

A test of altitude-related variation in aerobic metabolism of Andean birds

Gutierrez-Pinto, Natalia^{1*}, Gustavo A. Londoño², Mark A. Chappell³, and Jay F. Storz¹

1. School of Biological Sciences, University of Nebraska, Lincoln, NE 68588, USA

2. Departamento de Ciencias Biológicas, Facultad de Ciencias Naturales, Universidad Icesi, Cali, Colombia

3. Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, CA 92521, USA

*corresponding author: gutinata@gmail.com

Submitted to Journal of Experimental Biology

Summary statement

We tested for altitude-related variation in aerobic metabolism in species pairs with contrasting elevational ranges. Metabolic rates were significantly higher in most highland species but there was no uniform elevational trend.

Abstract

Endotherms at high altitude face the combined challenges of cold and hypoxia. Cold increases thermoregulatory costs, and hypoxia may limit both thermogenesis and aerobic exercise capacity. Consequently, in comparisons between closely related highland and lowland taxa, we might expect to observe consistent differences in basal metabolism (BMR), maximal metabolism (MMR), and aerobic scope. Broad-scale comparative studies of birds reveal no association between BMR and native elevation, and altitude effects on MMR have not been investigated. We tested for altitude-related variation in aerobic metabolism in 10 Andean passerines representing five pairs of closely related species with contrasting elevational ranges. Mass-corrected BMR and MMR were significantly higher in most highland species relative to their lowland counterparts, but there was no uniform elevational trend across all pairs of species.

Introduction

Endotherms that are native to high-altitude environments must contend with physiological challenges posed by the reduced partial pressure of O₂ (PO_2) and low ambient temperature (T_a). Depending on acclimatization history, reduced PO_2 may compromise the maximum capacities for aerobic exercise (MMR; maximum metabolic rate) due to the reduced availability of O₂ to fuel ATP synthesis (Chappell et al., 2007; Hayes, 1989a; McClelland and Scott, 2019; Storz and Scott, 2019; Storz et al., 2010). BMR may be elevated in highland species due to increased thermoregulatory demands or due to a correlated response to changes in MMR (Hayes and Garland, 1995; Portugal et al., 2016; Rezende et al., 2004). Non-proportional changes in BMR and MMR entail changes in absolute aerobic scope, defined as the difference between the two rates (MMR – BMR), which reflects an animal's capacity to increase its rate of aerobic metabolism above maintenance levels (Bennett, 1991; Hochachka, 1985).

In comparison with lowland relatives, mammals native to high-altitude often have higher mean MMR in hypoxia and suffer a smaller decrement in MMR with increasing hypoxia (Chappell and Dlugosz, 2009; Cheviron et al., 2012; Cheviron et al., 2014; Lau et al., 2017; Lui et al., 2015; Schippers et al., 2012; Storz et al., 2019; Tate et al., 2017; Tate et al., 2020). In addition, when measured at their native elevations, BMR is consistently higher in high-altitude deer mice (*Peromyscus maniculatus*) relative to lowland conspecifics (Hayes, 1989a; Hayes, 1989b), although it is not known to what extent the elevated BMR reflects an evolved change or a reversible acclimatization response. Available evidence for birds suggests that BMR does not vary with elevation, and it is unknown whether MMR exhibits a consistent pattern of altitudinal variation among species.

Studies of highland and lowland populations of rufous-collared sparrows (*Zonotrichia capensis*), measured at their native altitudes, found no differences in BMR (Castro et al., 1985), thermogenic capacity (Novoa et al., 1990), or field metabolic rates (Novoa et al., 1991). A recent study involving a phylogenetically diverse set of more than 250 neotropical bird species found

no significant association between BMR and native elevation (Londoño et al., 2015). However, altitude effects may be more readily detectable in fine-grained comparisons between pairs of closely related species that are native to different elevations but that are otherwise ecologically similar. Moreover, it remains unclear if exercise-induced MMR and aerobic scope exhibit consistent patterns of altitude variation.

We measured BMR, MMR, and aerobic scope in 10 Andean passerines, representing five pairs of closely related species with contrasting elevational ranges (Figure 1A, B). We used a paired-lineage design (Felsenstein, 2004) such that the five pairwise comparisons were phylogenetically independent (Figure 1A).

Materials and Methods

Experimental design

Birds were captured between June and August from 2017 to 2019 at several field sites in the western Andes of Colombia (figure 1A; supplementary Table 1). We compared closely related species that had contrasting elevation ranges (figure 1B) but which are otherwise similar ecologically (supplementary table 2). High elevation species were captured between 2300 and 2500 m; low elevation species were captured between 500 and 1400 m. Annual mean temperature differed between localities by approximately 6°C and ambient pO₂ differed by 1.9 - 4.5 kPa (mean 3 kPa).

Field protocol

We mist-netted birds (aided by playback of vocalizations) during the day (9:00 to 18:00 h). We released juveniles (identified on the basis of plumage or color of bill gape) and adults with brood patches. We measured MMR immediately after capture and subsequently kept birds inside cloth bags, taking them out every two hours to provide water and food, until 4 hours before the onset of BMR measurements (after sunset, around 19:00h), to ensure that the birds were post-absorptive (Karasov and del Rio, 2007). Body mass was measured immediately after capture and also before and after BMR measurements. All procedures were approved by the University of Nebraska IACUC (project ID 1499) and Colombian research permits granted to G.A.L. (Permit 536, May 20/2016).

Respirometry procedures

We used a flow-through respirometry system to measure metabolic rates. Incurrent air was dried with silica gel and the flow was then divided into four metered channels using a FlowBar (Sable Systems, Las Vegas, NV, USA). One channel was used for reference air. The other three supplied air continuously to three metabolic chambers made of acrylic, each equipped with a

thermocouple that measured excurrent air temperature. Another thermocouple measured T_a in the incubator. We measured one to three birds per night and matched incurrent airflow into chambers with the body mass of each tested bird (200–1000 mL min⁻¹ STPD). Excurrent air flows were sampled sequentially by a multiplexer (Sable Systems RM-8). Subsamples of excurrent airflow (50–200 mL min⁻¹) were scrubbed of CO₂ and H₂O (using soda lime and silica gel, respectively) and routed through a Sable Systems FoxBox to measure O₂ content. Each bird was monitored for 15 min and reference air was measured for 2.5 min before switching between individuals; this pattern was repeated until the measurements were finished. T_a was kept constant ($\pm 0.5^\circ\text{C}$) using a PELT-5 controller (Sable Systems). Birds were first measured at $T_a = 34^\circ\text{C}$ and then at $T_a = 30^\circ\text{C}$, remaining at each stable temperature for at least one hour. We recorded T_a , flow rate and O₂ content every second using Warthog LabHelper (www.warthog.ucr.edu) interfaced to a Sable UI-2 A-D converter.

We elicited MMR using forced exercise on a hop-flutter wheel (Chappell et al., 1999). This method reliably elicits behavioral exhaustion, with repeatable MMR (Chappell et al., 1996), but may not elicit maximum power output of the flight muscles. Accordingly, VO₂ values measured with this technique are usually lower than those measured in wind tunnels (Chappell et al., 2011; McKechnie and Swanson, 2010). During exercise trials, incurrent air flow was 750 – 1000 mL min⁻¹, and no multiplexer was used. The chamber was manually rotated until birds reached their maximum rates of oxygen consumption, typically at the beginning of the experiment. All tested birds attained similar levels of behavioral exhaustion during the exercise trials and no birds were injured during experiments.

Data analysis

We calculated metabolic rates using LabAnalyst (www.warthog.ucr.edu). After baseline correction, we used the flow rate (FR; mL min⁻¹ STPD), and the incurrent (FiO₂; 0.2095) and excurrent (FeO₂) oxygen concentrations to obtain VO₂ (mL O₂ min⁻¹), applying the ‘Mode 1’ formula:

$$VO_2 = FR(FiO_2 - FeO_2) / (1 - FeO_2).$$

BMR was computed as the lowest continuous average VO_2 over 5 min during periods of low and stable VO_2 , and the lowest value of the two temperature measurements per bird (30 and 34 °C) was chosen for subsequent analyses. Before obtaining MMR (VO_{2max}), we applied the instantaneous correction (Bartholomew et al., 1981) to compensate for the mixing characteristics of the system (i.e., the blunted response to rapid changes in O_2 concentration). We calculated MMR as the highest continuous averaged VO_2 over 1 minute during periods of high and stable VO_2 values. Finally, we calculated the absolute aerobic scope for each bird as the difference between MMR and BMR.

Statistics

We used log-transformed metabolic rates in interspecific comparisons and we included log-transformed body mass as a covariate in the analyses. For each species and metabolic measurement, we discarded data points that fell outside ± 2 standard deviations from the mean, resulting in the removal of two data points for BMR, six for MMR, and five for aerobic scope.

To evaluate whether the allometric association between mass and VO_2 differed between closely related species, we tried fitting standardized major axis (SMA) regressions between mass and VO_2 for each species using the *smatr* package (Warton et al., 2012) for R v. 3.3.2 (R Core Team, 2012). Since most of the regressions were not significant (results not shown), we followed two different approaches to evaluate the influence of body mass on measured metabolic rates. First, we adjusted linear models to account for the joint influences of elevation and mass on VO_2 . For this, we fitted a linear mixed model (package *lme4*; Bates et al., 2014) with VO_2 as the response variable, elevation (categorical; 1 for high elevation, 2 for low elevation) as a fixed effect, and mass (continuous) per species pair (categorical; 5 levels) as a random effect:

$$VO_2 \sim \text{elevation} + (\text{mass} \mid \text{species pair})$$

This model allows the slope of the relationship between mass and VO_2 to be different for each species group. To further explore whether the effect of elevation differs between species pairs after accounting for mass, we also ran a nested ANCOVA, where the variation in VO_2 is explained by mass and by elevation per group:

$$VO_2 \sim \text{mass} + (\text{species pair} : \text{elevation})$$

Second, in order to better understand the trends observed in the linear models, we mass-corrected each of our observed VO_2 values (BMR, MMR, aerobic scope) by dividing them by M_b^S (Gillooly et al., 2001), where M_b is body mass for each individual and S is the allometric scaling coefficient obtained by McKechnie and Wolf (2004) for passerine birds ($S = 0.667$). We then used t -tests to evaluate the statistical significance of the difference in mass-corrected VO_2 between high- and low-elevation species within each pair. We also ran a *post-hoc* power analysis (package *pwr* v1.3; Champely, 2020) using Hedges' G (Hedges, 1983) to estimate effect sizes with a significance level of 0.05.

Finally, to assess the influence of other variables on metabolic rates, we built linear mixed models that included various combinations of mass and the interaction between species group (categorical; 5 levels) and elevation (categorical; high, low) as fixed factors, and age (categorical; immature, adult), sex (categorical; male, female), molt state (categorical; absent if bird had no developing feathers, moderate if few, abundant if several), and year of capture (2017, 2018, 2019) as random factors. In all mixed effect models the variance explained by any variable other than mass was negligible (usually $\ll 0.1$), and the model that included mass as the only predictor had the lowest AIC ($\Delta AIC = 46$; results not shown).

Results and Discussion

We measured 96 wild-caught birds, with sample sizes of 8-12 individuals per species (Figure 2; supplementary table 3), except for *Turdus serranus* ($n=4$). Both the linear mixed model and the ANCOVA explained a high percentage of variation in the data (average R^2 for mixed model: 0.98; average adjusted R^2 for ANCOVA: 0.69; supplementary tables 4 and 5). Elevation was correlated with BMR, MMR, and aerobic scope, but only after accounting for the effects of species pair and body mass, as evidenced by the difference between the average marginal R^2 (0.006; variance explained by the fixed effect) and the average conditional R^2 (0.98; variance explained by the full model) (supplementary table 4). Likewise, after accounting for mass, elevation also had a significant effect on variation in BMR, MMR, and aerobic scope in each species pair (supplementary table 5), with the exception of BMR in thrushes. In all species pairs other than thrushes, high-elevation species had higher metabolic rates than their lowland counterparts, as indicated by the negative slopes estimated for the effect of elevation on each species group (supplementary table 5).

Our pairwise comparisons of mass-corrected metabolic rates revealed significant differences between high- and low- elevation species in most but not all cases. BMR was significantly higher in high-elevation wrens, tanagers, and warblers (Figure 3, left; supplementary table 3), MMR was significantly higher for high-elevation wrens, tanagers and warblers (Figure 3, middle), and aerobic scope was significantly higher for high-elevation thrushes, tanagers, and warblers (Figure 3, right). Spinetails did not exhibit significant elevational differences in BMR, MMR, or aerobic scope.

In our small sample of Andean passerines, we found a significant effect of elevation on BMR, MMR, and aerobic scope after accounting for effects of body mass (supplementary tables 3, 4, and 5). In most cases, high-elevation species had higher BMR and MMR than closely related and ecologically similar low-elevation species. Overall, we observed significant differences in rates of aerobic metabolism between individual pairs of species, but we did not document a

uniformly consistent elevational trend. Our study and most others to date have investigated elevational variation in aerobic metabolism by measuring wild-caught birds in their native habitat (e.g. Jones et al., 2020; Londoño et al., 2015; Londoño et al., 2017). In the future, common-garden or reciprocal-transplant experiments that control for acclimatization effects should help reveal whether bird species native to high elevations have generally evolved increased aerobic performance capacities in cold, hypoxic conditions.

Acknowledgements

We wish to thank all the students whose invaluable help in the field made this project possible, especially to Jorge Lizarazo, Isabel Cifuentes, Valentina Echeverry, Jose Riascos, and Maria Laura Mahecha. For facilitating logistics at fieldwork sites in Colombia we thank the staff at ICESI University, staff at CELSIA, Alirio Bolivar, Ana Tulia Montes, and Gustavo Giraldo. Additional help with fieldwork preparation was provided by Andrew Crawford and Santiago Herrera. Gwen Bachman offered vital guidance while processing metabolic rate data. We are thankful with Kate Lyons, John DeLong, Graham Scott, and Grant McClelland for discussions and assistance with the statistical analyses. Finally, this work greatly benefited from discussions with Gwen Bachman, Chris Witt, Kristi Montooth, Colin Meiklejohn, and Jamilynn Poletto.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.G.P., J.F.S.; Methodology: N.G.P., J.F.S.; Formal analysis: N.G.P.; Investigation: N.G.P.; Data curation: N.G.P.; Writing – original draft: N.G.P.; Writing – review & editing: N.G.P, G.A.L., M.A.C., J.F.S.; Supervision: G.A.L, J.F.S.; Funding acquisition: N.G.P, G.A.L., J.F.S.

Funding

This research was supported by grants from the National Institutes of Health (HL087216) and the National Science Foundation (OIA-1736249 and IOS-1927675) to J. F. S.; R. C. Lewontin Graduate Research Excellence Grant and University of Nebraska SBS Special Funds to N. G. P.; and the research agreement between Icesi University and EPSA-Celsia signed in 2016 to G. A. L.

Figure legends

Figure 1. A. Phylogenetic relationships among the ten study species. Terminal branches (in black) connect pairs of high- and low-elevation species. Branch lengths are proportional to estimated divergence times (Barker et al., 2015; Batista et al., 2020; Cadena et al., 2019; Derryberry et al., 2011) **B.** Approximate elevational distribution of the study species in the northern Andes (Hilty and Brown, 1986). White circles represent the elevation at which measurements were made for each species (see supplementary table 1). Illustrations from Ayerbe-Quiñones (2018).

Figure 2. Relationships between mass and BMR (top row), MMR (middle row), and absolute aerobic scope (AAS; bottom row) for each pair of high- and low-elevation species. SMA regression lines are shown in cases where the tested association was statistically significant. In each pair, high elevation species are shown in light blue and low elevation species in dark blue. Boxplots depict the variation for each species in metabolic rate (left) and mass (bottom).

Figure 3. Mass-corrected values ($\text{ml O}_2 \text{ min}^{-1} \text{ g}^{-1}$) for BMR (top), MMR (middle), and absolute aerobic scope (AAS; bottom) of Andean passerines. Alternating light grey and white columns denote the species pairs being compared; within each pair, light and dark blue boxes represent high and low elevation species, respectively. Three significance levels of the pairwise *t*-tests comparing mass-corrected metabolic values between high- and low- elevation species are indicated on top of each graph: non-significance (n.s; $p > 0.05$), $p < 0.05$ (*), and $p < 0.01$ (**); *p*-values and differences in group means can be found in supplementary table 3.

Literature cited

- Ayerbe-Quiñones, F.** (2018). Guía ilustrada de la avifauna colombiana. *Wildlife Conservation Society, Bogotá, Colombia*.
- Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M. and Lovette, I. J.** (2015). New insights into New World biogeography: An integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers, and allies. *The Auk* **132**, 333-348.
- Bartholomew, G. A., Vleck, D. and Vleck, C. M.** (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *Journal of Experimental Biology* **90**, 17-32.
- Bates, D., Mächler, M., Bolker, B. and Walker, S.** (2014). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1-48.
- Batista, R., Olsson, U., Andermann, T., Aleixo, A., Ribas, C. C. and Antonelli, A.** (2020). Phylogenomics and biogeography of the world's thrushes (Aves, Turdus): new evidence for a more parsimonious evolutionary history. *Proceedings of the Royal Society B: Biological Sciences* **287**, 20192400.
- Bennett, A. F.** (1991). The evolution of activity capacity. *Journal of Experimental Biology* **160**, 1-23.
- Cadena, C. D., Pérez-emán, J. L., Cuervo, A. M., Céspedes, L. N., Epperly, K. L. and Klicka, J. T.** (2019). Extreme genetic structure and dynamic range evolution in a montane passerine bird: implications for tropical diversification. *Biological Journal of the Linnean Society* **126**, 487-506.
- Castro, G., Carey, C., Whittembury, J. and Monge, C.** (1985). Comparative responses of sea level and montane rufous-collared sparrows, *Zonotrichia capensis*, to hypoxia and cold. *Comparative Biochemistry and Physiology Part A: Physiology* **82**, 847-850.
- Champely, S.** (2020). pwr: Basic Functions for Power Analysis. In *R package*
- Chappell, M. A., Bech, C. and Buttemer, W. A.** (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *The Journal of Experimental Biology* **202**, 2269-2279.

Chappell, M. A. and Dlugosz, E. M. (2009). Aerobic capacity and running performance across a 1.6 km altitude difference in two sciurid rodents. *Journal of Experimental Biology* **212**, 610-619.

Chappell, M. A., Hammond, K. A., Cardullo, R. A., Russell, G. A., Rezende, E. L. and Miller, C. (2007). Deer mouse aerobic performance across altitudes: effects of developmental history and temperature acclimation. *Physiological and Biochemical Zoology* **80**, 652-662.

Chappell, M. A., Savard, J.-F., Siani, J., Coleman, S. W., Keagy, J. and Borgia, G. (2011). Aerobic capacity in wild satin bowerbirds: repeatability and effects of age, sex and condition. *Journal of Experimental Biology* **214**, 3186-3196.

Chappell, M. A., Zuk, M. and Johnsen, T. S. (1996). Repeatability of Aerobic Performance in Red Junglefowl: Effects of Ontogeny and Nematode Infection. *Functional Ecology* **10**, 578-585.

Cheviron, Z. A., Bachman, G. C., Connaty, A. D., McClelland, G. B. and Storz, J. F. (2012). Regulatory changes contribute to the adaptive enhancement of thermogenic capacity in high-altitude deer mice. *Proceedings of the National Academy of Sciences* **109**, 8635-8640.

Cheviron, Z. A., Connaty, A. D., McClelland, G. B. and Storz, J. F. (2014). Functional genomics of adaptation to hypoxic cold-stress in high-altitude deer mice: transcriptomic plasticity and thermogenic performance. *Evolution* **68**, 48-62.

Derryberry, E. P., Claramunt, S., Derryberry, G., Chesser, R. T., Cracraft, J., Aleixo, A., Pérez-Emán, J., Remsen Jr, J. and Brumfield, R. T. (2011). Lineage diversification and morphological evolution in a large-scale continental radiation: the neotropical ovenbirds and woodcreepers (Aves: Furnariidae). *Evolution* **65**, 2973-2986.

Felsenstein, J. (2004). *Inferring Phylogenies*. Sunderland, MA: Sinauer Associates, Inc.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of Size and Temperature on Metabolic Rate. *Science* **293**, 2248-2251.

Hayes, J. P. (1989a). Altitudinal and seasonal effects on aerobic metabolism of deer mice. *Journal of Comparative Physiology B* **159**, 453-459.

Hayes, J. P. (1989b). Field and maximal metabolic rates of deer mice (*Peromyscus maniculatus*) at low and high altitudes. *Physiological Zoology* **62**, 732-744.

Hayes, J. P. and Garland Jr, T. (1995). The evolution of endothermy: testing the aerobic capacity model. *Evolution* **49**, 836-847.

Hedges, L. V. (1983). A random effects model for effect sizes. *Psychological Bulletin* **93**, 388-395.

Hilty, S. L. and Brown, W. L. (1986). A Guide to the Birds of Colombia. Princeton: Princeton University Press.

Hochachka, P. (1985). Exercise limitations at high altitude: the metabolic problem and search for its solution. In *Circulation, respiration, and metabolism*, pp. 240-249: Springer.

Jones, S. E. I., Suanjak, M., Tobias, J. A., Freeman, R. and Portugal, S. J. (2020). Comparative physiology of five tropical montane songbirds reveals differential seasonal acclimatisation and cold adaption. *bioRxiv*, 2020.05.22.111328.

Karasov, W. H. and del Rio, C. M. (2007). Physiological ecology: how animals process energy, nutrients, and toxins: Princeton University Press.

Lau, D. S., Connaty, A. D., Mahalingam, S., Wall, N., Cheviron, Z. A., Storz, J. F., Scott, G. R. and McClelland, G. B. (2017). Acclimation to hypoxia increases carbohydrate use during exercise in high-altitude deer mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*.

Londoño, G. A., Chappell, M. A., Castañeda, M. d. R., Jankowski, J. E. and Robinson, S. K. (2015). Basal metabolism in tropical birds: latitude, altitude, and the 'pace of life'. *Functional Ecology* **29**, 338-346.

Londoño, G. A., Chappell, M. A., Jankowski, J. E. and Robinson, S. K. (2017). Do thermoregulatory costs limit altitude distributions of Andean forest birds? *Functional Ecology* **31**, 204-215.

Lui, M. A., Mahalingam, S., Patel, P., Connaty, A. D., Ivy, C. M., Cheviron, Z. A., Storz, J. F., McClelland, G. B. and Scott, G. R. (2015). High-altitude ancestry and hypoxia acclimation have distinct effects on exercise capacity and muscle phenotype in deer mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **308**, R779-R791.

McClelland, G. B. and Scott, G. R. (2019). Evolved mechanisms of aerobic performance and hypoxia resistance in high-altitude natives. *Annual Review of Physiology* **81**, 561-583.

McKechnie, A. E. and Swanson, D. L. (2010). Sources and significance of variation in basal, summit and maximal metabolic rates in birds. *Current Zoology* **56**, 741-758.

McKechnie, A. E. and Wolf, B. O. (2004). The allometry of avian basal metabolic rate: good predictions need good data. *Physiological and Biochemical Zoology* **77**, 502-521.

Novoa, F. F., Bozinovic, F. and Rosenmann, M. (1990). Maximum metabolic rate and temperature regulation in the rufous-collared sparrow, *Zonotrichia capensis*, from central Chile. *Comparative Biochemistry and Physiology Part A: Physiology* **95**, 181-183.

Novoa, F. F., Rosenmann, M. and Bozinovic, F. (1991). Physiological responses of four passerine species to simulated altitudes. *Comparative Biochemistry and Physiology Part A: Physiology* **99**, 179-183.

Portugal, S. J., Green, J. A., Halsey, L. G., Arnold, W., Careau, V., Dann, P., Frappell, P. B., Grémillet, D., Handrich, Y. and Martin, G. R. (2016). Associations between resting, activity, and daily metabolic rate in free-living endotherms: no universal rule in birds and mammals. *Physiological and Biochemical Zoology* **89**, 251-261.

Rezende, E. L., Bozinovic, F., Garland, T. and Merilä, J. (2004). Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. *Evolution* **58**, 1361-1374.

Schippers, M.-P., Ramirez, O., Arana, M., Pinedo-Bernal, P. and McClelland, Grant B. (2012). Increase in Carbohydrate Utilization in High-Altitude Andean Mice. *Current Biology* **22**, 2350-2354.

Storz, J. F., Cheviron, Z. A., McClelland, G. B. and Scott, G. R. (2019). Evolution of physiological performance capacities and environmental adaptation: insights from high-elevation deer mice (*Peromyscus maniculatus*). *Journal of Mammalogy* **100**, 910-922.

Storz, J. F. and Scott, G. R. (2019). Life Ascending: Mechanism and Process in Physiological Adaptation to High-Altitude Hypoxia. *Annual Review of Ecology, Evolution, and Systematics*.

Storz, J. F., Scott, G. R. and Cheviron, Z. A. (2010). Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *The Journal of Experimental Biology* **213**, 4125-4136.

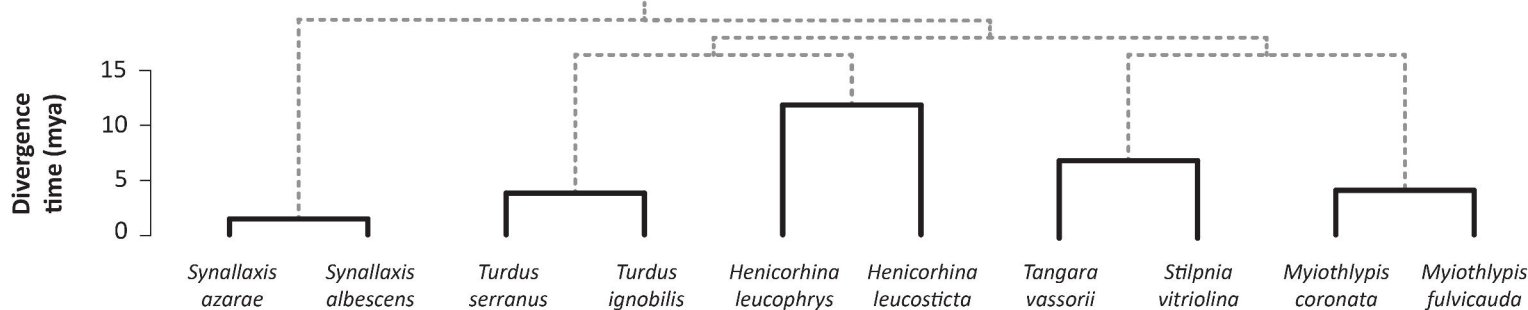
Tate, K. B., Ivy, C. M., Velotta, J. P., Storz, J. F., McClelland, G. B., Cheviron, Z. A. and Scott, G. R. (2017). Circulatory mechanisms underlying adaptive increases in thermogenic capacity in high-altitude deer mice. *Journal of Experimental Biology* **220**, 3616-3620.

Tate, K. B., Wearing, O. H., Ivy, C. M., Cheviron, Z. A., Storz, J. F., McClelland, G. B. and Scott, G. R. (2020). Coordinated changes across the O₂ transport pathway underlie adaptive increases in thermogenic capacity in high-altitude deer mice. *Proceedings of the Royal Society B* **287**, 20192750.

Team, R. C. (2012). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Warton, D. I., Duursma, R. A., Falster, D. S. and Taskinen, S. (2012). smatr 3— an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution* **3**, 257-259.

A



B

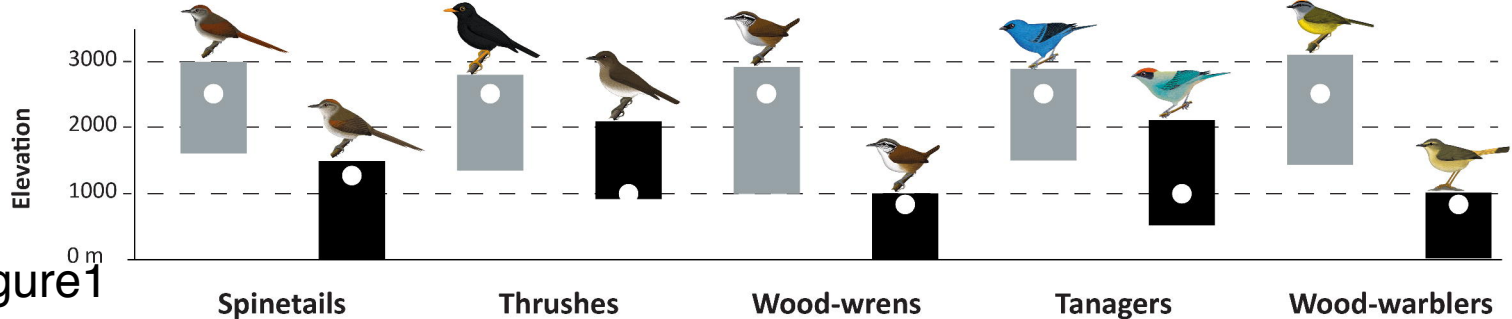


Figure 1

Figure 2

Spinetails

Thrushes

Wrens

Tanagers

Warblers

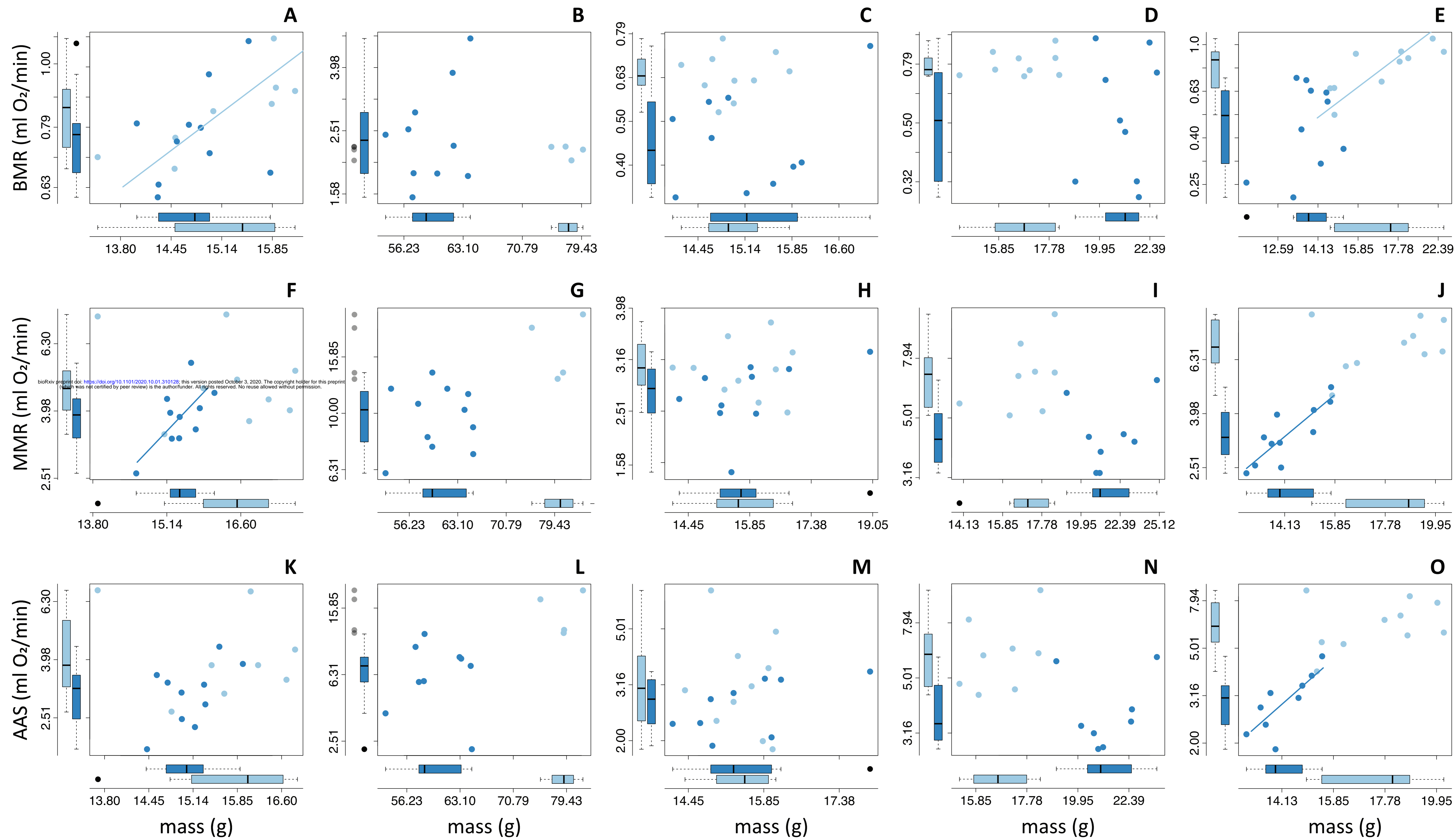
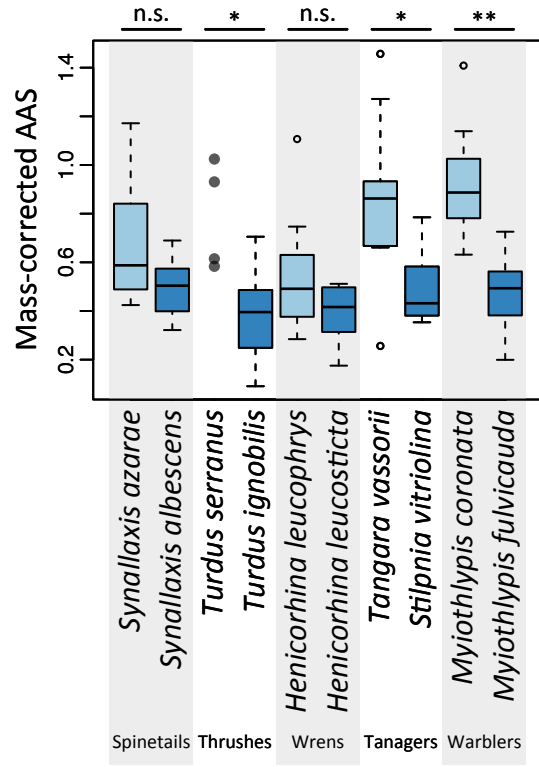
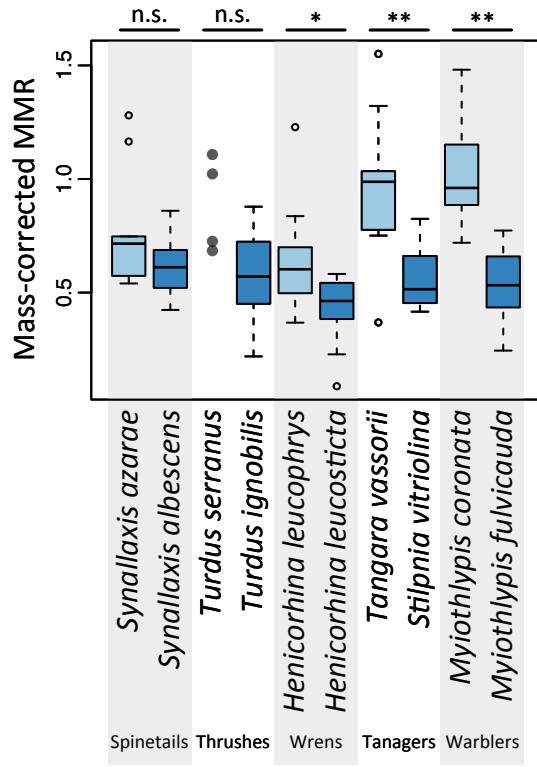
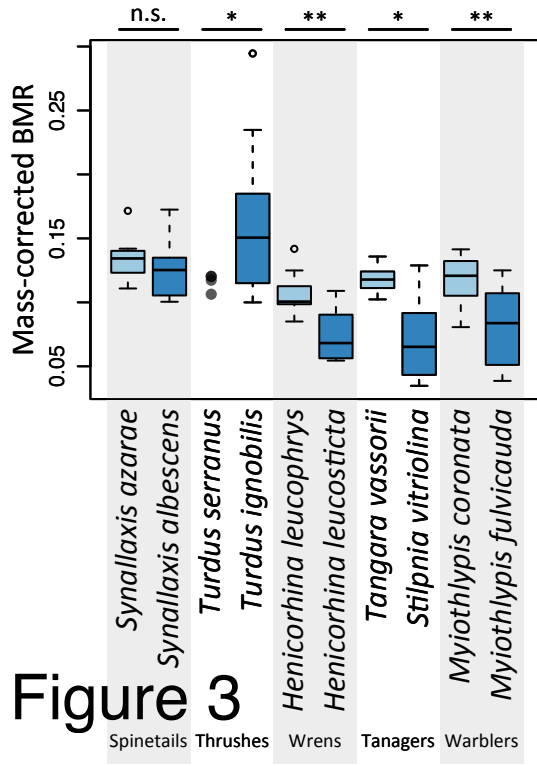


Figure 3



Supplementary Tables

Supplementary table 1. Information about geographical coordinates, elevation (m), weather, and landscape matrix of the localities visited for this study. All localities visited are located in the Valle del Cauca department, Colombia. Annual mean temperature and precipitation were extracted from the WorldClim database (BIO1 and BIO12, respectively; Hijmans et al, 2005^a). Also included are the species captured in each site and the year(s) each locality was visited.

Name	Coordinates	Elevation (m)	Annual Mean Temperature (°C)	Annual precipitation (mm)	Ambient pO ₂ (kPa)	Landscape matrix	Species captured	Years visited
Estación Biológica Zygia-ICESI	3.442°N, 76.662°W	2311-2553	17.5	2260	15.6-16.1	Primary and secondary forests	<i>Synallaxis azarae</i> <i>Turdus serranus</i> <i>Henicorhina leucophrys</i> <i>Tangara vassorii</i> <i>Myiothlypis coronata</i>	2017-2019
Alto Anchicayá	3.576°N, 76.880°W	525-889	24.2	2093	19.2-20.0	Primary forests and road sides	<i>Henicorhina leucosticta</i> <i>Myiothlypis fulvicauda</i>	2017-2018
Universidad ICESI	3.341°N, 76.529°W	1014-1021	23.6	1490	18.9	Urban university campus	<i>Turdus ignobilis</i> <i>Tangara vitriolina</i>	2017-2018
Ecoparque el Embudo	3.345°N, 76.553°W	1099	23.2	1557	18.7	Secondary forests and cleared areas	<i>Tangara vitriolina</i>	2019
Finca el Pasatiempo	4.677°N, 75.739°W	1420-1445	20.4	2251	17.9	Coffee farms and road sides	<i>Synallaxis albescens</i>	2019
Finca el Tabacal	4.681°N, 75.764°W	1266-1276	21.3	1973	18.3	Coffee farms and road sides	<i>Synallaxis albescens</i>	2019
Rural Alcala	4.676°N, 75.772°W	1276	21.4	1927	18.3	Coffee farms and road sides	<i>Synallaxis albescens</i>	2019

Finca Tipitapa	4.662°N, 75.761°W	1334	21.3	1965	18.2	Coffee farms and road sides	<i>Synallaxis albescens</i>	2019
Finca Villa Ramona	4.661°N, 75.754°W	1337	21.0	2052	18.2	Coffee farms and road sides	<i>Synallaxis albescens</i>	2019

^aHijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**, 1965-1978.

Supplementary table 2. General information of the biology of the species selected for this study. We paired closely related species that had contrasting elevation ranges but were similar in terms of morphology, life history, and foraging ecology.

Species	Size	Habitat	Diet	Foraging	Breeding	Behavior	Sources
Azara's Spinetail <i>Synallaxis azarae</i>	15-18 cm, 12-18 g.	Dense undergrowth of forest edges secondary forest, and overgrown roadsides, usually above 1600 m.	Mostly arthropods, occasionally small seeds.	Captures prey from foliage, small branches and dead leaves, within 1–2 m of ground.	Throughout the year in Colombia.	Sedentary and territorial, usually in pairs, rarely joining mixed species flocks.	Hilty and Brown (1986); Remsen (2020a)
Pale-breasted Spinetail <i>Synallaxis albescens</i>	13-16 cm, 9-17 g.	Open areas with grassland, second growth scrub and overgrown roadsides, usually below 1500 m.	Mostly invertebrates.	Captures prey from foliage, grass and small branches, within 1–2 m of ground.	February to November in Colombia.	Sedentary and territorial, usually in pairs.	Hilty and Brown (1986); Remsen (2020b)
Glossy-black Thrush <i>Turdus serranus</i>	23-25 cm, 70-90 g.	Primary humid montane forest, forest borders, occasionally in open areas, mainly within 1400 to 2800 m.	Fruits and berries.	Mainly forages arboreally, sometimes along roads.	March to August in Colombia.	Sedentary, usually single or in pairs, may gather at fruiting trees. Rarely in mixed-species flocks.	Hilty and Brown (1986); Collar (2020)
Black-billed Thrush <i>Turdus ignobilis</i>	18-24 cm, 48-81 g.	Semi-open to open areas, forest borders, mainly within 900 to 2100 m.	Mainly invertebrates and fruits, occasionally seeds.	Mainly forages arboreally, also often on ground, usually near or in forests.	December to August in Colombia.	Sedentary, usually single or in pairs, may gather at fruiting trees.	Hilty and Brown (1986); Collar et al (2020)
Gray-breasted Wood-wren <i>Henicorhina leucophrys</i>	10-11 cm, 16-17 g.	Undergrowth of humid mountain forest, usually above 1500 m.	Mostly invertebrates.	Captures prey in dense vegetation, usually below 2 m.	December to June in Colombia.	Sedentary and territorial, usually in pairs.	Hilty and Brown (1986); Kroodsma et al (2020)
White-breasted Wood-wren <i>Henicorhina leucosticta</i>	10-11 cm, 15 g.	Undergrowth of wet lowland forest, from sea-level up to 1300 m.	Mostly invertebrates.	Captures prey in tangles around fallen trees and dense vegetation, from the ground up to 2–3 m.	January to July in most of its range.	Sedentary and territorial, usually in pairs, rarely joining ant follower flocks.	Hilty and Brown (1986); Kroodsma and Brewer (2020)

Blue-and-black Tanager <i>Tangara vassorii</i>	14-18 cm, 15-21 g.	Primary and secondary humid montane forest, and forest borders, from 1500 to 3500 m.	Mostly fruits and some insects.	Feeds in all forest levels but prefers the canopy, especially around fruiting trees.	February to August in Colombia.	Very active, usually seen in pairs or small groups, and often joins large mixed-species flocks.	Hilty and Brown (1986); Bernabe and Burns (2020)
Scrub Tanager <i>Stilpnia vitriolina</i>	14 cm, 18-26 g	Scrubby or open areas with sparse trees and bushes, mostly between 500 to 2200 m.	Mostly fruits and arthropods.	Feeds in all forest levels but prefers the canopy, especially around fruiting trees.	Throughout the year in Colombia.	Very active, usually seen alone, in pairs or small groups, rarely found with mixed-species flocks.	Hilty and Brown (1986); Hilty (2020)
Russet-crowned Warbler <i>Myiothlypis coronata</i>	14 cm, 13-19 g.	Humid montane forests, forest borders, and secondary growth, between 1300 to 2500 m.	Mostly invertebrates.	Captures prey at low to middle levels and dense areas, mainly at 1 to 6 m but can be seen higher.	May to June in Colombia.	Very active, usually seen in pairs or small groups, and often joins mixed-species flocks.	Hilty and Brown (1986); Curson and Bonan (2020); personal observations
Buff-rumped Warbler <i>Myiothlypis fulvicauda</i>	13 cm, 14 g	Primary and secondary forest areas, mostly around water bodies and forest edges. Usually below 1000 m.	Mostly invertebrates.	Captures prey close to the ground, along stream edges and damp areas, but can be seen higher.	February to April in Colombia.	Very active, usually seen in pairs.	Hilty and Brown (1986); Curson (2020); personal observations

Supplementary table 3. Average mass-corrected BMR, MMR, and aerobic scope values, standard deviations (SD) and sample size (n) for each species analyzed. Also shown, the percentage of change in the highland mass-corrected metabolic rates in relation to lowland (positive values indicate higher values in the highland species), the respective pairwise t-tests evaluating the difference in means, the size of the effect, and the power of each statistical comparison.

	High elevation species			Low elevation species			% change	t-test	Power analysis	
	Average (mlO ₂ min ⁻¹ g ⁻¹)	SD	n	Average (mlO ₂ min ⁻¹ g ⁻¹)	SD	n		p-value	effect size (Hedges' G)	power
BMR										
Spinetails	0.135	0.018	8	0.127	0.023	10	6.3%	0.4239	0.378	0.197
Thrushes	0.114	0.006	4	0.162	0.062	10	-29.5%	0.0383	0.888	0.619
Wrens	0.107	0.016	11	0.074	0.019	10	44.4%	0.0005	1.857	1.000
Tanagers	0.119	0.011	9	0.074	0.034	9	60.4%	0.0039	1.770	0.999
Warblers	0.117	0.019	10	0.082	0.032	10	43.5%	0.0085	1.357	0.987
MMR										
Spinetails	0.771	0.269	9	0.610	0.120	10	26.5%	0.1265	0.789	0.658
Thrushes	0.896	0.230	4	0.587	0.191	12	52.5%	0.0661	1.540	0.988
Wrens	0.637	0.241	11	0.430	0.155	11	48.1%	0.0283	1.022	0.912
Tanagers	0.974	0.339	9	0.563	0.152	8	73.1%	0.0069	1.534	0.991
Warblers	1.024	0.219	10	0.538	0.144	12	90.3%	0.0000	2.674	1.000
AAS										
Spinetails	0.679	0.276	8	0.493	0.112	10	37.7%	0.1071	0.926	0.770
Thrushes	0.794	0.230	4	0.389	0.193	10	103.8%	0.0283	1.989	0.999
Wrens	0.542	0.244	10	0.398	0.109	10	36.3%	0.1122	0.764	0.654
Tanagers	0.865	0.351	9	0.491	0.161	8	76.1%	0.0146	1.339	0.966
Warblers	0.927	0.224	10	0.474	0.153	10	95.5%	0.0001	2.363	1.000

Supplementary table 4. Results of the linear mixed models that explained the variation in each metabolic parameter (logBMR, log MMR, and log AAS) as a function of elevation (fixed effect) and the interaction between species group and mass (random effects).

<i>Predictors</i>	BMR			MMR			AAS		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(Intercept)	-0.11	-0.19 – -0.04	0.004	0.73	0.63 – 0.82	<0.001	0.65	0.56 – 0.73	<0.001
Elevation	-0.07	-0.13 – -0.01	0.022	-0.15	-0.20 – -0.10	<0.001	-0.2	-0.26 – -0.13	<0.001
Random Effects	<i>Variance</i>			<i>Variance</i>			<i>Variance</i>		
Residuals	0.02			0.01			0.02		
Species group	1.76			1.41			0.66		
Mass	1.16			0.95			0.49		
Sample size	90			90			84		
Marginal R ² / Conditional R ²	0.001 / 0.991			0.004 / 0.992			0.013 / 0.970		

Supplementary table 5. Results of the ANCOVA that explained the variation in each metabolic parameters (logBMR, log MMR, and log AAS) as a function of mass, and elevation per group.

<i>Predictors</i>	BMR			MMR			AAS		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(Intercept)	-0.53	-0.93 – -0.13	0.009	-0.04	-0.44 – 0.35	0.837	-0.23	-0.73 – 0.27	0.362
Mass	0.46	0.16 – 0.76	0.003	0.77	0.48 – 1.06	<0.001	0.9	0.52 – 1.27	<0.001
Spinetails : elevation	-0.06	-0.13 – -0.00	0.043	-0.16	-0.22 – -0.09	<0.001	-0.18	-0.26 – -0.10	<0.001
Thrushes : elevation	0.04	-0.06 – 0.14	0.38	-0.17	-0.27 – -0.08	<0.001	-0.29	-0.41 – -0.16	<0.001
Wrens : elevation	-0.18	-0.25 – -0.12	<0.001	-0.23	-0.29 – -0.17	<0.001	-0.23	-0.31 – -0.15	<0.001
Tanagers : elevation	-0.17	-0.23 – -0.11	<0.001	-0.15	-0.21 – -0.09	<0.001	-0.17	-0.25 – -0.09	<0.001
Warblers : elevation	-0.17	-0.23 – -0.10	<0.001	-0.15	-0.21 – -0.08	<0.001	-0.15	-0.23 – -0.07	0.001
Observations	90			91			85		
R ² / R ² adjusted	0.799 / 0.784			0.741 / 0.723			0.603 / 0.572		