

# Disentangling environmental drivers of metabolic flexibility in birds: the importance of temperature extremes versus temperature variability

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Examining physiological traits across large spatial scales can shed light on the environmental factors driving physiological variation. For endotherms, flexibility in aerobic metabolism is especially important for coping with thermally challenging environments and recent research has shown that aerobic metabolic scope [the difference between maximum thermogenic capacity ( $M_{\text{sum}}$ ) and basal metabolic rate (BMR)] increases with latitude in mammals. One explanation for this pattern is the climatic variability hypothesis, which predicts that flexibility in aerobic metabolism should increase as a function of local temperature variability. An alternative explanation is the cold adaptation hypothesis, which predicts that cold temperature extremes may also be an important driver of variation in metabolic scope. To determine the thermal drivers of aerobic metabolic flexibility in birds, we combined data on metabolic scope from 40 bird species sampled across a range of environments with several indices of local ambient temperature. Using phylogenetically-informed analyses, we found that minimum winter temperature was the best predictor of variation in avian metabolic scope, outperforming all other thermal variables. Additionally,  $M_{\text{sum}}$  was a better predictor of latitudinal patterns of metabolic scope than BMR, with species inhabiting colder environments exhibiting increased  $M_{\text{sum}}$  over their counterparts in warmer environments. Taken together, these results suggest that cold temperature extremes drive latitudinal patterns of metabolic scope via selection for enhanced thermogenic performance in cold environments, supporting the cold adaptation hypothesis. Temperature extremes may therefore be an important selective pressure driving macrophysiological trends of aerobic performance in endotherms.

Macrophysiology – the study of physiological trait variation over large spatial and temporal scales (Chown et al. 2004, Gaston et al. 2009) – can provide key insights into both pattern and process in physiological adaptation. A central question in macrophysiology is how geographic variation in abiotic conditions can shape patterns of physiological trait variation within and among species (Gaston et al. 2009, Bozinovic et al. 2011). Temperature variation is one abiotic selective pressure that is frequently invoked to explain macrophysiological patterns. For instance, latitudinal variation in temperature can drive spatial patterns of variation in metabolic rates (Kendeigh and Blem 1974, Lovegrove 2003, Wiersma et al. 2007). Many metabolic parameters increase with latitude in both birds and mammals, suggesting that variation in the thermal environment is an important selective pressure driving the evolution of metabolic traits in

endotherms (Lovegrove 2000, 2003, Rezende et al. 2004, Anderson and Jetz 2005, Wiersma et al. 2007, Swanson and Garland 2009).

In response to environmental variation, individuals often make reversible changes in phenotypic traits to maximize survival and fitness. This phenomenon, known as phenotypic flexibility, is ubiquitous and important in determining organismal resilience to changing environmental conditions (Piersma and Drent 2003). Phenotypic flexibility in aerobic metabolism is likely to be critical for endotherms coping with thermally challenging environments. Endotherms dynamically adjust their metabolic rates to maintain a constant body temperature (McNab 2002). As a result, phenotypic flexibility in aerobic metabolism underlies both short-term metabolic changes in response to acute thermal stressors (Cortés et al. 2015) and metabolic acclimatization to longer-term temperature changes (Swanson 2010).

Flexibility in aerobic metabolism may be particularly important for organisms inhabiting thermally variable environments

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(Swanson 2010, Sgueo et al. 2012). Both birds (Swanson and Garland 2009) and mammals (Rezende et al. 2004) exhibit seasonal increases in summit metabolic rate ( $M_{\text{sum}}$ ; a metric of thermogenic capacity), which enhances their tolerance of cold temperatures; however, these seasonal increases in  $M_{\text{sum}}$  are often accompanied by concomitant increases in basal metabolic rate (BMR; minimum metabolic rate). These increases in BMR may represent a cost of maintaining the metabolic machinery required for increased thermogenic capacity (Dutenhoffer and Swanson 1996). Maintaining a high  $M_{\text{sum}}$  and its associated increases in BMR may thus be energetically inefficient during less thermally challenging periods. Phenotypic flexibility in aerobic performance may therefore allow endotherms to optimize their thermoregulatory capacities by minimizing the costs associated with thermogenic organ maintenance.

Consistent with these expectations, recent empirical studies have documented macrophysiological trends in the flexibility of aerobic performance. Metabolic scope – the difference between  $M_{\text{sum}}$  and BMR – provides a snapshot of the capacity of an organism to rapidly adjust metabolic output to meet an energetic challenge. In rodents, metabolic scope has been found to increase with latitude (Naya et al. 2012), which, in turn, is likely related to latitudinal variation in ambient temperature (Swanson and Olmstead 1999, Lovegrove 2003, Rezende et al. 2004, Swanson and Garland 2009).

Two prominent hypotheses have been proposed to explain latitudinal variation in metabolic scope in relation to ambient temperature: the climatic variability hypothesis and the cold adaptation hypothesis. The climatic variability hypothesis predicts that an organism's physiological flexibility will increase as a means of tolerating increased climatic variability (Janzen 1967, Ghalambor et al. 2006). Thus, thermally variable climates associated with high latitudes should select for greater organismal flexibility in metabolic rate (Bozinovic et al. 2011). Alternatively, the cold adaptation hypothesis posits that winter temperature extremes select for increased thermogenic performance in species that inhabit cold climates (Swanson and Garland 2009). This hypothesis predicts that  $M_{\text{sum}}$  will increase with decreasing ambient temperatures, and consequently, that BMR will also increase as a byproduct of the enhanced metabolic machinery required to maintain a higher  $M_{\text{sum}}$  (Swanson 2010). If cold-induced increases in  $M_{\text{sum}}$  outpace correlated increases in BMR, they could drive expansions in metabolic scope, leading to latitudinal trends in metabolic flexibility. Consistent with this hypothesis, temperate-zone birds (Wiersma et al. 2007) and mammals (Bozinovic and Rosenmann 1989) exhibit higher  $M_{\text{sum}}$  values than their tropical counterparts, and BMR also increases in cold climates in endotherms (Lovegrove 2000, 2003, Rezende et al. 2004, Wiersma et al. 2007). Similarly,  $M_{\text{sum}}$  is most strongly correlated with minimum ambient temperature in a suite of rodent species (Rezende et al. 2004). Nonetheless, patterns of geographic variation in avian metabolic scope have not been thoroughly explored.

Related questions regarding the physiological and mechanistic underpinnings of latitudinal patterns in metabolic scope also remain unanswered. Specifically, it is not clear whether geographic variation in avian metabolic scope is driven primarily by modifications to BMR,  $M_{\text{sum}}$  or both.

Decomposing metabolic scope into its individual component traits can provide further insight into its thermal drivers. For example, while the climatic variability hypothesis predicts that metabolic scope should increase with thermal variability, it makes no specific predictions about whether these changes are driven by modifications to  $M_{\text{sum}}$ , BMR, or both. In contrast, if  $M_{\text{sum}}$  is the best predictor of variation in metabolic scope, it would support the cold adaptation hypothesis and suggest that cold temperature extremes are selecting for increased  $M_{\text{sum}}$  (Swanson and Garland 2009, Swanson 2010), which in turn would generate the observed latitudinal increases in metabolic scope. Under this scenario, BMR would also increase with latitude due to its functional linkage with  $M_{\text{sum}}$  (Dutenhoffer and Swanson 1996, Rezende et al. 2004), but would be a poorer predictor of variation in metabolic scope compared to metrics of minimum temperature extremes.

To understand the thermal and metabolic drivers of geographic variation in avian metabolic scope, we examined associations between metabolic scope and 1) indices of ambient temperature and 2) its component traits (BMR and  $M_{\text{sum}}$ ) in 40 bird species. Using these data, we show that 1) minimum temperature is the best predictor of variation in avian metabolic scope and 2) that patterns of geographic variation in metabolic scope are primarily driven by increases in  $M_{\text{sum}}$  in species that occur at high latitudes. Together, these results suggest that minimum temperature extremes are an important selective pressure driving variation in avian metabolic scope.

## Methods

### Metabolic data collection

We performed an exhaustive search of the literature for publications including avian basal and cold-induced summit metabolic rates using ISI Web of Science and Google Scholar for combinations of the following search terms: 'bird', 'avian', 'metabolic scope', 'summit metabolic rate', and 'thermogenic capacity'. When we located publications that reported raw data for these measures, we applied the following criteria for inclusion within our dataset: 1) in accordance with McKechnie and Wolf (2004), we characterized BMR as the minimum metabolic rate of a post-absorptive individual in its rest phase and within its thermoneutral zone; 2) in accordance with Swanson et al. (1996), we characterized  $M_{\text{sum}}$  as the maximum cold-induced oxygen consumption elicited by either sliding cold exposure (birds exposed to a decreasing series of temperatures) or static cold exposure (birds exposed to a single temperature within a range of temperatures) in a helium-oxygen atmosphere (the higher convective capacity of helium facilitates rapid heat loss at moderate ambient temperatures) resulting in hypothermia (Supplementary material Appendix 1, Table A1); 3) we restricted our analysis to datasets wherein both BMR and  $M_{\text{sum}}$  were measured within the same study (and often within the same individual); and 4) we restricted our analysis to datasets wherein metabolic measurements were made within 48 h of capture, as captivity can influence metabolic rate (Swanson and King 2013). This resulted in 20 published studies, which included

40 avian species from 13 localities spanning 82° of absolute latitude (48°N–34°S). From these studies, we also extracted data on body mass ( $M_b$ ), sampling locality, and sampling period (the span of dates during which birds were assayed; Supplementary material Appendix 1, Table A1). All metabolic rates are reported in  $\text{ml O}_2 \times \text{min}^{-1}$ . When metabolic rates were reported in watts, we converted watts to  $\text{ml O}_2 \times \text{min}^{-1}$  using equations provided in Schmidt-Nielsen (1997).

## Scaling

Avian metabolic rates (MR) typically scale allometrically with  $M_b$ . This relationship is described by the equation  $\text{MR} = aM_b^b$ , where  $a$  is the y-intercept and  $b$  is the scaling exponent. In interspecific analyses, reported values of  $b$  are usually within the range of 0.65–0.75 for BMR and 0.56–0.83 for  $M_{\text{sum}}$  (McKechnie and Swanson 2010). The allometric exponents for our dataset were  $b = 0.54$  for BMR and  $b = 0.53$  for  $M_{\text{sum}}$  (derived from non-linear regressions of BMR or  $M_{\text{sum}}$  against  $M_b$ , respectively). These values were likely outside the typical ranges of  $b$  because our dataset was comprised almost exclusively of passerine bird species whose  $M_b$  range (~9–80 g) spanned only one order of magnitude.

To control for the effects of body mass on metabolic rates, a common practice is to regress metabolic rate on body mass and use the residuals of this relationship as data in subsequent analyses. However, when body mass and other variables of interest vary collinearly, this practice can both bias parameter estimates and reduce power to detect significant associations (Darlington and Smulders 2001, Freckleton 2002, 2009). Given the well-established relationship between body mass, latitude, and temperature, we opted to simply analyze mass-specific BMR and  $M_{\text{sum}}$  values, which were calculated by dividing each metabolic measure by  $M_b^b$  using the corresponding  $M_b$  and the above scaling exponent for either BMR or  $M_{\text{sum}}$  (e.g. mass-specific BMR =  $\text{BMR}/M_b^{0.54}$ ). Similarly, we calculated metabolic scope as the absolute difference between uncorrected measures of the upper and lower metabolic limits and divided by  $M_b^b$  using the average  $M_b$  across the two measures and the scaling exponent estimated from these data [ $\text{scope} = (M_{\text{sum}} - \text{BMR})/M_{b\text{Ave}}^{0.50}$ , where  $M_{b\text{Ave}}$  is the mean body mass for individuals used in the BMR and  $M_{\text{sum}}$  trials]. Mass-specific values were used in all subsequent analyses and we refer to them simply as BMR,  $M_{\text{sum}}$  and metabolic scope.

## Climate data

To determine the influence of ambient temperature on variation in metabolic scope, we obtained historic climate data from the NOAA National Climatic Data Center's Climate Data Online (CDO; <[www.ncdc.noaa.gov/cdo-web/](http://www.ncdc.noaa.gov/cdo-web/)>; Supplementary material Appendix 1, Table A1). We retrieved daily thermal data throughout the given sampling period for each study from weather stations located within 50 miles of the respective sampling locality. Although we obtained thermal data from weather stations situated as close as possible to the sampling localities, these data can only approximate the ambient temperature conditions experienced by birds at

the capture sites. Additionally, even within sites, variation in microclimate may be substantial and birds may select microclimates that differ significantly from average climatic conditions of the site (Walsberg 1993), although recent studies have found limited evidence of microclimatic selectivity in birds (Champlin et al. 2009, Pollock et al. 2015). Since we were limited by the historic data that are available, we acknowledge that the thermal data that we used are only an approximation of the operative environmental temperatures (sensu Bakken 1992) that birds actually experienced prior to metabolic measurements.

The weather stations included data for an average of 96% of the complete dates within the sampling period (range = 61–100%). In many cases the original sources cited coarse sampling dates. If the sampling period was not specified to the day, but rather the month (e.g. 'March') or a portion of the month (e.g. 'early March'), we included all dates within the month or the first half of the month, respectively. If the year was not specified, we contacted the original authors to obtain this information.

From these daily temperature data, we calculated absolute minimum temperature ( $T_{\text{min}}$ ; the lowest of the daily minimum temperatures), mean minimum temperature ( $T_{\text{Umin}}$ ; the average of the daily minimum temperatures), absolute maximum temperature ( $T_{\text{max}}$ ; the highest of the daily maximum temperatures), mean maximum temperature ( $T_{\text{Umax}}$ ; the average of the daily maximum temperatures), and mean temperature ( $T_{\text{med}}$ ; the average of the daily mean temperatures) across the sampling period in which the birds were assayed. Additionally, we included several metrics of temperature variability: absolute latitude ( $\text{Lat}_{\text{abs}}$ ); corrected latitude ( $\text{Lat}_{\text{cor}}$ ; absolute latitude + (elevation/76.25); Price et al. 1998); seasonal temperature range (TSR; the difference between  $T_{\text{max}}$  and  $T_{\text{min}}$ ); daily temperature range (TDR; the mean difference between the daily minimum and maximum temperatures across the sampling period); standard deviation ( $T_{\text{sd}}$ ; the standard deviation of  $T_{\text{med}}$ ); and standard error ( $T_{\text{se}}$ ; the standard error of  $T_{\text{med}}$ ) in order to control for inherent differences in the lengths of each sampling period.

## Phylogeny construction

We constructed a phylogeny for the sampled taxa (Fig. 1) using gene sequences for three mitochondrial and six nuclear loci available on Genbank (Supplementary material Appendix 1, Table A2). Sequences were aligned in MUSCLE (Edgar 2004), checked by eye, and then concatenated with SequenceMatrix (Vaidya et al. 2011). Gaps resulting from uneven sequence lengths and missing loci were coded as missing data. Three species (*Euplectes orix*, *Ramphocelus dimidiatus*, and *Vireo flavoviridis*) were data deficient, with short sequences and few loci, so three closely related species with more sequence data (*Ploceus cucullatus*, *Ramphocelus carbo*, and *Vireo olivaceus*, respectively) were added to the dataset to strengthen their placement in the tree. Model selection run in TOPALI ver. 2.5 (Milne et al. 2009) using PhyML (Guindon and Gascuel 2003) selected GTR+gamma as the most appropriate model of molecular evolution for the dataset by hierarchical likelihood ratio tests and the Bayesian information criterion. We then estimated the phylogeny

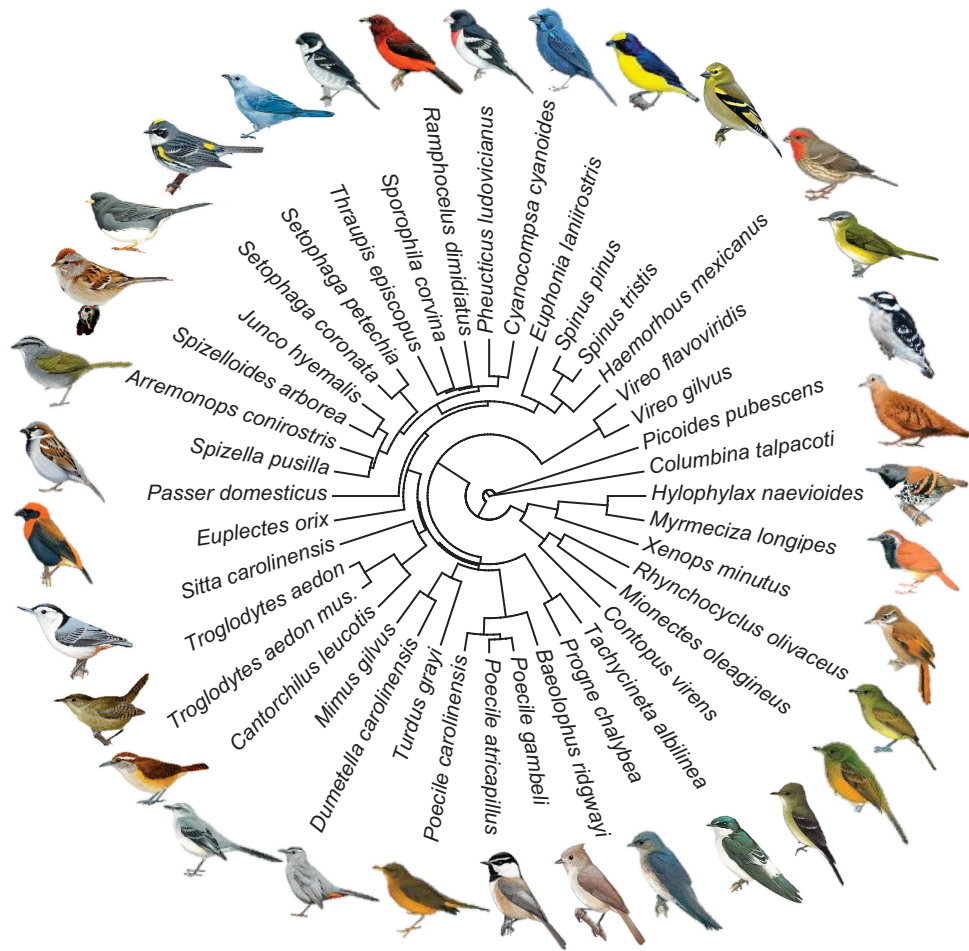


Figure 1. Phylogeny of the 40 avian species used in the analysis and illustrations of representative taxa. Illustrations are reproduced with permission from Handbook of the birds of the World alive (del Hoyo et al. 2014).

using maximum likelihood with the program RAXML (Stamatakis 2006) as implemented in raxmlGUI (Silvestro and Michalak 2012). Using the GTR+ gamma model, we assigned each locus to a separate partition, performed rapid bootstrapping with 1000 pseudoreplicates, and retained the best-scoring tree for our analyses.

The best-scoring tree recovered relationships that are well supported in the literature (Hackett et al. 2008, McCormack et al. 2013, and Jarvis et al. 2014 for higher relationships; Barker et al. 2013 and Ericson et al. 2014 for inter-family relationships in Passeriformes), with two main exceptions. First, relationships among five major clades of Passeriformes (Certhioidea, Muscicapoidae, Sylvioidea, Paroidea, and Passeroidea) were poorly resolved in our tree, with very short internodes and low bootstrap support. Phylogenetic comparative methods are robust to topological error when the branch lengths involved are short (Stone 2011), and there is no consensus in the literature for the relationships among these groups (short internodes with low support are commonly found; Barker et al. 2004, Irestedt and Ohlson 2008, Johansson et al. 2008, Nabholz et al. 2010, Ericson et al. 2014), so we used the recovered relationships in our analyses. Second, our tree also failed to recover a monophyletic Thraupidae (sensu Burns et al. 2014). Phylogenetic regression methods are sensitive to topological error near the tips

of the tree (Symonds 2002), so we reanalyzed the data in RAXML using identical run parameters except with a monophyletic Thraupidae enforced by a constraint tree. Except for the changes enforced by the constraint tree, the resulting best-scoring tree (Supplementary material Appendix 1, Fig. A1) had the same topology as the unconstrained phylogeny. We then used this constrained RAXML topology to generate an ultrametric tree in the software r8s ver. 1.80 (Sanderson 2003) by fixing the root to an arbitrary age of 1, then using penalized likelihood with the TN algorithm and an optimal smoothing parameter of 1 (as determined by cross-validation). This ultrametric tree was used in subsequent analyses.

## Statistics

We employed our phylogeny in phylogenetically generalized least squares (PGLS) analyses to examine associations between temperature variables and metabolic measures (scope, BMR, or  $M_{sum}$ ) while controlling for phylogenetic non-independence. We repeated the same procedure to independently test for associations between scope and  $M_{sum}$  and BMR to gain insight into the relative importance of each metabolic trait in driving patterns of metabolic scope variation. To do this,

we performed PGLS regressions with sampling error using the function `ppls.Ives` (in package `phytools`; Revell 2012) in program R (ver. 3.0.2 – R Development Core Team), which accounts for intraspecific variation resulting from multiple trait values for a given tip taxon (Ives et al. 2007). We first standardized all variables by subtracting the mean and dividing by two standard deviations (Gelman 2008) to enable direct comparisons among variables and eliminate any potential scaling problems [e.g. given that  $M_{sum}$  is several times greater than BMR, without standardization it would be expected to have a higher correlation with metabolic scope than BMR]. We then used standardized variables in bivariate PGLS regressions to test for associations between each response and predictor variable independently. We did not perform multivariate regressions due to high correlations between many of the predictor variables (e.g.  $T_{min}$  and  $T_{med}$ :  $r = 0.98$ ), in addition to the fact that, to our knowledge, PGLS with sampling error has not yet been implemented for multivariate regression in R. We estimated Pagel's  $\lambda$  (a metric of phylogenetic signal; Pagel 1999) in the residual error of each regression model while simultaneously estimating regression parameters (Revell 2010), took the average value over 50 iterations, and then scaled models using this optimized value of  $\lambda$ . We compared competing models using a corrected Akaike information criterion ( $AIC_c$ ) for small sample sizes (Hurvich and Tsai 1989) using the formula  $AIC_c = -2(\log\text{-likelihood}) + 2k(n/(n - k - 1))$ , where  $k = 4$  parameters and  $n = 73$  metabolic measures. Methods for the corresponding ordinary least squares (OLS) regressions, as well as complimentary OLS models including mass as a covariate, are presented in the Supplementary material Appendix 1, Table A3 and A4. Because many of the original sources did not report error estimations for metabolic data, we did not attempt to use meta-analytic techniques to estimate the effect sizes of each study.

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.8qk1v>> (Stager et al. 2015b).

Table 1. Comparison of PGLS regressions. Each of the standardized environmental variables (Var.) was used in a bivariate PGLS regression with one of the three metabolic measures (Scope,  $M_{sum}$ , BMR) while simultaneously estimating phylogenetic signal ( $\lambda$ ) in the residuals. Additionally, the explanatory powers of  $M_{sum}$  and BMR were tested on variation in metabolic scope. The slope ( $\beta$ ), corrected AIC ( $AIC_c$ ), and delta  $AIC_c$  ( $\Delta$ ) are listed for each. Best model fits indicated in bold.

Var.	Scope				$M_{sum}$				BMR			
	$\lambda$	$\beta$	$AIC_c$	$\Delta$	$\lambda$	$\beta$	$AIC_c$	$\Delta$	$\lambda$	$\beta$	$AIC_c$	$\Delta$
Lat <sub>abs</sub>	0.42	0.66	96.74	27.65	0.33	0.66	94.78	31.96	0.17	0.40	109.71	52.45
Lat <sub>cor</sub>	0.54	0.60	100.15	31.06	0.51	0.60	98.66	35.84	0.45	0.33	111.33	54.07
Long	0.95	-0.13	92.00	22.91	0.95	-0.12	90.00	27.18	0.86	-0.18	84.86	27.60
Elev	0.36	0.27	114.97	45.88	0.39	0.26	113.34	50.52	0.33	0.08	109.87	52.61
$T_{min}$	1.00	-0.99	<b>69.09</b>	<b>0.00</b>	0.97	-0.96	<b>62.82</b>	<b>0.00</b>	0.87	-0.47	78.41	21.15
$T_{umin}$	0.96	-1.05	<b>70.69</b>	<b>1.60</b>	0.91	-1.01	<b>64.58</b>	<b>1.76</b>	0.82	-0.36	76.74	19.48
$T_{max}$	0.75	-1.10	83.82	14.73	0.75	-1.11	78.43	15.61	0.00	-0.61	74.54	17.28
$T_{umax}$	0.92	-1.01	80.00	10.91	0.89	-1.00	73.55	10.73	0.68	-0.60	74.75	17.49
$T_{med}$	0.95	-1.08	74.92	5.83	0.91	-1.06	68.57	5.75	0.73	-0.46	76.21	18.95
TSR	1.00	0.79	80.45	11.36	1.00	0.75	77.95	15.13	0.84	0.28	93.69	36.43
TDR	0.70	1.88	94.02	24.93	0.77	1.88	93.16	30.34	0.63	-0.02	95.40	38.14
$T_{sd}$	0.98	0.66	78.59	9.50	0.96	0.64	74.60	11.78	0.88	0.26	81.65	24.39
$T_{se}$	0.67	1.40	75.88	6.79	0.75	1.33	72.09	9.27	0.85	1.17	<b>57.26</b>	<b>0.00</b>
$M_{sum}$	0.77	1.04	<b>-67.17</b>	<b>0.00</b>								
BMR	0.99	1.06	86.94	154.11								

## Results

As predicted by the cold adaptation hypothesis, variation in metabolic scope was most strongly associated with indices of minimum temperature ( $T_{min}$  and  $T_{umin}$ ; Table 1). Phylogenetically controlled analyses showed a negative correlation between scope and  $T_{min}$  (Fig. 2). This association was primarily driven by increases to  $M_{sum}$  in birds inhabiting colder environments rather than reductions in BMR ( $\Delta AIC_c = 154.11$ ; Table 1; Fig. 3a, b). Similarly, changes in  $T_{min}$  were also strongly negatively correlated with variation in  $M_{sum}$  (Table 1). In contrast, the best predictor of variation in BMR was the standard error of the mean temperature ( $T_{se}$ ), an index of temperature seasonality across the sampling period. Phylogenetic signal was relatively high in the residuals of all temperature variables (Table 1), indicating that phylogenetic correlation was present in the data and thereby validating the use of PGLS regressions. As a result, we restrict the presentation of the OLS analyses to the supplemental material. The PGLS results were largely corroborated in conventional OLS analyses using either mass-corrected metabolic values or mass as a covariate (Supplementary material Appendix 1, Table A3 and A4).

## Discussion

Macrophysiological approaches can shed light on large-scale patterns of physiological trait variation, and the underlying processes that generate them. Recent macrophysiological studies of flexibility in aerobic performance suggest that increased flexibility may be adaptive at higher latitudes (Naya et al. 2012, 2013), but the abiotic selective pressures driving this pattern remain unclear. We used empirical measurements of metabolic scope from 40 bird species to explore the thermal drivers of geographic variation in aerobic metabolic flexibility. Although we found some support for the climatic variability hypothesis – metabolic scope was correlated with some indices of climatic variability – our results

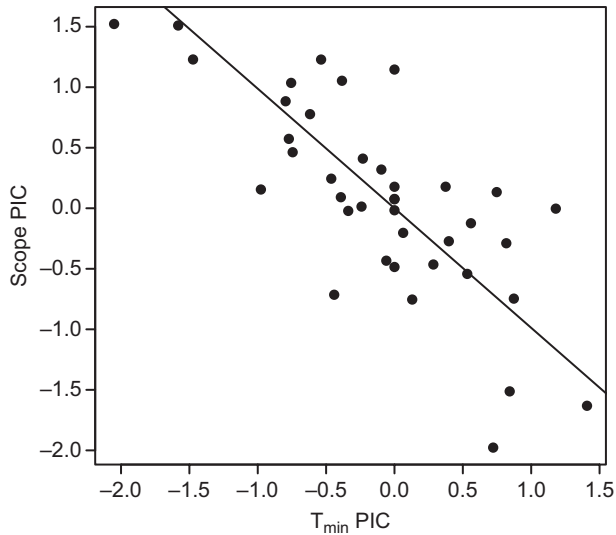


Figure 2. Metabolic scope increases with decreasing temperature. Phylogenetically independent contrasts (PIC) derived from standardized values of metabolic scope and  $T_{\min}$  shown. Contrasts and slope fit using `pgls.Ives` (Revell 2012) in R.

indicate that indices of minimum temperature ( $T_{\min}$  and  $T_{\mu\min}$ ) were the best predictors of variation in avian metabolic scope across latitude, supporting the cold adaptation hypothesis (Swanson and Garland 2009). Despite the high level of covariation between variables,  $T_{\min}$  was the best predictor of both  $M_{\text{sum}}$  and scope, suggesting that minimum temperature extremes are a primary driver of latitudinal patterns in avian metabolic flexibility. However,  $T_{\min}$  was a poor predictor of BMR, which was instead best predicted by an index of temperature seasonality (standard error of the mean temperature;  $T_{\text{se}}$ ). Geographic patterns of metabolic trait variation are therefore likely the result of a complex interplay of environmental factors, including both temperature variability and temperature extremes.

Testing for associations between temperature variables and metabolic parameters is challenging. Such analyses are complicated by covariance among thermal indices and latitude, and by the fact that measures of temperature variability often incorporate temperature extremes. However, it is still possible to decouple these complex relationships through the use of broad geographic sampling. In an effort to differentiate among the various temperature variables that can drive variation in metabolic scope, our analysis included all of the available studies on avian metabolic scope that meet our criteria. Nonetheless,  $\sim 60\%$  of the measurements (44 of 73 scope measures) are derived from two primary localities (Panama;  $9^{\circ}\text{N}$  and South Dakota;  $42^{\circ}\text{N}$ ), which could reduce the explanatory power of latitude as a predictor variable. Additionally, because many of the original studies did not include local temperature data, we had to rely on environmental data gathered at nearby weather stations. As a result, we could not account for small-scale microclimatic variation, and these temperature data only approximate the operative temperatures that birds experienced prior to metabolic measurements. However, this problem applies to all of the thermal variables included in this study. The fact that  $T_{\min}$  still outperforms all other indices of temperature

variability corroborates a clear pattern emerging from recent studies (Naya et al. 2012, 2013): minimum temperature is one of the most important environmental variables influencing  $M_{\text{sum}}$  and metabolic scope in endotherms.

Moreover, our results are consistent with a large body of literature suggesting that cold temperature extremes are a strong selective pressure driving metabolic adaptation in birds. For example, Root (1988a) found that 60% of bird species overwintering in North America had northern range limits associated with minimum mean January temperature (but see Castro 1989, Repasky 1991 for a critique of this analysis) and temperate-zone bird species are expanding their range limits northward in response to increasing temperatures (Hitch and Leberg 2007, La Sorte and Thompson 2007), implying that cold temperatures may constrain their winter distributions. While food availability may ultimately determine an organism's ability to persist in an environment (Root 1988b), many bird species exhibit marked seasonal

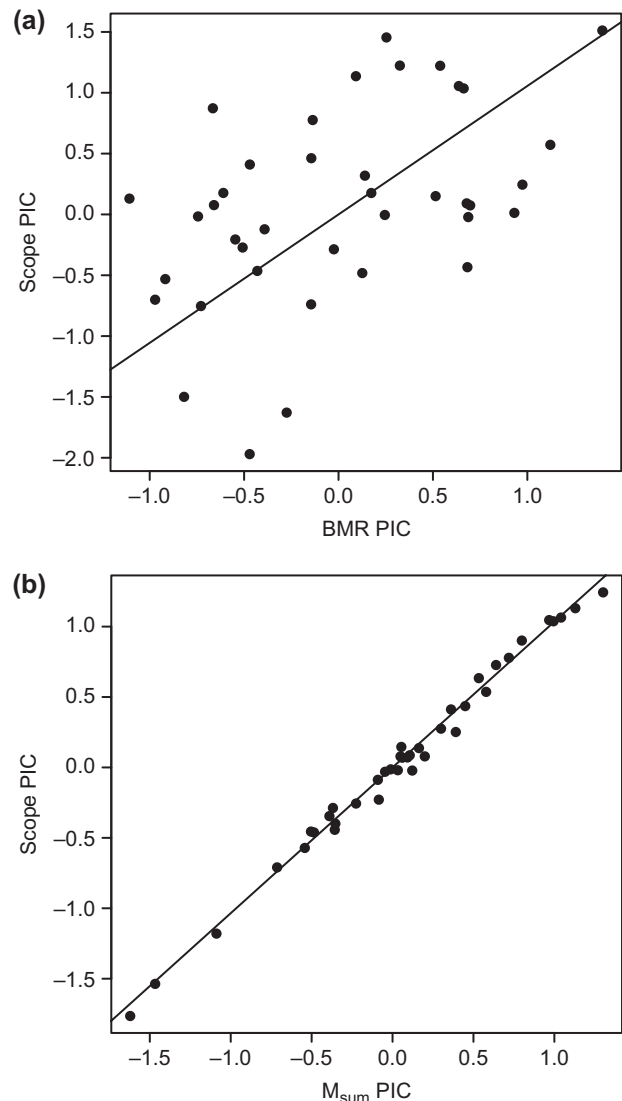


Figure 3. Increases to  $M_{\text{sum}}$  drive increases in metabolic scope. Phylogenetically independent contrasts (PIC) derived from standardized values of metabolic scope and (a) BMR or (b)  $M_{\text{sum}}$  shown. Contrasts and slope fit using `pgls.Ives` (Revell 2012) in R.

increases in  $M_{\text{sum}}$  in response to reduced winter temperatures (Swanson 2010). Given that  $M_{\text{sum}}$  is highly correlated with cold tolerance (Swanson and Garland 2009), these enhancements are likely essential for birds to persist under harsh winter conditions (Swanson 2010). Our analyses of individual trait components suggest that geographic variation in avian metabolic scope is driven by increases to  $M_{\text{sum}}$  in birds that inhabit cold environments. We found a highly significant positive linear relationship between  $M_{\text{sum}}$  and metabolic scope, and interspecific variation in  $M_{\text{sum}}$  was strongly, negatively associated with  $T_{\text{min}}$ .

We also found that BMR increased in colder climates and was positively correlated with metabolic scope, although to a much lesser degree than  $M_{\text{sum}}$ . Parallel increases in  $M_{\text{sum}}$  and BMR could leave metabolic scope unchanged or even reduced. However, our analysis revealed that the relative increase of  $M_{\text{sum}}$  in colder climates greatly outpaced the increase in BMR, resulting in broader metabolic scopes in birds that occupy colder environments. The smaller, concomitant increase in BMR may represent a by-product of the enhancement of metabolic machinery required to sustain high  $M_{\text{sum}}$  (Dutenhoffer and Swanson 1996, Rezende et al. 2004). Taken together, these results suggest that latitudinal trends in avian metabolic scope are driven by a necessary balance between elevated thermogenic capacity (represented by  $M_{\text{sum}}$ ) to cope with cold temperature extremes on one hand, and the pressures to reduce excess maintenance costs (represented by BMR) in colder, less productive environments on the other.

Despite the strong association between minimum temperature and metabolic scope, we did not account for the potential influence of ecological and/or life-history traits that may help to explain residual variation in metabolic scope. In principle, variation in traits such as torpor ability, foraging guild, and activity patterns could influence patterns in metabolic scope. For example, in a recent study across rodent species, metabolic scope was negatively correlated with both  $T_{\text{min}}$  and torpid body temperature, suggesting that selection on the depth and duration of torpor may influence latitudinal patterns of metabolic scope (Careau 2013). However, there are considerable practical limitations to including such traits in macrophysiological studies. First, many life-history traits are continuous variables that often must be simplified into categorical variables for the sake of analysis (e.g. nectarivore vs insectivore), which diminishes their explanatory power. Additionally, we lack detailed knowledge of life-history traits for many species across space and time. In light of these shortcomings – and given the well-established relationship between temperature and  $M_{\text{sum}}$  in endotherms (Rezende et al. 2004, Swanson 2010) – we considered life-history traits to be secondary in importance to temperature variables in explaining geographic variation in scope and did not include them in our analysis. Nevertheless, it is possible that variation in ecological/life-history traits could explain a fraction of the residual variation in interspecific patterns of metabolic scope, and careful consideration of these factors could be a fruitful avenue for future studies.

Metabolic scope is a flexible trait, and the degree to which an individual's scope varies with recent acclimatization history has not been systematically investigated. Ultimately, acclimation studies are needed to determine not only the

physiological and regulatory mechanisms that underlie macrophysiological patterns and their environmental drivers (Stager et al. 2015a), but also to determine the limits of acclimatization capacity in flexible physiological traits like metabolic scope. Determining these limits is of fundamental importance to interpretation of broad-scale macrophysiological patterns in an era of rapid global change. The climatic variability hypothesis has been a prominent conceptual framework for investigating which species may be vulnerable to climatic change (Bozinovic et al. 2011). However, in addition to climatic variability, climatic extremes have been implicated as a driver of both ecological and evolutionary change in natural populations (Parmesan et al. 2000, Hoffmann et al. 2002). Given that climate change is expected to increase the frequency and intensity of extreme weather events (Meehl and Tebaldi 2004), understanding the physiological effects of climatic extremes is becoming increasingly important. Although climatic variability undoubtedly contributes to the evolution of physiological tolerances in a wide range of taxa (Sunday et al. 2011, Khaliq et al. 2014, Sheldon and Tewksbury 2014), our results suggest that in some cases the magnitude of temperature extremes rather than temperature variability per se may be the most important selective pressure driving the evolution of physiological traits that are important in coping with thermal stress.

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Supplementary material (Appendix ECOG-01465 at <[www.ecography.org/appendix/ecog-01465](http://www.ecography.org/appendix/ecog-01465)>). Appendix 1.