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SHORT COMMUNICATION

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

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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 metabolic rate (BMR), maximal metabolic rate (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. Our results suggest that there is no simple explanation regarding the ecological and physiological causes of elevational variation in aerobic metabolism.

KEY WORDS: Andes, Elevation, Metabolic rates, Passerines

INTRODUCTION

Endotherms that are native to high-altitude environments must contend with physiological challenges posed by the reduced partial pressure of O_2 (P_{O_2}) and low ambient temperature (T_a). Reduced P_{O_2} may compromise the maximum capacities for aerobic exercise (MMR; maximum metabolic rate) and shivering thermogenesis because of the reduced availability of O_2 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 as a result of increased thermoregulatory demands or as a correlated response to changes in MMR that entail higher maintenance costs (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, 2014; Lau et al., 2017; Lui et al., 2015; Schippers et al., 2012; Storz et al., 2019; Tate et al., 2017, 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,b), although it is not known to what extent the elevated BMR reflects an evolved change or a reversible acclimatization response. Available evidence for birds is not conclusive on whether BMR varies with elevation, and it is unknown whether MMR exhibits a consistent pattern of altitudinal variation among species.

Studies evaluating changes in BMR with elevation show mixed results. Highland and lowland populations of rufous-collared sparrows (*Zonotrichia capensis*), measured at their native altitudes, had no differences in BMR (250–4540 m; Castro et al., 1985), thermogenic capacity (600–3000 m; Novoa et al., 1990) or field metabolic rates (600–3000 m; Novoa et al., 1991). High elevation amethyst sunbirds (*Chalcomitra amethystina*) had higher BMR even after acclimatization to low elevation, but the effect was statistically significant only in winter (540–1550 m; Lindsay et al., 2009a,b). In contrast, high elevation fiscal shrikes (*Lanius collaris*) showed lower BMR after acclimatization (130–1800 m; Soobramoney et al., 2003). Interspecific comparisons of birds of paradise found that high elevation (>1000 m) species had higher BMR but only after accounting for diet (McNab, 2003). However, a recent study involving a phylogenetically diverse set of more than 250 Neotropical bird species found no significant association between BMR and native elevation (400–3000 m; Londoño et al., 2015). The effects of altitude 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 whether 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 (Fig. 1A,B). We used a paired-lineage design (Felsenstein, 2004) such that the five pairwise comparisons were phylogenetically independent (Fig. 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 (Fig. 1A; Table S1). We compared closely related species that had contrasting elevation ranges (Fig. 1B) but which are otherwise similar ecologically (Table S2). High elevation species were captured between 2300 and 2500 m; low elevation species were captured between 500 and 1400 m. Annual mean temperature and ambient P_{O_2} differed, respectively, by approximately 3.6°C and 2.3 kPa for the spinetails; 6.1°C and 3 kPa for the thrushes; 6.7°C and 3.7 kPa for the wood wrens and the warblers; and 5.9°C and 2.9 kPa for the tanagers.

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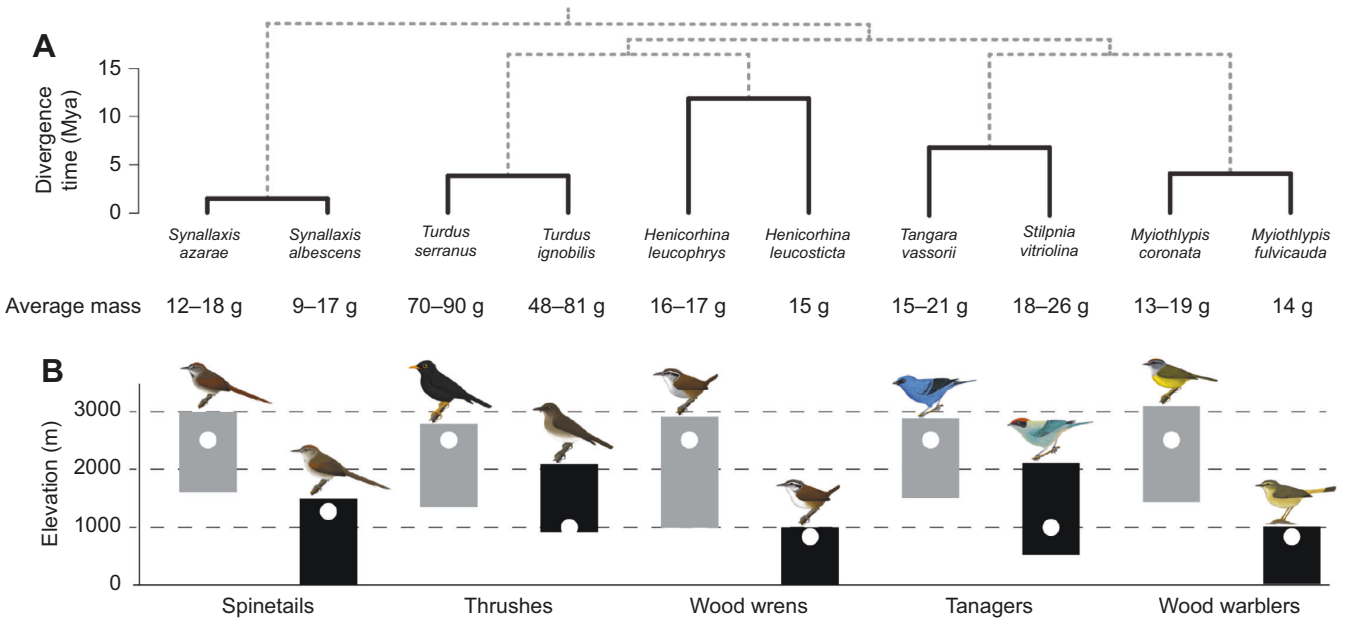

Fig. 1. Phylogenetic relationships and distribution of the 10 Andean passerines studied. (A) Terminal branches (in black) connect pairs of high- and low-elevation species. Branch lengths are proportional to estimated divergence times (Barker et al., 2015; Batista et al., 2020; Cadena et al., 2019; Derryberry et al., 2011). Also shown is the average body mass per species. (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 Table S1). Illustrations from Ayerbe-Quiñones (2018). mya, million years ago.

Field protocol

We mist-netted birds (aided by playback of vocalizations) during the day (09:00 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 (~20×30 cm) in a dark and isolated room to minimize stress. Keeping birds inside cloth bags will cause stress; however, most individuals remained calm for the time they were restrained and seemed less agitated than when we tried retrieving them from cages immediately before BMR experiments. We took the birds out of the bags every 2 h to provide water and food (banana for fruit-eating birds, mealworm larvae and adults for insectivorous birds) offered *ad libitum* while they were hand-held for a few minutes. To ensure that the birds were post-absorptive (Karasov and del Rio, 2007), we stopped providing food and water 4 h before the onset of BMR measurements. All BMR measurements were conducted after sunset, between 19:00 h and 01:00 h. Body mass was measured immediately after capture and also before and after BMR measurements. Birds were euthanized for tissue collection after all measurements were taken, and deposited at the ICESI zoological collection. 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 positive-pressure 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 acrylic metabolic chambers (2.7 liters), 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 (200–1000 ml min^{-1} at standard temperature and pressure (STP)) with the body mass of each tested bird. Excurrent air flows were sampled sequentially by a multiplexer (Sable Systems RM-8). Subsamples (50–200 ml min^{-1}) of excurrent airflow were scrubbed of CO_2 and H_2O (using soda lime and silica gel, respectively) and routed through a Sable Systems FoxBox to measure O_2 content, which was calibrated against atmospheric air (20.95% O_2). O_2 content deflections ranged between 0.5% and 1.5%. Flow rates were adjusted to STP conditions, as the mass flow meters in the FlowBar compensated for variations in atmospheric pressure and temperature. Birds were allowed at least 30 min of acclimation to experimental conditions before the measurements. 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 (±0.5°C) using a PELT-5 controller and a PTC-1 cabinet (Sable Systems). Birds were first measured at T_a=34°C and then at T_a=30°C, remaining at each stable temperature for at least 1 h. We recorded T_a, flow rate and O_2 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 in 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, rates of oxygen consumption ($\dot{V}_{O_2}$) values measured with this technique are usually lower than those measured in wind tunnels (Chappell et al., 2011; McKechnie and Swanson, 2010). We ran these experiments at ambient temperature, between 15 and 18°C at high elevation and at 20–26°C at low elevation. During exercise trials, incurrent air flow was 750–1000 ml min^{-1} and no multiplexer was used. The chamber (14.5 liters) was manually rotated for a maximum of 10 min or until birds showed signs of exhaustion (coordination loss and heavy breathing) and

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we verified that they reached their maximum rates of oxygen consumption – typically shortly after the beginning of the exercise bout. All tested birds attained similar levels of behavioral exhaustion during the exercise trials and no birds were injured during experiments. Birds were then fed and allowed to rest for at least 4 h before the onset of the BMR experiments (see field protocol section).

Data analysis

We calculated metabolic rates using LabAnalyst (www.warthog.ucr.edu). After baseline correction, we used the flow rate (FR; ml min^{-1} STP) and the incurrent ($F_{I_{O_2}}$; 0.2095) and excurrent ($F_{E_{O_2}}$) oxygen concentrations to obtain $\dot{V}O_2$ (ml O_2 min^{-1}), applying the 'Mode 1' formula:

$$\dot{V}_{O_2} = \mathrm{FR}(F\mathrm{I}_{O_2} - F\mathrm{E}_{O_2})/(1 - F\mathrm{E}_{O_2}). \quad (1)$$

BMR was computed as the lowest continuous average $\dot{V}_{O_2}$ over 5 min during periods of low and stable $\dot{V}_{O_2}$, and the lowest value of the two temperature measurements per bird (30 and 34°C) was chosen for subsequent analyses. Before obtaining MMR $\dot{V}_{O_2,max}$, 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 $\dot{V}_{O_2}$ over 1 min during periods of high and stable $\dot{V}_{O_2}$ 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 $\dot{V}_{O_2}$ differed between closely related species, we tried fitting standardized major axis (SMA) regressions between mass and $\dot{V}_{O_2}$ for each species using the *smatr* package (Warton et al., 2012) for R v.3.3.2 (https://www.r-project.org/). We used the mass values obtained before each experiment to analyze MMR and BMR data (after capture and before BMR, respectively), and an average between both values to analyze aerobic scopes. 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 $\dot{V}O_2$. For this, we fitted a linear mixed model (package *lme4*; Bates et al., 2014) with $\dot{V}O_2$ 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:

$$\dot{V}_{O_2} \sim \mathrm{elevation} + (\mathrm{mass}|\mathrm{species\ pair}). \quad (2)$$

This model allows the slope of the relationship between mass and $\dot{V}_{O_2}$ to be different for each species group. To further explore

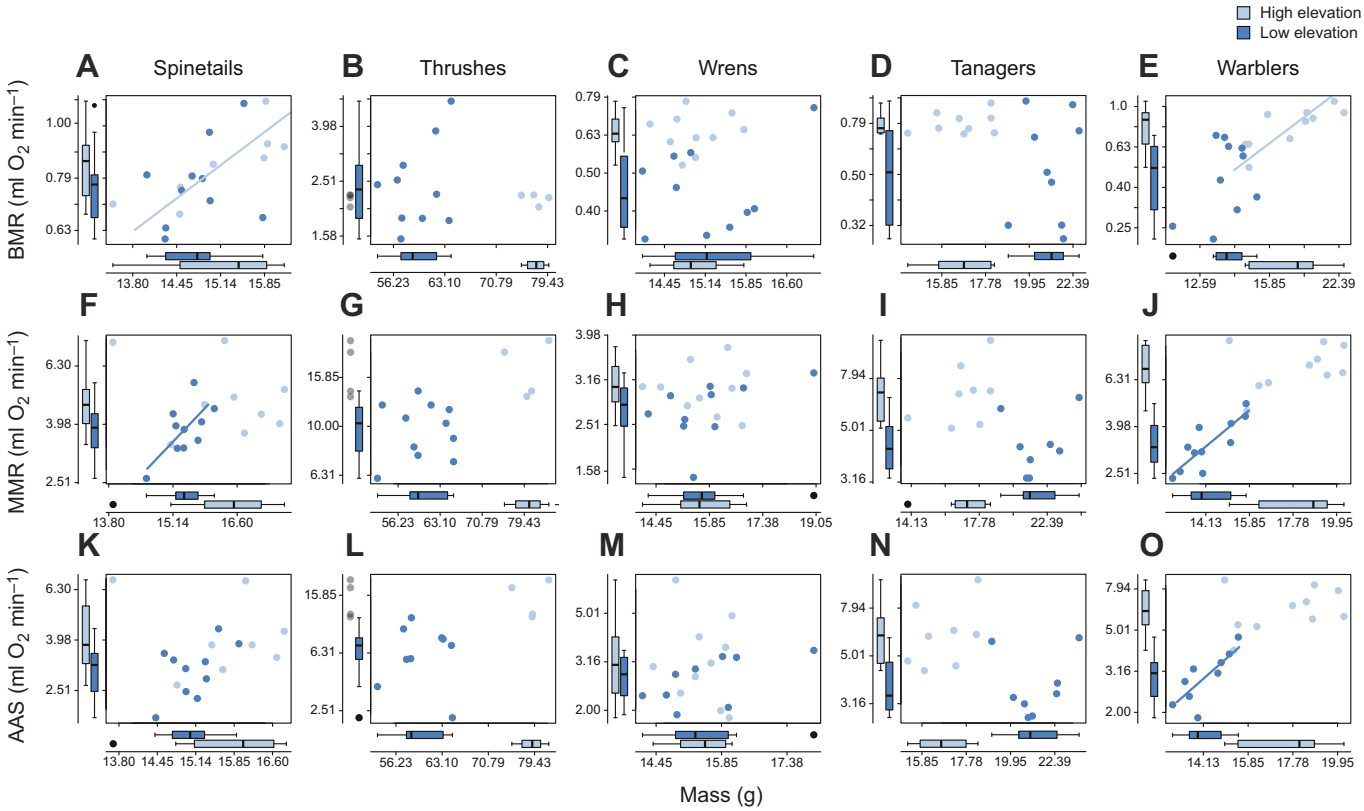

Fig. 2. Relationships between mass and BMR, MMR and absolute aerobic scope (AAS) for each pair of high- and low-elevation species. (A–E) BMR, (F–J) MMR and (K–O) AAS in the indicated pairs of birds. SMA regression lines are shown in cases where the tested association was statistically significant. In 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) and show median, 5th and 95th centiles and range.

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whether the effect of elevation differs between species pairs after accounting for mass, we also ran a nested ANCOVA, where the variation in $\dot{V}_{O_2}$ is explained by mass and by elevation per group:

$$\dot{V}_{O_2} \sim \text{mass} + (\text{species pair} : \text{elevation}). \quad (3)$$

Second, in order to better understand the trends observed in the linear models, we mass-corrected each of our observed $\dot{V}_{O_2}$ 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 $\dot{V}_{O_2}$ between high- and low-elevation species within each pair. Considering that scaling coefficients for BMR, MMR, and aerobic scope may vary, we ran additional analyses using the coefficients reported by McKechnie and Swanson (2010), but the overall patterns remained unchanged (results not shown). We also ran a *post-hoc* power analysis (package *pwr* v1.3; https://cran.r-project.org/web/packages/pwr/) 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<<0.1), and the model that included mass as the only predictor had the lowest AIC (ΔAIC=46; results not shown).

RESULTS AND DISCUSSION

We measured 96 wild-caught birds, with sample sizes of 8–12 individuals per species (Fig. 2; Table S3), 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; Tables S4 and S5). 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 mean marginal R^2 (0.006; variance explained by the fixed effect) and the mean conditional R^2 (0.98; variance explained by the full model) (Table S4). Likewise, after accounting for mass, elevation also had a significant effect on variation in BMR, MMR and aerobic scope in each species pair (Table S5), 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 (Table S5). These results are noteworthy considering the relatively small elevational difference (around 1500 m) between our sampling localities.

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 (Fig. 3A; Table S3), MMR was significantly higher for high-elevation wrens, tanagers and warblers (Fig. 3B) and aerobic scope was significantly higher for high-elevation thrushes, tanagers, and warblers (Fig. 3C). Spinetails did not exhibit significant elevational differences in BMR, MMR or aerobic scope. Our results are similar to previous

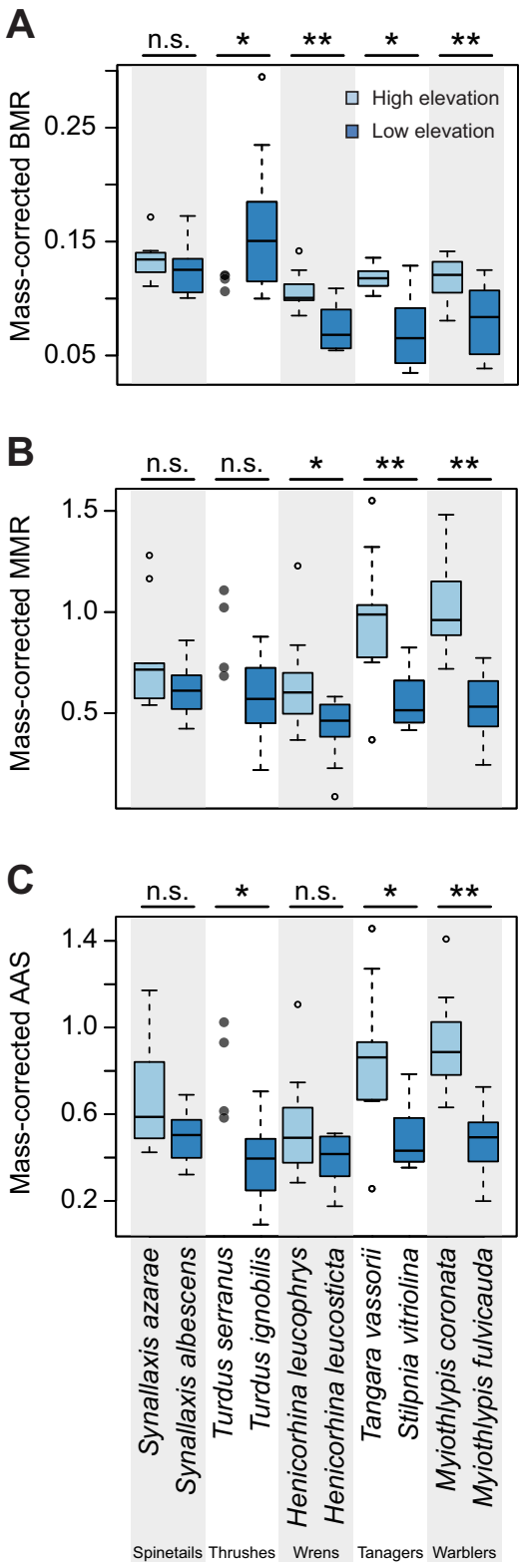

Fig. 3. Mass-corrected $\dot{V}_{O_2}$ (ml O_2 min^{-1} g^{-1}) for BMR, MMR and AAS of Andean passerines. Body mass corrected (A) BMR, (B) MMR and (C) AAS. Alternating grey and white shading denote the species pairs being compared; within each pair, light and dark blue boxes represent high and low elevation 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 Table S3.

studies in birds that found either no differences in BMR with elevation (Castro et al., 1985; Londoño et al., 2015) or higher BMR in high elevation populations (Lindsay et al., 2009a,b; McNab, 2003).

The available evidence suggests that the relationship between elevation and avian metabolic rates likely depends on each species' ecology. It has been hypothesized that BMR may depend on food quality, availability and predictability (food habits hypothesis; McNab, 1988, 2019). Previous studies have shown that changes in BMR with elevation are more apparent in species with more frugivorous diets (McNab, 2003), similar to the case of the tanagers in our study, although a direct test of this hypothesis did not support it (Sabat et al., 2010). Similarly, McNab (2019) found that BMR variation in birds is correlated with the frequency and intensity of flight, with higher rates in more active birds. In our set of sampled taxa, comparisons between species with greater dispersal propensity (i.e. tanagers and warblers) revealed larger elevation-related differences in metabolic rates than in comparisons between pairs of more sedentary species (i.e. spinetails and wrens) (Sheard et al., 2020). Our results and those of other comparative studies suggest that there is no simple, unitary explanation regarding causes of elevational variation in aerobic metabolism, and it seems equally clear that relevant interactions between ecological and physiological factors vary among taxa.

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 (Tables S3–S5). 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 preprint; Londoño et al., 2015, 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. Such experiments would complement results of comparative studies by revealing the genetic and environmental components of variation in aerobic metabolism within and among species.

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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.; Visualization: N.G.-P.; Supervision: G.A.L., J.F.S.; Funding acquisition: N.G.-P., G.A.L., J.F.S.

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