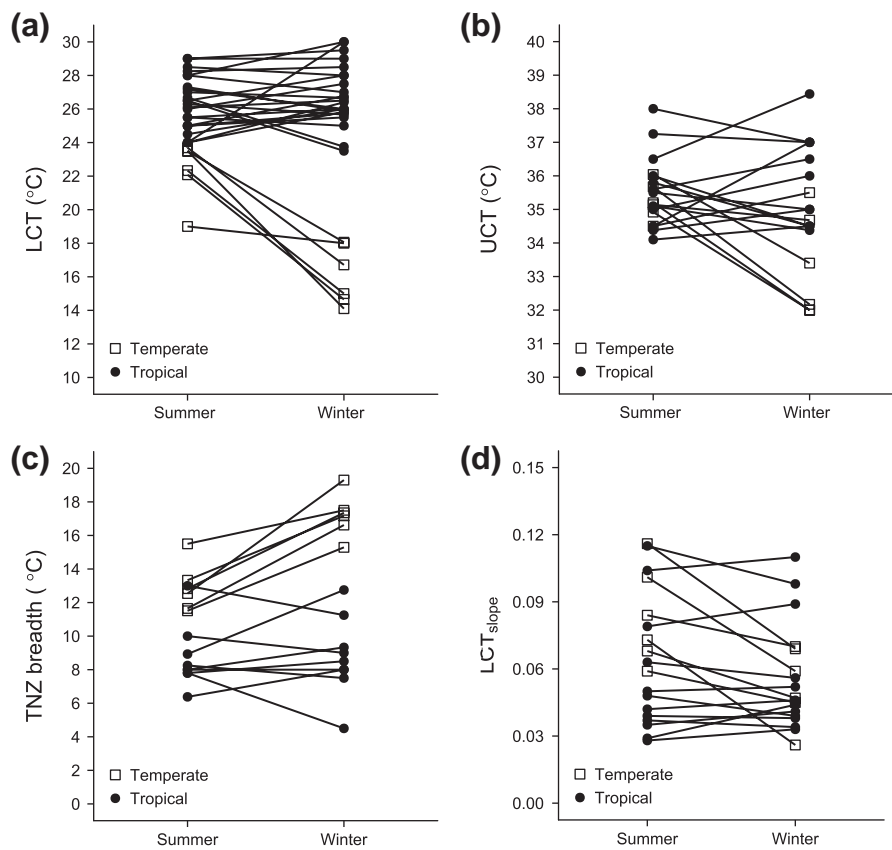

Figure 3. Seasonal change (i.e. winter trait value–summer trait value) in (a) lower critical temperature (LCT; n = 30 species), (b) upper critical temperature (UCT; n = 18 species), (c) thermoneutral zone breadth (TNZ; n = 15 species) and (d) LCT_{slope} (the slope of the metabolic rate below the LCT; units = ml O_2 min^{-1}/°C) of temperate (white squares) and tropical (black circles) species. Means are shown for each species – SE are not shown for clarity's sake but are included in Supplementary material Appendix 1 Table A2.

ratio = 1.11, range = 0.89–1.28; mass-adjusted BMR: mean W/S ratio = 1.15, range = 0.95–1.31). In contrast, tropical species exhibited much greater variance in their seasonal metabolic responses, with some increasing and some decreasing trait values, resulting in mean W/S ratios very close to 1, which is indicative of no seasonal change (M_b: mean W/S ratio = 0.98, range = 0.83–1.14; whole-animal BMR: mean W/S ratio = 0.99, range = 0.71–1.33; mass-adjusted BMR: mean W/S ratio = 1.07, range = 0.65–1.44). As a result of their consistent seasonal responses, the magnitude of seasonal change tended to be higher in temperate species for all three traits (Fig. 2d–f). Nevertheless, because certain tropical species exhibited large seasonal changes in M_b and both metrics of BMR, W/S ratios did not differ significantly between temperate and tropical species (Table 1).

Relationship between seasonal flexibility in LCT, UCT and TNZ breadth

Seasonal acclimatization in TNZ breadth was associated with seasonal change in LCT; UCT was less variable across seasons in both temperate and tropical species. Temperate species in particular exhibited large reductions to LCT in winter that outpaced the reductions to UCT and resulted in a net increase in TNZ breadth. We therefore found a significant linear relationship between ΔLCT and ΔTNZ ($p < 0.0001$, $R^2 = 0.79$; Fig. 4a) but not between ΔUCT and ΔTNZ ($p = 0.06$, $R^2 = 0.13$; Fig. 4b). As a result, ΔLCT was a stronger predictor of ΔTNZ than ΔUCT (ΔAICc = 21.64; Table 2). We also found a moderately strong positive correlation between ΔLCT and ΔUCT ($\lambda = 0.60$, $R^2 = 0.51$, $t = 3.66$, $p = 0.003$) – therefore, species that exhibited larger ΔLCT also tended to exhibit larger ΔUCT.

Discussion

We present the first temperate-tropical comparison of seasonal variation in avian TNZ breadth and vastly expand the data currently available in the literature on seasonal changes in avian M_b and BMR. We found that temperate species exhibited consistent seasonal changes in some thermoregulatory traits, including winter increases in TNZ breadth and reductions to LCT_{slope}, a proxy for thermal conductance. The magnitude of these seasonal changes was greater in temperate species than tropical species, consistent with predictions of the CVH. Patterns of seasonal changes to M_b and BMR were qualitatively similar, although the magnitude of seasonal change did not differ significantly between temperate and tropical species. Overall, the seasonal adjustments made by temperate birds appear to be primarily related to heat conservation. These changes serve to reduce heat loss and are presumably driven by acclimatization to cold winter temperatures, a selective pressure that tropical birds do not face in their relatively aseasonal environments. Nevertheless, tropical birds do appear capable of making seasonal adjustments to thermoregulatory traits (particularly M_b and BMR) and may have more phenotypic flexibility than previously expected (McKechnie et al. 2015). Future studies that explore these patterns in traits directly related to metabolic heat production and cold tolerance (e.g. summit metabolic rate, M_{sum}) will be necessary to provide an integrated picture of seasonal acclimatization in thermoregulatory traits (Fristoe et al. 2015). Furthermore, controlled acclimation experiments will ultimately be needed to fully assess whether differences in seasonal acclimatization between tropical and temperate species represent evolutionary differences in capacity for phenotypic flexibility of thermoregulatory traits.

Geographic variation in seasonal acclimatization of thermoregulatory traits

Temperate species exhibited large (up to 9°C) reductions in LCT and moderate (~2–3°C) reductions in UCT in winter, resulting in a net increase in winter TNZ breadth (~2–7°C). In contrast, tropical species exhibited idiosyncratic patterns of seasonal variation in LCT, UCT and TNZ breadth. Consequently, the magnitude of seasonal change (as indicated by W/S ratio) in TNZ breadth was significantly greater in temperate species, supporting the central prediction of the
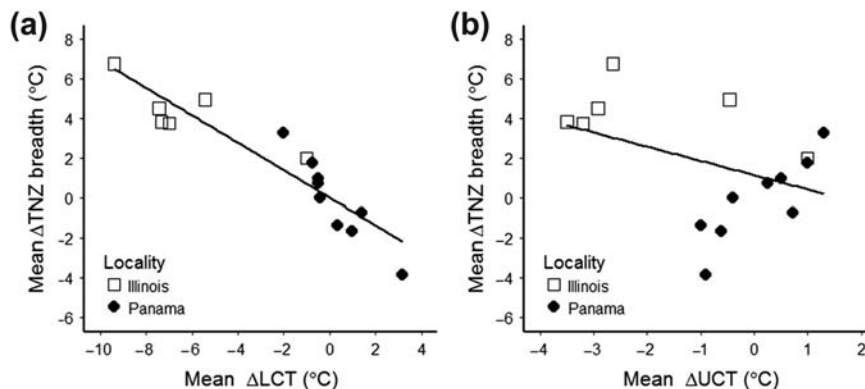

Figure 4. Linear regressions of seasonal change in thermoneutral zone (TNZ) breadth (i.e. ΔTNZ = winter TNZ–summer TNZ) as a function of seasonal change in (a) lower critical temperature (ΔLCT = winter LCT–summer LCT) and (b) upper critical temperature (ΔUCT = winter UCT–summer UCT) of temperate (white squares; n = 6) and tropical (black circles; n = 9) species.

Table 2. Results of PGLS regression models testing for associations between ΔTNZ breadth and (a) ΔLCT and (b) ΔUCT using the Akaike information criterion corrected for small sample sizes (AICc) to rank each model. The F-value, p-value, corrected AIC score (AICc), ΔAICc, model weight (Wt.) and log-likelihood score (LL) are listed for each model. Top model fit indicated in bold.

Trait	λ	F-value (df_1, df_2)	p-value	R^2	AICc	ΔAICc	Wt.	LL
ΔLCT	**0.59**	**50.22 (1.13)**	**< 0.0001**	**0.79**	**53.52**	**0.00**	**1.0**	**−24.26**
ΔUCT	0.68	1.94 (1.13)	0.06	0.13	75.16	21.64	0.0	−35.08

CVH (Bozinovic et al. 2011). Previous studies investigating seasonal change in TNZ breadth or its component traits have yielded mixed results. For example, several studies have found seasonal acclimatization in TNZ breadth of certain bird species (Maddocks and Geiser 2000, Bush et al. 2008), including winter reductions to LCT (Bush et al. 2008, Thompson et al. 2015, Wu et al. 2015), while other studies have found little seasonal variation in TNZ breadth (Nzama et al. 2010). Our data suggest that the avian TNZ is a flexible trait and that the magnitude of seasonal change in TNZ breadth is correlated with the magnitude of temperature seasonality that birds experience.

Increases in TNZ breadth should reduce the energetic costs of thermoregulation by extending the range of ambient temperatures at which an organism does not need to expend energetic resources to maintain temperature homeostasis (McNab 2002). We thus interpret winter increases in TNZ breadth of temperate species as an energy-savings mechanism that functions to reduce the energetic costs of thermoregulation during winter. This interpretation is bolstered by the fact that reductions to LCT were largely responsible for winter increases in TNZ breadth in temperate species. Although there were also moderate winter reductions to UCT in temperate species, they were far less pronounced that seasonal reductions to LCT. We propose that there is a functional linkage between ΔLCT and ΔUCT, which is supported by the significant ($R^2 = 0.51$) correlation that we found between ΔUCT and ΔLCT, and posit that winter reductions to UCT are therefore a byproduct of corresponding reductions to LCT.

The mechanism for this functional linkage is likely related to thermal conductance, a measure of ease of heat transfer between an organism and the environment (Scholander et al. 1950, McNab 1980). We found that temperate species exhibited large seasonal reductions in LCT_{slope}, a proxy for thermal conductance, in winter. The coordinated seasonal changes in TNZ breadth and LCT_{slope} presumably are likely a function of changes in feather insulation that serve to reduce heat loss and promote energy savings in cold winter temperatures. Indeed, a recent study found that total feather mass and the density of downy feathers was greater in species inhabiting colder environments (Osváth et al. 2018), suggesting that changes in feather insulation is a key part of tolerating low temperatures in temperate birds. Nevertheless, rigorous estimation of thermal conductance requires measuring body temperature during thermal trials (Herreid and Kessel 1967), which we were unable to do due to space constraints within the temperature cabinet. Therefore, although our results suggest that reductions in thermal conductance are a key part

of the seasonal acclimatization response in temperate birds, body temperature data will be necessary to validate this trend. Furthermore, winter increases in TNZ breadth and decreases in LCT_{slope} are primarily related to heat loss, and we did not investigate traits related to metabolic heat production. For example, such as M_{sum} have been directly linked to fitness in endotherms (Hayes and O'Connor 1999, Petit et al. 2017) and are an important part of the seasonal acclimatization response that we did not investigate here. The only study to compare seasonal acclimatization in M_{sum} between temperate and tropical birds found that temperate species had larger W/S ratios (McKechnie et al. 2015), consistent with our results. However, more data are needed to assess the generality of these conclusions, as they are based on data from fewer than 10 tropical species.

Most temperate species exhibited modest increases in winter M_b and both metrics of BMR, consistent with previous studies of seasonal acclimatization in temperate-zone birds (McKechnie 2008). Mean winter increases of both whole-animal (10.5%) and mass-adjusted (14.5%) BMR of temperate species suggest that the mechanism of seasonal change is related to increases in both metabolically active tissue (i.e. central organ masses) and cellular aerobic capacity (Swanson 2010). However, there was substantial variation among both temperate (W/S ratio range: 0.89–1.31) and tropical (W/S ratio range: 0.71–1.33) species, perhaps because BMR is a complex trait influenced by a suite of life history and environmental variables (reviewed by Glazier 2015) including precipitation (White et al. 2007), net primary productivity (Mueller and Diamond 2001), and diet (Naya et al. 2013). Our results corroborate a recent meta-analysis (McKechnie et al. 2015) that found that W/S ratios for BMR did not differ between temperate and tropical bird species, contrary to previous analyses that suggested that temperate species had greater seasonal flexibility in BMR (Smit and McKechnie 2010). Firstly, our data demonstrate that not all temperate species exhibit seasonal acclimatization in BMR (Dawson and O'Connor 1996, McKechnie 2008). Instead, increases in BMR of temperate species in winter are potentially a byproduct of the enhanced thermogenic machinery required to maintain a higher M_{sum} (Vézina et al. 2007, Swanson 2010, Stager et al. 2016, Swanson et al. 2017). Secondly, we confirm that tropical bird species are capable of seasonal flexibility in thermoregulatory and metabolic traits (McKechnie et al. 2015). It is unclear why tropical species exhibit greater variability in BMR, although McKechnie et al. (2015) suggested a greater diversity of metabolic niches at lower latitudes as the mechanistic basis for this pattern. Regardless of the mechanism, our data clearly

show that ambient temperature is not the sole driver of seasonal responses in metabolic traits, and further investigation of seasonal metabolic adjustments in tropical birds will be necessary to validate this hypothesis.

Overall, our findings indicate that tropical birds have narrower TNZs and exhibit more modest seasonal acclimatization than their temperate counterparts. In general, species with narrow thermal tolerances (Deutsch et al. 2008, Huey et al. 2009, 2012) and low physiological flexibility (Calosi et al. 2008, Somero 2010) are predicted to be most sensitive to environmental change, and our study indicates that both of these characteristics may apply to tropical birds. However, we were unable to control the environmental conditions to which focal species were exposed. The patterns we observed could be attributed simply to acclimatization to different temperature regimes, and it remains possible that tropical species possess an equivalent capacity for seasonal acclimatization as temperate species. For example, Maldonado et al. (2009) found no seasonal differences in BMR of rufous-collared sparrows *Zonotrichia capensis* captured in winter and summer, but wild-caught birds that were then acclimated to 15°C in the laboratory exhibited elevated BMR, indicating a capacity for flexibility in BMR that was not revealed from field experiments. Conversely, laboratory experiments may recapitulate patterns documented in field-acclimatized birds – for example, populations of rufous-collared sparrows *Zonotrichia capensis* from stable environments had less flexibility in BMR in controlled acclimation experiments than populations from more variable environments (Cavieres and Sabat 2008), suggesting that differences in the field may be maintained in the laboratory setting. Therefore, acclimation and common garden experiments will be necessary to directly measure the acclimatization capacity of thermoregulatory traits. Such data will be critically important for accurately predicting whether tropical birds are under greater thermal constraints, and as a result, more vulnerable to future climate change (Şekercioğlu et al. 2012).

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Supplementary material (available online as Appendix jav-02067 at <www.avianbiology.org/appendix/jav-02067>). Appendix 1.