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Authors

Londoño, Gustavo A
Chappell, Mark A
Jankowski, Jill E
[et al.](#)

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1 DO THERMOREGULATORY COSTS LIMIT ALTITUDE DISTRIBUTIONS OF ANDEAN
2 FOREST BIRDS?

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7 Gustavo A. Londoño^{1,2}, Mark A. Chappell², Jill E. Jankowski³, and Scott K. Robinson⁴

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11 ¹ Departamento de Ciencias Biológicas, Universidad Icesi, Cali, Colombia

12 ² Biology Department, University of California, Riverside, CA 92521 USA

13 ³ Biodiversity Research Centre, University of British Columbia, BC V6T 1Z4 Canada

14 ⁴ Florida Museum of Natural History, University of Florida, Gainesville, FL 32611 USA

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20 *Corresponding author: Gustavo A. Londoño*

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25 Running headline: Thermal physiology and altitude in tropical birds

26

27 Keywords: Andes, elevation, thermal conductance, lower critical temperature, body

28 temperature

29 **Summary**

- 30 1. Along tropical mountains, species often occupy narrow altitude ranges. Numerous
31 biotic and abiotic factors have been proposed as determinants of altitude occupancy.
- 32 2. We measured several aspects of thermal physiology of 215 bird species across a 2.6 km
33 altitude gradient in the Peruvian Andes. We predicted that highland species would show
34 adaptation to the colder high-altitude climate, and that energy costs of thermoregulation
35 might limit up-slope dispersal of lowland natives.
- 36 3. We found reductions of thermal conductance, body temperature, and lower critical
37 temperature in highland birds compared to lowland species. These combine to make
38 highland natives more resistant to heat loss.
- 39 4. We did not find convincing evidence that acute thermal limits or energy costs of
40 thermoregulation constrained altitude distributions. Heat budget models predicted low
41 to moderate long-term costs at native altitudes. Costs increased for lowland natives
42 modeled in the highland climate, but for all but a few species, costs remained within
43 putative expenditure limits.
- 44 5. Although we did not test heat tolerances, we measured all species at temperatures
45 similar to the hottest air temperatures at the lowland site. There was no evidence that
46 high lowland temperatures preclude down-slope movements of highland birds.
- 47 6. While thermal tolerances probably do not directly determine altitude occupancy by
48 most species, the additional energy cost of thermoregulation experienced by lowland
49 species moving up-slope may trade off against investment in important life history
50 components such as breeding, and thereby affect altitude range limits.

51

52 **Introduction**

53 The tropical Andes are among the most species-rich regions on earth, particularly for birds
54 (Rahbek & Graves 2001, Cadena *et al.* 2012). That diversity reflects both high species numbers
55 within habitats and rapid turnover across elevations (Melo, Rangel & Diniz-Filho 2009), with
56 most Andean birds restricted to narrow elevation zones (e.g., Jankowski *et al.* 2013).

57 Understanding the mechanisms underlying that zonation has long been a focus of research.
58 Many studies implicate competition or aggression as barriers to occupancy of particular
59 altitudes (e.g., Terborgh & Weske 1975; Jankowski, Robinson & Levey 2010). Strong habitat

60 preferences, coupled with abrupt habitat transitions across altitudes, may also affect
61 distributions (Terborgh 1971; Jankowski *et al.* 2009, 2013). Other studies suggest predation,
62 parasites, or pathogens as limiting factors (e.g., van Riper III *et al.* 1986; Boyle 2008; Atkinson
63 & Samuel 2010; G.A. Londoño, *unpublished data*).

64 Abiotic factors may also be important. Janzen (1967) proposed that stable tropical
65 climates, coupled with minimal temperature overlap among elevations separated by more than
66 ~600 m, lead to thermal physiologies optimized for specific altitudes. This results in low
67 tolerance for temperatures outside the optimal range, inhibiting up- or downslope dispersal.
68 Some tropical ectotherms fit Janzen's predictions: these species have narrow temperature
69 optima within which performance is maximal, and their thermal tolerances are often closely
70 matched to environmental temperatures (Huey *et al.* 2009). Therefore, species adapted to cool
71 mid- and high altitudes risk lethal hyperthermia if they move downslope, whereas warm-
72 adapted lowland species risk compromised performance if they move upslope.

73 Whether similar constraints apply to endotherms – which almost by definition are more
74 tolerant of temperature variation than ectotherms – is unclear (Ghalambor *et al.* 2006). For
75 Andean birds, altitude distributions are unavoidably associated with temperature due to the
76 adiabatic lapse rate of 5-6 °C per 1000 m of elevation (Rapp & Silman 2012). However,
77 distribution models based on occupancy generally cannot reveal the causal factors underlying
78 altitude limits, and hence do not indicate if a species can tolerate conditions at other elevations.
79 That requires knowledge of relevant physiological traits in combination with microclimate.

80 For endotherms such as birds, thermal constraints could range from acute physiological
81 limits to indirect limits derived from life-history tradeoffs. Acute limits arise from
82 environmental temperatures that exceed a species' ability to keep body temperature within
83 tolerable bounds. Indirect limits result from energy costs or behavioral restrictions imposed by
84 stressful but nonlethal thermal conditions. At temperatures outside of thermoneutrality,
85 homeothermic endotherms increase metabolism above basal metabolic rate (BMR) to power
86 thermogenesis (in cold) or evaporative cooling (in heat). Those costs reduce the energy
87 available for crucial life history functions (growth, maintenance, reproduction) and may be
88 accompanied by behavioral shifts (e.g., microhabitat use or activity time) that impose further
89 trade-offs. Acute limits for maximal heat production have been described for a number of
90 tropical and temperate birds (McKechnie & Swanson 2010), but temperature tolerances *per se*

91 are less studied. Little is known about long-term limits, particularly how regulatory costs or
92 thermal constraints on behavior impact life history. However, daily energy expenditures during
93 the breeding season (presumably a time of high energy demand) seldom exceed 4X BMR
94 (Drent & Daan 1980), and this is often interpreted as an upper limit to energy flux over
95 extended periods. Costs of thermoregulation can comprise only part of that total and still allow
96 for energy allocation to other functions; therefore, long-term limits for thermoregulatory
97 expenditures are likely closer to 2 - 2.5X BMR. Estimated metabolic rates at the northern
98 boundaries of winter ranges are similar to these values (Canterbury 2002).

99 In this study of Andean birds, we had two main objectives. The first was to determine
100 if thermal physiology varied along a 2.6 km altitude gradient encompassing a 12 °C range of
101 annual temperature means (11- 23 °C; Londoño *et al.* 2014). Most birds maintain body
102 temperatures (T_b) of 38-41 °C (Prinzinger, Pressmar & Schleucher 1991) and small species
103 typically have lower critical temperatures (LCT; below which elevated heat production is
104 necessary) of 25-30 °C (Calder & King 1974). Therefore, a decrease in environmental
105 temperature from 23 °C to 11 °C should greatly increase costs of regulatory thermogenesis.
106 Given the selection that should impose, we expected physiological differentiation across
107 altitudes. Previous work revealed no effect of altitude on BMR (Londoño *et al.* 2014), but
108 BMR is largely irrelevant to thermoregulatory costs at temperatures below the LCT. We
109 hypothesized that thermal conductance, LCT, and T_b would be reduced in high-altitude natives,
110 because those changes would decrease heat loss rates.

111 Our second goal was to test if thermal biology restricts altitude ranges. To do this we
112 combined physiological and microclimate data in a heat-budget model to produce species- and
113 habitat-specific estimates of energy costs of thermoregulation. Our hypothesis was that
114 lowland species exposed to the high-altitude climate would require thermoregulatory
115 expenditures exceeding the putative maxima for acute exposure (4 - 7 X BMR) or over
116 extended periods (2 - 4X BMR). Finally, we considered potential barriers to down-slope
117 movements of highland birds.

118

119 **Materials and methods**

120 FIELD SITES

121 We worked at three sites near Parque Nacional del Manu in southeastern Peru. Pantiacolla
122 (mean elevation ~400 m; 12° 39' S, 71° 13' W) is in lowland rainforest. San Pedro (1500 m;
123 13° 03' S, 71° 32' W) is in montane cloud forest. Wayqecha (3000 m; 13° 10' S, 71° 35' W) is
124 in highland cloud forest. San Pedro and Wayqecha are on steep mountain slopes; Pantiacolla is
125 on more level terrain. The sites are roughly aligned along a northeast-southwest transect
126 extending about 70 km.

127 Physiological data were obtained between June and December in 2011 - 2014;
128 environmental temperatures were measured between September 2011 and December 2014.

129

130 BIRD CAPTURE AND HANDLING

131 We mist-netted birds in the afternoon (15:00 - 17:30 local time). Individuals in reproductive
132 condition were released; others were held in quiet cloth-covered cages with water but no food.
133 After dusk, birds were weighed and placed in metabolism chambers. Small nectar-feeding
134 species (flowerpiercers, hummingbirds) were fed to repletion with sugar water (~ 25% sucrose)
135 prior to insertion in chambers to reduce stress. Metabolic measurements did not begin until 1-2
136 h after birds were placed in chambers, sufficient time for sugar to be absorbed (Karasov *et al.*
137 1986). We first tested at 10 °C; subsequently all individuals were tested at 30 °C; most at 32-35
138 °C and some at 20 °C. Ambient temperatures (T_a) were maintained for at least 1 h before
139 sampling began. Immediately after the 10 °C test, T_b was measured within 1 min of initial
140 disturbance with a fine-gauge thermistor inserted at least 5-10 mm into the cloaca.
141 Measurements at warm T_a , intended to elicit basal metabolism, occurred > 5 h after the last
142 feeding opportunity; accordingly, we assume birds were postabsorptive. A final T_b and body
143 mass were obtained at the conclusion of measurements; we used the mean mass in calculations.
144 Birds were banded and released at the site of capture the following morning.

145 Procedures were approved by the Institutional Animal Care and Use Committees at the
146 University of California Riverside (protocol # 0408026, 20070023, 20100048) and the
147 University of Florida (Protocol #: 201106068), and by permits from the government of Peru
148 (0239-2013 MINAGRI-DGFFS/DGEFFS 2013).

149

150 THERMAL PHYSIOLOGY

151 We used open-circuit respirometry to measure rates of oxygen consumption ($\dot{V}O_2$) as
152 previously described (Londoño *et al.* 2014; Supplementary Material). We converted $\dot{V}O_2$ to
153 metabolic heat production (MHP; watts) using an oxycaloric value of 19.8 Joules/ml O_2 .

154 Barometric pressure averaged at 521 torr at Wayqecha and 723 torr at Pantiacolla. To
155 determine if lower O_2 partial pressure at Wayqecha affected thermoregulation of lowland birds,
156 we tested a subset of Pantiacolla species in acute hypobaria. We used a small vacuum pump to
157 pull air through the chamber against the resistance of the upstream flow controller. Chamber
158 pressure was measured with a manometer (Sper Scientific 840081, Scottsdale, Arizona). We
159 adjusted pump power and the flow controller until the desired pressure differential and flow
160 were achieved. Other aspects of the system were as in Londoño *et al.* (2014). During tests, we
161 cooled the chamber to 10 °C at ambient pressure, measured $\dot{V}O_2$ after 1 h of acclimation, then
162 quickly reduced pressure by 200 torr and measured $\dot{V}O_2$ again. Results were compared with
163 paired t-tests.

164

165 THERMAL CONDUCTANCE AND LOWER CRITICAL TEMPERATURE

166 We calculated thermal conductance (C; watts/°C) at 10 °C from MHP and the gradient between
167 body and ambient temperatures:

$$168 \quad C = \text{MHP} / (T_b - T_a) \quad 1$$

169 We estimated lower critical temperature (LCT) from C, basal metabolic rate (BMR, watts), and
170 body temperature at BMR:

$$171 \quad \text{LCT} = T_b - (\text{BMR}/C) \quad 2$$

172 In a few species we could not compute C or LCT because there was no increase in MHP at 10
173 °C (i.e. 10 °C was within thermoneturity).

174 These calculations assume that T_b is constant and C is minimal at temperatures below
175 the LCT, as in the classic “Scholander-Irving model” (Scholander *et al.* 1950). In our species,
176 the change in T_b across T_a of 10 °C to 30-34 °C averaged 0.9 °C (see Results). Assuming a
177 linear change, the T_b at the LCT would differ from that at BMR by ~0.5 °C or less.

178 Conductance at 20 °C did not differ from conductance at 10 °C (N = 435, P = .28;
179 Supplementary Materials). Accordingly we believe the computed LCT are acceptably
180 accurate.

181

182 ENVIRONMENTAL TEMPERATURES

183 Shade temperatures (T_a) were monitored for 38 months with Hobo dataloggers (Pro v2; Onset
184 Computers, Massachusetts, USA). At each site, several loggers were simultaneously deployed
185 in different habitat types. There was little difference in T_a among habitats, so we computed a
186 mean T_a for each site at intervals of 30 min.

187 The thermal environment of a bird exposed to wind and sun is more complex than can
188 be represented by T_a (Bakken 1976). There is little wind below the canopy in the densely
189 forested habitats at our sites (averaging < 0.3 m/s; unpublished data), but depending on
190 microhabitat, birds may experience sun exposure and in open areas, some wind. We used gray-
191 painted hollow aluminum spheres with internal thermistors to estimate the integrated effects of
192 T_a , wind, and sun (operative environmental temperature, T_e). Spheres yield reliable T_e data
193 over extended periods (but not short intervals; Walsberg & Weathers 1986). We deployed two
194 spheres simultaneously (5 and 7 cm diameter) in open areas at each site in 2012 and 2013 (408
195 days at Pantiacolla, 383 days at San Pedro, 159 days at Wayqecha), with T_e recorded every 30
196 min. There was little T_e difference between spheres so we used the higher value (assuming the
197 cooler sphere might have been shaded).

198 T_e and T_b specify the gradient driving heat flow, but do not account for the effect of
199 wind on conductance (Bakken 1976; Chappell, Morgan & Bucher 1990). Heat transfer
200 increases as wind speed increases, but that relationship is complex and dependent on size,
201 posture, turbulence, and plumage characteristics. Because wind speed was low inside the
202 canopy, where most of our species live, we did not estimate its effects in cost calculations.

203

204 ENERGY COSTS OF THERMOREGULATION

205 The energy an endotherm spends to thermoregulate is a function of physiology (C , T_b , BMR),
206 environment (T_a , wind, sun), behavior (microhabitat, posture, activity, use of torpor), and other
207 factors (e.g., plumage wetting or damage). Precise calculation of thermoregulatory costs
208 requires detailed data on all these factors, many of which were unavailable. We modeled
209 expenditures with the ‘Thermoregulatory Costs’ heat budget algorithm in LabAnalyst. For each
210 microclimate datum (i.e., every 30 min), this computes the heat production necessary to
211 maintain T_b (Supplementary Materials; Fig. S1). Over the duration of microclimate records, it

212 provides each species' mean cost of thermoregulation, highest single 30 min value, highest 6,
213 12, and 24 hour costs, and the fraction of time in thermoneutrality. Because the quantitative
214 details of sun use and torpor are unknown in the species we studied, we modeled heat budgets
215 with and without these factors to estimate upper and lower bounds of thermoregulatory costs.

216

217 MINIMUM TOLERABLE T_e

218 Maximal cold-induced metabolic rate ('summit metabolism', MR_{sum} ; McKechnie & Swanson
219 2010) is a common index of cold resistance. Together, C, minimum T_b , and MR_{sum} are the
220 main determinants of an endotherm's lowest tolerable T_e ($T_{e,min}$). At our sites, power and
221 logistical constraints precluded measurement of MR_{sum} . However, in 19 lowland bird species
222 from Panama, MR_{sum} averaged 4.5 ± 0.3 X higher than BMR (Wiersma, Chappell & Williams
223 2007). Panamanian and Peruvian birds have similar BMR (Londoño *et al.* 2014), so we
224 assumed PMR_C was also similar and estimated $T_{e,min}$ as:

$$225 \quad T_{e,min} = T_b - ((BMR * 4.5)/C) \quad 3$$

226 We measured C and T_b during the rest phase (night). Because active-phase C is about 1.4-fold
227 higher than rest-phase C (Aschoff 1981; Schleucher & Withers 2001), and active-phase T_b
228 averages 2 °C higher than rest-phase T_b (Prinzinger, Pressmar & Schleucher 1991), we
229 estimated daytime minimal T_e as:

$$230 \quad T_{e,min} = (T_b + 2) - ((BMR * 4.5)/(C * 1.4)) \quad 4$$

231 In some small non-passerines, circadian T_b changes may exceed 2 °C (Aschoff 1982). If
232 applicable to Peruvian birds, this would affect estimates of daytime LCT and $T_{e,min}$. The
233 impact on heat budgets is minor, because the highest costs occur at night, when T_e is lowest
234 (Fig. 1) and we directly measured T_b . Accordingly, and because circadian T_b variance in
235 Peruvian birds is unknown, we did not include mass scaling of T_b changes in our analyses.

236

237 ANALYSIS AND STATISTICS

238 Species means were used for all analyses except hypobaric and seasonality tests. Body mass
239 (M_b), BMR, and conductance were log-transformed to account for allometric scaling. To
240 normalize distributions, we converted $T_{e,min}$ to K and applied either a log or square-root
241 transform, and if necessary a further Box-Cox transform. Comparisons were performed with

242 ordinary least squares Analysis of Covariance (OLS ANCOVA) with altitude as a fixed factor
243 and M_b as the covariate. Time in thermoneutrality was analysed with nonparametrics
244 (Wilcoxon and Tukey-Kramer HSD). Tests were performed with JMP Pro 11 (SAS Institute,
245 Cary, North Carolina, USA).

246 We estimated phylogenetic signal (Pagel 1999; Blomberg, Garland & Ives 2003) based
247 on the avian ‘tree of life’ (Jetz *et al.* 2012; Londoño *et al.* 2014). When phylogenetic signal
248 was present, we used phylogenetic independent contrasts (Felsenstein 1985; Garland, Harvey
249 & Ives 1992) and Phylogenetic Generalized Least Squares (PGLS) as implemented in R (R
250 Core Team 2013; Supplementary Materials). For all tests, the significance level was 0.05.

251

252 **Results**

253 ENVIRONMENTAL TEMPERATURES

254 Seasonal variation was small (Fig. 1), but air and sphere temperatures and high temperature
255 maxima averaged about 12 °C cooler at Wayqecha than at Pantiacolla (Table S1).

256 Temperatures at San Pedro were intermediate. Differences were smaller for low temperature
257 minima (Table S1). Air and sphere temperatures differed substantially when sunlight was
258 present, and daily temperature cycles were larger for spheres than for T_a (Fig. 1).

259

260 SEASONAL EFFECTS

261 Because seasonality may affect thermoregulatory traits, (Swanson 1991; McKechnie 2008), we
262 tested for effects of sample date. Date could not be normalized, so we employed nonparametric
263 methods (Kendall’s τ). To avoid bias from small sample sizes or clustered capture dates, we
264 restricted analysis to 16 species with at least 10 (mean 22.6) individuals sampled across periods
265 of at least 4 months (usually 5-6 months). There was no effect of date on BMR ($P = 0.634$), C
266 ($P = 0.319$), T_b at BMR ($P = 0.120$), T_b at 10 °C ($P = 0.272$), or LCT ($P = 0.153$). Accordingly,
267 we did not incorporate seasonality in analyses.

268

269 THERMAL PHYSIOLOGY

270 For T_b and LCT, we included data from birds with stable $\dot{V}O_2$ and $T_b \geq 35$ °C. Individuals
271 with lower T_b were considered to be in torpor (e.g., Bech *et al.* 1997). For calculation of C we

272 used data from birds with stable $\dot{V}O_2$ and $T_b \geq 33$ °C. Applying these criteria, we obtained
273 BMR, BMR T_b , LCT, C, and minimum tolerable T_e for 215 species, and 10 °C T_b for 206
274 species. In all analyses, phylogenetic signal was present in M_b ($\lambda = 1.00$, $P < 0.0001$) and most
275 physiological variables (Table 1).

276 We found no effect of altitude on BMR with either OLS ANCOVA ($P = 0.27$) or PGLS
277 ($P = 0.91$; Table 1; Londoño *et al.* 2014).

278 OLS ANCOVA indicated that C was affected by both M_b ($F_{1,214} = 978$, $P < 0.0001$) and
279 altitude ($F_{2,213} = 4.09$, $P = 0.018$; Fig. 2A). Mass-adjusted C (least-square mean) was about 9%
280 higher at 400 m than at 3000 m. There was no M_b X altitude interaction ($P = 0.28$). Post-hoc
281 Tukey-Kramer comparisons revealed a difference between high- and low-altitude sites ($P =$
282 0.0146); no other comparison was significant ($P > 0.2$). The pooled C data were correlated
283 with M_b : C (watts/ °C) = $0.00359 \cdot M_b^{.515 \pm .015 \text{ SE}}$ ($r^2 = 0.85$, $P < 0.0001$). That scaling is
284 consistent with previous analyses of avian conductance (mass exponent 0.48 to 0.52;
285 Schleucher & Withers 2001), and there was no difference in C ($P = 0.265$) between the Peru
286 species and rest-phase measurements of 35 temperate zone species from Aschoff (1981),
287 recommended as the best predictor of avian C by Schleucher & Withers (2001).

288 Accounting for phylogeny, conductance and M_b were positively correlated ($r^2 = 0.58$; P
289 < 0.0001 ; 95% CI = 0.49 - 0.66) and PGLS revealed effects of M_b and altitude on C ($P <$
290 0.0001; Table 1), with lower C at high altitude and at large M_b .

291 In OLS ANCOVA, body temperature at $T_a = 10$ °C (T_b10) was positively correlated
292 with M_b ($F_{1,205} = 22.6$, $P < 0.0001$) and varied among altitudes ($F_{2,204} = 7.74$, $P = 0.0006$; Fig.
293 2C). There was no M_b X altitude interaction ($P = 0.96$). Tukey-Kramer tests revealed
294 differences between high and low altitude sites ($P = 0.0004$), but not for other altitude
295 comparisons ($P > 0.07$). Mass-adjusted T_b10 at high altitude was 37.7 ± 0.16 °C (mean \pm SE),
296 or 0.8 °C less than that of lowland species (38.5 ± 0.14 °C); the value for the mid-altitude site
297 was intermediate (38.0 ± 0.17 °C).

298 Accounting for phylogeny, body mass and T_b10 were positively correlated ($r^2 = 0.16$; P
299 $= 0.015$; 95% CI = 0.03 - 0.30). PGLS revealed the same qualitative results as OLS: T_b10
300 varied with both M_b and altitude ($P = 0.0009$ and $P = 0.0029$, respectively; Table 1), increasing
301 as M_b increased and declining as altitude increased.

302 Body temperatures at BMR were obtained at T_a between 30 °C and 34 °C. There was
303 no effect of T_a on T_b at BMR ($P = 0.86$, ANCOVA with M_b and altitude as covariates);
304 therefore, we pooled these data as ' T_b BMR'. OLS ANCOVA revealed effects of M_b and
305 altitude on T_b BMR, with higher T_b BMR at low altitude ($F_{2,210} = 9.99$, $P < 0.0001$) and at large
306 M_b ($F_{1,211} = 33.3$, $P < 0.0001$; Fig. 2D). The M_b X altitude interaction did not reach significance
307 ($F_{2,210} = 2.9$, $P = 0.056$). Tukey-Kramer comparisons revealed differences between high- and
308 low-altitude sites ($P < 0.0001$) and low- and mid-altitude sites ($P = 0.0047$), but not mid- and
309 high-altitude sites ($P = 0.69$). Mass adjusted T_b BMR at high altitude averaged 38.5 ± 0.15 °C
310 (mean \pm SE), or 0.9 °C cooler than at low altitude (39.4 ± 0.13 °C); T_b BMR at the mid-altitude
311 site was intermediate (38.7 ± 0.16 °C).

312 PGLS revealed the same qualitative results as OLS: T_b BMR was positively correlated
313 with body mass ($r^2 = 0.19$; $P = 0.006$; 95% CI = 0.05 - 0.32) and decreased with increasing
314 altitude ($P < 0.0001$; Table 1).

315 OLS ANCOVA indicated that LCT was not affected by M_b ($F_{1,211} = 0.20$, $P = 0.65$) but
316 decreased with increasing altitude ($F_{2,210} = 12.9$, $P < 0.0001$; Fig. 2B) with no M_b X altitude
317 interaction ($P = 0.19$). Tukey-Kramer tests revealed differences between high- and low-altitude
318 sites ($P < 0.0001$) and low- and mid-altitude sites ($P = 0.0002$) but not mid- and high-altitude
319 sites ($P = 0.98$). Mass-adjusted LCT at high altitude ($19.4 \pm .51$ °C; mean \pm SE) averaged 3.0
320 °C lower than at low altitude (22.4 ± 0.44 °C); the LCT at San Pedro (19.6 ± 0.53 °C) was
321 close to that at high altitude.

322 As with OLS results, PGLS indicated that LCT was not correlated with M_b but declined
323 with increasing altitude ($P = 0.78$ and $P = 0.0001$, respectively; Table 1).

324

325 PRESSURE EFFECTS ON LOWLAND SPECIES

326 In 86 individuals of 57 Pantiacolla species tested at 10 °C at both the *in situ* barometric
327 pressure and the Wayqecha barometric pressure, there was no difference in $\dot{V}O_2$ ($P = 0.84$,
328 paired t-test; Supplementary Fig. S2).

329

330 MINIMUM TOLERABLE T_e

331 Minimum tolerable T_e ($T_{e,min}$) lacked phylogenetic signal. Nighttime $T_{e,min}$ was inversely
332 related to altitude ($F_{2,210} = 5.61$; $P = 0.0042$) and declined as M_b increased ($F_{1,211} = 4.96$; $P =$

333 0.0269). Mass-adjusted $T_{e\min}$ was $-47.0 \text{ }^\circ\text{C} \pm 2.4$ (SE) at the mid-altitude site, $-45.5 \pm 3.0 \text{ }^\circ\text{C}$ at
334 the high-altitude site, and $-37.6 \pm 2.0 \text{ }^\circ\text{C}$ at the low altitude site. There was no $M_b \times$ altitude
335 interaction ($P = 0.06$). Daytime $T_{e\min}$ (where T_b and C were higher) was affected by altitude
336 ($F_{2,210} = 6.01$; $P = 0.0029$) and M_b ($F_{1,211} = 4.32$; $P = 0.0389$), with no interaction ($P = 0.07$).
337 Daytime mass-adjusted $T_{e\min}$ was $-20.0 \pm 1.7 \text{ }^\circ\text{C}$ at the mid-altitude site, $-19.5 \pm 1.6 \text{ }^\circ\text{C}$ at the
338 high-altitude site, and $-13.6 \pm 1.4 \text{ }^\circ\text{C}$ at the low altitude site.

339 At night, distributions of shade T_a had essentially no overlap with $T_{e\min}$ at any altitude
340 (Fig. 3). During the day, $T_{e\min}$ was roughly $23 \text{ }^\circ\text{C}$ higher, and for a few species, shade T_a at
341 Wayqecha was, on rare occasions, below $T_{e\min}$ (Fig. 3).

342

343 HEAT TOLERANCE

344 We exposed all species to T_a of $30 \text{ }^\circ\text{C}$ and most to $32\text{-}35 \text{ }^\circ\text{C}$ for several hours when measuring
345 BMR. Those T_a s are similar to the highest T_a at the low-altitude site (Fig. 1), and therefore
346 simulate the warmest conditions likely to be experienced in this habitat (exclusive of sun
347 exposure, which can be avoided behaviorally). Chamber relative humidity during BMR
348 measurements (mean $44.9 \pm 16.4\%$ SD) varied between stations ($F_{2,834} = 109$, $P < 0.0001$),
349 ranging from $34.6 \pm 10.5\%$ at San Pedro to $51.4 \pm 16.6\%$ at Pantiacolla. There was no indication
350 of heat stress (panting, escape attempts, hyperthermia) in any mid- or high-altitude species and
351 only one instance of T_b higher than $44 \text{ }^\circ\text{C}$ ($44.4 \text{ }^\circ\text{C}$ in *Syndactyla ucayalae*, a lowland native).

352

353 THERMOREGULATORY COSTS AT NATIVE ALTITUDES

354 Phylogenetic signal was significant for all cost estimates ($P < 0.038$). Most costs, with or
355 without sun exposure and torpor, increased with increasing altitude and (expressed as multiples
356 of BMR) decreased with increasing M_b (Table 2). The fraction of time in thermoneutrality was
357 not affected by M_b , but declined as altitude increased (Table 2).

358 At all altitudes, annual mean costs averaged below 2X BMR (Fig. 4, Table 2). The
359 highest 24 h, 12 h, and 6 h costs averaged below 2.4X BMR at Pantiacolla and were somewhat
360 greater at higher elevations. A few small species at Wayqecha had predicted costs exceeding
361 4X BMR and one exceeded 5X BMR for the highest 30 min. Pantiacolla birds experienced
362 thermoneutral conditions almost 50% of the time, declining to 15% at Wayqecha.

363 Exclusion of sun exposure and torpor had little effect (Table 2). Differences were
364 substantial only for time in thermoneutrality, which decreased considerably.

365

366 THERMOREGULATORY COSTS IN THE HIGH-ALTITUDE CLIMATE

367 Thermoregulatory costs in the Wayqecha climate showed no phylogenetic signal and no effect
368 of M_b , but increased for low- and mid-altitude species (Figs. 1, 5; Tables 2, 3). Native altitude
369 affected costs, which averaged 11-20% larger for lowland species than for highland natives
370 (Table 3). Nevertheless, annual mean costs averaged less than 2.1X BMR across all native
371 altitudes, and even the highest 30 min costs averaged less than 3X BMR (Fig. 4). No species'
372 annual mean exceeded 4X BMR; only 5 species exceeded 4X BMR for intervals of 6 or 12 h
373 (one a high-altitude native), and only 3 species, including one high-altitude native, exceeded
374 4X BMR over 24 h. Highest 30-min costs equaled or exceeded 5X BMR for four species (one
375 a high-altitude native).

376 Exclusion of sun exposure and torpor had minor effects on costs (Table 3). Differences
377 were substantial only for time in thermoneutrality.

378

379 **Discussion**

380 The goals of our study are aligned with Janzen's (1967) concept that the striking altitudinal
381 zonation characteristic of tropical mountains occurs because up- or down-slope range
382 expansion is challenging for tropical animals. Janzen assumed that: (1) temperature
383 differences are effective barriers to dispersal; (2) seasonal stability in the tropics minimizes the
384 temperature 'overlap' between altitudes (i.e., temperatures differ across altitudes but have little
385 variation within altitudes); (3) organisms are unlikely to evolve tolerances to temperature they
386 do not experience. Compared to high-latitude species that experience strong seasonality,
387 tropical organisms should have narrow ranges of thermal tolerances and limited capacities for
388 acclimatization. Accordingly, dispersing up-or down-slope would bring them into intolerable
389 temperature regimes. Climates at our sites conform to expectations of seasonal stability within
390 altitudes but differences across altitudes (Fig. 1), and we hypothesized that (1) species native to
391 different altitudes (and hence different T_e) will show adaptive specialization in thermal
392 physiology, and (2) such specialization, combined with the ~12 °C across-altitude temperature
393 gradient, precludes occupation of non-native altitudes.

394 Our results support the first hypothesis: we found significant differences across
395 altitudes in several important indices of thermal physiology (C , T_b and LCT; Table 1). These
396 ‘match’ the thermal gradient, with highland birds more resistant to heat loss (Fig. 2). The
397 potential energetic benefit is shown in cost calculations for the highland climate (Table 3): the
398 combination of lower T_b and lower C in highland species produced savings of 11-20%
399 compared to lowland natives. In the context of a bird’s overall energy budget (daily energy
400 expenditure; DEE), benefits are smaller because DEE includes substantial activity costs in
401 addition to expenditures for BMR and thermoregulation (Weathers & Sullivan 1993), but even
402 a 5-10% reduction in DEE could be ecologically important.

403 Those findings make intuitive sense, but there are caveats. First, trait differences
404 between altitudes may not be genetic adaptations, but instead may result from phenotypic
405 plasticity or flexibility. If so, lowland species moving up-slope would acclimatize and attain
406 physiological phenotypes similar to those of highland natives. Resolving that question requires
407 controls (common-garden breeding) beyond the scope of this project. Similarly, we have not
408 excluded seasonal acclimatization, which can induce substantial changes in cold tolerance
409 (e.g., Cooper & Swanson 1994, Piersma, Cadée & Daan 1995, Liknes, Scott & Swanson 2002,
410 McKechnie 2008, McKechnie & Lovegrove 2002). Many of those studies concerned species
411 that experience strong seasonality; in contrast, the Peruvian birds are exposed to little annual T_e
412 change (Fig. 1). We found no seasonal physiological variation in 16 species for which we have
413 measurements across 4-6 months (winter to late spring), but because we lack data for almost
414 half the year (January - May), some changes may not have been detected.

415 Second, our study had technical constraints due to difficult access and lack of mains
416 electricity, and the need to minimize stress on birds and release them the morning following
417 capture. Therefore we could not test across the complete range of T_a at our sites. About 15% of
418 Wayqecha T_a were below our minimum test temperature of 10 °C, although less than 0.5%
419 were colder than 5 °C (Fig. 1). At San Pedro, less than 0.1% of T_a were lower than 10 °C; no T_a
420 at Pantiacolla were that cold. BMR testing (at 30-35 °C) included nearly all of the high T_a we
421 observed: at Pantiacolla, 0.5% of T_a exceeded 30 °C (none exceeded 33 °C); no T_a exceeded
422 30 °C at San Pedro or Wayqecha.

423 Also, we could not measure summit metabolism (MR_{sum}), which generally requires
424 near- or sub-zero temperatures and a helium-oxygen atmosphere (Cooper & Swanson 1994;

425 Liknes, Scott & Swanson 2002). This was not feasible at our sites, but it seems questionable if
426 that level of thermogenesis is relevant to the majority of our species. Estimated cold tolerances
427 ($T_{e\min}$), based on C , T_b , and the MR_{sum} of 4.5 X BMR for Panamanian forest birds (Wiersma,
428 Chappell & Williams 2007), are generally far colder than nighttime T_a , even at Wayqecha (Fig.
429 3). Paradoxically, there was a small overlap between daytime $T_{e\min}$ and shade T_a (Fig. 1), due
430 to higher T_b and C during the daytime active phase. Such overlap occurred rarely in a few
431 species in the Wayqecha climate, and for many of these, higher T_e were achievable
432 behaviorally via sun exposure (Fig. 1).

433 Finally, high-altitude hypoxia could limit the power output of lowland birds, rendering
434 them unable to generate sufficient heat to withstand T_e they tolerate at their native altitude.
435 Ambient oxygen partial pressure (pO_2) in dry air was about 38% higher at Pantiacolla than at
436 Wayqecha (152 versus 109 torr); the difference was 43% for moisture-saturated gas at lung
437 temperatures of ~ 38 °C (140 torr at Pantiacolla; 98 torr at Wayqecha). However, we found no
438 difference in 10 °C $\dot{V}O_2$ in Pantiacolla species tested at both pO_2 (Fig. S2). Since most
439 Wayqecha T_e are warmer than 10 °C and nearly all are warmer than 5 °C (Fig. 1), that suggests
440 the highland pO_2 would not be a barrier to up-slope movement of lowland species.

441 To summarize, we found altitude variation in all measured thermophysiological traits
442 except BMR. While the differences are not large and their causality (genetic adaptation versus
443 phenotypic plasticity) is unknown, they combine to provide highland species substantial
444 savings in costs of regulatory thermogenesis. One unexpected finding was absence of an effect
445 of mass on LCT. Other factors being equal, the surface to volume ratio decreases and,
446 presumably, plumage thickness increases at larger M_b (Calder & King 1974). Both factors
447 reduce the potential for heat loss per unit mass, so we expected an inverse relationship between
448 M_b and LCT. However, LCT depends on T_b and BMR as well as C (equation 2). Body
449 temperature varies only slightly with M_b (Fig. 2D), while in our dataset BMR scales to $M_b^{0.551}$
450 and C scales to $M_b^{0.515}$. Therefore, LCT should scale approximately to $M_b^{0.036}$. Given the
451 variance in LCT (Fig. 2B), that did not statistically differ from scaling to M_b^0 (no effect of
452 mass). Presumably, LCT would decline at larger body sizes than the ~ 300 g maximum M_b in
453 our data (Calder & King 1974).

454 We found little support for our second hypothesis: that thermal physiology is so
455 specialized that birds cannot disperse to non-native altitudes. As expected, thermoregulatory

456 costs increase with increasing altitude (Table 2), but even at Wayqecha, these costs are
457 generally below levels expected to be restrictive. In birds from all latitudes, short-term limits to
458 metabolic heat production are usually 4-7 X BMR (McKechnie & Swanson 2010). Among the
459 Peruvian species, very few approached that limit even for brief periods (the largest estimated
460 cost, about 5X BMR for the highest 30 min, was for a small highland hummingbird). Similarly,
461 estimated minimum tolerable temperatures, with rare exceptions, were much colder than
462 measured T_e (Fig. 3). We conclude that limits to acute cold tolerance are unlikely to preclude
463 up-slope movement for all but a few lowland natives, and even for these, intolerably low
464 temperatures occur infrequently.

465 Long-term constraints on metabolic power output (over days or weeks) may be more
466 important than acute cold tolerance in determining altitude occupancy, but are poorly
467 understood. Based largely on measurements during breeding, long-term maxima are often
468 assumed to be ~4X BMR (e.g., Drent & Daan 1980; Peterson, Nagy & Diamond 1990).
469 Because thermoregulatory costs cannot comprise the entire energy budget, we assumed they
470 can be at most 2.5 X BMR, similar to cost estimates for wintering temperate-zone birds
471 (Canterbury 2002). Some insight into the possibility that long-term costs preclude occupation
472 of non-native altitudes comes from modeling lowland birds in the Wayqecha climate (Table 3,
473 Fig. 4). Lowland species have less resistance to heat loss than highland birds, so it is
474 unsurprising that their costs were greater than those of highland natives (Table 3), and that
475 costs for lowland species increased considerably in the highland climate (e.g., 60% greater
476 annual means than at their native altitude). Nevertheless, maximal costs for lowland species
477 exceeded 2.5 X BMR only for short intervals (12 h or less), and annual mean costs averaged
478 only slightly above 2X BMR – again, inconsistent with the hypothesis that lowland species
479 cannot tolerate Wayqecha temperatures. Moreover, if high regulatory costs inhibit upward
480 migration, then lowland species should be disproportionately represented among species with
481 the highest long-term costs in the Wayqecha climate. This was not the case. In species for
482 which costs were 2.5X BMR or greater, there was no effect of native altitude on mean annual
483 costs ($N = 23$; $P = 0.86$), maximal 24 h costs ($N = 54$; $P = 0.40$), maximal 12 h costs ($N = 77$; P
484 $= 0.61$), or maximal 6 hr costs ($N = 86$, $P = 0.49$).

485 It is worth noting that our proposed limit of 2.5 X BMR is subject to several potentially
486 important ameliorating factors. First, activity is a substantial portion of a typical bird's energy

487 budget (Weathers & Sullivan 1993), and exercise-generated heat can be substituted for
488 thermogenesis (Webster & Weathers 1990), lowering overall costs. Second, heat released
489 during digestion is also substitutive for thermogenesis, at least for high-protein foodstuffs
490 (Chappell, Bachman & Hammond 1997; Bech & Praesteng 2004). Third, birds may save
491 energy behaviorally during the day by sun-basking or selecting warm microhabitats and at
492 night by using sheltered roosts (Buttemer 1985; Buttemer *et al.* 1987). Fourth, in species that
493 undergo torpor, the minimum observed T_b , which we used in calculations, may not be the
494 lowest defended T_b in natural conditions, since we did not subject birds to torpor-inducing
495 temperatures for more than a few hours. Our heat budgets did not account for most of these
496 factors, so our cost estimates are probably worst-case scenarios.

497 We emphasize that while strong physiological constraints on upward dispersal are
498 unlikely, an ability to tolerate highland thermal conditions is only part of the challenge lowland
499 natives face if they move up-slope. Higher heat-production costs would probably trade off
500 against investment in critical life history traits – for example, reducing the energy available for
501 maintenance or reproduction, or requiring increased foraging effort. That is a much more
502 complex issue than thermal biology *per se*, and a robust understanding of these interactions
503 requires extensive knowledge of numerous factors, such as food availability and distribution,
504 foraging efficiency, predation intensity, and reproductive physiology and behavior.

505 Three additional points merit discussion. First, are lowland temperatures a barrier to
506 down-slope movement? We did not measure heat tolerance but our data speak to this
507 possibility. Exposure to 30-35 °C for several hours during BMR tests did not elicit indications
508 of heat stress in mid- or high-altitude species. After BMR testing, T_b averaged 0.9 °C cooler in
509 highland natives than in lowland natives (suggesting these T_a were not more stressful to
510 highland birds) (Fig. 2D). Test temperatures encompassed essentially all T_a at the lowland site
511 (Fig 1). While hotter T_e occur in direct sunlight (Fig. 1), shaded refugia are readily available in
512 these forested habitats. Because we perfused metabolism chambers with dry air, evaporative
513 cooling may have been facilitated compared to natural conditions. Chamber humidity during
514 BMR tests averaged 36-50 % (from ventilatory and transcutaneous water loss and evaporation
515 from feces), less than maximal humidity at Pantiacolla. However, daytime T_b is generally 40
516 °C or higher (Aschoff 1982) and because no Pantiacolla T_a exceeded 33 C, there was always a
517 gradient for heat loss of ~ 7 °C (> 10 °C more than 99% of the time) and a substantial vapor

518 pressure deficit between T_b and T_a (~ 2.3 kilopascals even at 100% RH at 33 °C). Therefore,
519 even assuming that across-altitude physiological differences are genetically fixed, we found no
520 indication that warm lowland temperatures prevent down-slope dispersal. Moreover, we
521 measured birds at night, when C is minimal (Aschoff 1981). During the day, when T_a are
522 highest, C and T_b are elevated, so birds lose heat more readily and are better able to tolerate hot
523 conditions (if T_e remains below T_b).

524 Second, our findings apply to adults, but thermal factors may have stronger effects on
525 other life stages. Altitude limits could arise from tolerances of eggs or nestlings, or indirectly
526 through impacts on the energy budgets of parents and offspring. For example, a cool climate
527 may reduce incubation or body temperatures and hence developmental and growth rates of
528 eggs and young ectothermic nestlings, or require more parental time spent incubating or
529 brooding. Later in development, energy tradeoffs from a thermally demanding climate may
530 slow the growth of endothermic nestlings or make it difficult for parents to find sufficient food
531 for both themselves and their offspring.

532 Third, our three-year climate sample showed high seasonal stability, but does not
533 exclude the possibility that rare weather events with extreme temperatures may impose
534 stronger selection than the routine thermal milieu. We cannot address this possibility with our
535 data, as the magnitude and duration of such events are difficult to predict, and it is unclear if
536 species from different altitudes diverge in their abilities to withstand them.

537 To our knowledge no previous analysis of altitudinal distributions of tropical birds has
538 integrated physiological data from a large number of species with extensive microclimate
539 records. We found across-altitude differences in several key thermophysiological traits in
540 Andean birds, consistent with thermal specialization as predicted by Janzen (1967). However,
541 with few exceptions among 200+ tested species, we did not find convincing evidence that
542 limits to thermal tolerance –acute or long-term – are by themselves sufficient to preclude
543 colonization of non-native altitudes. That said, we believe that thermoregulatory costs, even if
544 not directly limiting, have important interactions with crucial ecological and life history
545 parameters that together determine altitude ranges.

546

547

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563

564 **Data accessibility**

565 Data for this paper are deposited with the Dryad Digital Repository: (Londoño *et al.* 2014).

566

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686

687

688 **Table 1.** Phylogenetic signal and effects of body mass and native altitude on physiological
689 variables. Body mass and conductance were \log_{10} -transformed prior to analysis; a further
690 Box-Cox transform was needed to normalize body mass. Analyses were performed with
691 phylogenetic least squares methods (PGLS), with phylogenetic signal and its mass residuals
692 tested with Pagel's λ . Five evolutionary models were evaluated and the best fit was
693 determined using AIC (see text).

694

695

	Body mass	Native altitude	Phylogenetic signal	Phylogenetic signal (resids)	PGLS best fit model
BMR	$F_{1,211} = 626$ $P < 0.0001$	$F_{2,210} = 0.09$ $P = 0.91$	$\lambda = 0.953$ $P < 0.0001$	$\lambda = 0.304$ $P = 0.0004$	Pagel
C	$F_{1,211} = 957$ $P < 0.0001$	$F_{2,210} = 21.5$ $P < 0.0001$	$\lambda = 0.956$ $P < 0.0001$	$\lambda \sim 0$ $P \sim 1$	Ornstein-Uhlenbeck ($\alpha = 5$)
T_b10	$F_{1,202} = 11.4$ $P = 0.0009$	$F_{2,201} = 6.03$ $P = 0.0029$	$\lambda = 0.301$ $P = 0.0014$	$\lambda = 0.156$ $P = 0.062$	Grafen
T_bBMR	$F_{1,211} = 23.9$ $P < 0.0001$	$F_{2,210} = 11.7$ $P < 0.0001$	$\lambda = 0.219$ $P = 0.0012$	$\lambda = 0.068$ $P = 0.21$	Pagel
LCT	$F_{1,211} = 0.075$ $P = 0.78$	$F_{2,210} = 10.1$ $P = 0.0001$	$\lambda = 0.31$ $P = 0.0027$	$\lambda = 0.31$ $P = 0.0026$	Pagel

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698 **Table 2.** Estimated thermoregulatory costs and percentage of time in thermoneutral conditions for
699 Andean bird species from three altitudes (400, 1500, and 3000 meters) exposed to weather
700 conditions at their native altitudes in 2011-2014. In each cell the upper value incorporates thermal
701 effects of sun exposure and energy savings from use of torpor (in species that exhibited nocturnal
702 hypothermia) and the lower value excludes sun exposure and torpor. Costs are expressed as
703 factorial increases above basal metabolic rate and are shown as least-square mean \pm SE, obtained
704 from ANCOVA with log mass as covariate. Phylogenetic signal was present in all
705 thermoregulatory cost variables ($P < 0.039$) and P values for across-altitude cost comparisons are
706 from PGLS. The percentage of time thermoneutral is shown as unadjusted means \pm SE;
707 comparisons among different altitudes were performed with nonparametric methods (Tukey-
708 Kramer HSD); there was no effect of mass ($P > 0.4$).
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	Pantiacolla (400 m; $N = 90$)	San Pedro (1500 m; $N = 64$)	Wayqecha (3000 m; $N = 68$)	P (body mass)	P (native altitude)
Annual mean cost	1.29 \pm 0.037	1.47 \pm 0.045	1.86 \pm 0.042	0.042	< 0.0001
	1.31 \pm 0.044	1.55 \pm 0.052	2.01 \pm 0.049	0.0452	0.0049
Highest 24 h cost	1.97 \pm 0.057	2.07 \pm 0.069	2.26 \pm 0.064	0.0748	< 0.0001
	2.15 \pm 0.061	2.19 \pm 0.073	2.36 \pm 0.068	0.0324	0.0040
Highest 12 h cost	2.19 \pm 0.064	2.32 \pm 0.077	2.44 \pm 0.072	0.0684	< 0.0001
	2.41 \pm 0.067	2.41 \pm 0.080	2.55 \pm 0.075	0.0285	0.0472
Highest 6 h cost	2.27 \pm 0.066	2.38 \pm 0.066	2.54 \pm 0.073	0.0521	0.0001
	2.44 \pm 0.068	2.47 \pm 0.082	2.64 \pm 0.076	0.0048	0.126
Highest 30 min cost	2.34 \pm 0.072	2.47 \pm 0.086	2.97 \pm 0.080	0.0734	< 0.0001
	2.50 \pm 0.072	2.57 \pm 0.087	2.91 \pm 0.081	0.0419	< 0.0001
% of time thermoneutral	47.6 \pm 3.2	25.3 \pm 2.4	15.4 \pm 1.0		< 0.0001
	43.5 \pm 3.4	13.7 \pm 2.7	1.7 \pm 0.5		< 0.0001

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713 **Table 3.** Estimated thermoregulatory costs and percentage of time in thermoneutral conditions for
714 Andean bird species from three native altitudes (400, 1500, and 3000 meters) exposed to weather
715 conditions at Wayqecha, Peru (3000 m) in 2011-2014. In each cell the upper value incorporates
716 effects of sun exposure and energy savings from use of torpor (in species that exhibited nocturnal
717 hypothermia) and the lower value excludes sun exposure and torpor. Costs are expressed as
718 factorial increases above basal metabolic rate and are shown as least-square mean \pm SE, obtained
719 from ANCOVA with log mass as covariate. Significant phylogenetic signal was absent. The
720 percentage of time thermoneutral is shown as unadjusted means \pm SE; comparisons among
721 different altitudes were performed with nonparametric methods (Tukey-Kramer HSD); there was
722 no effect of mass ($P > 0.4$).

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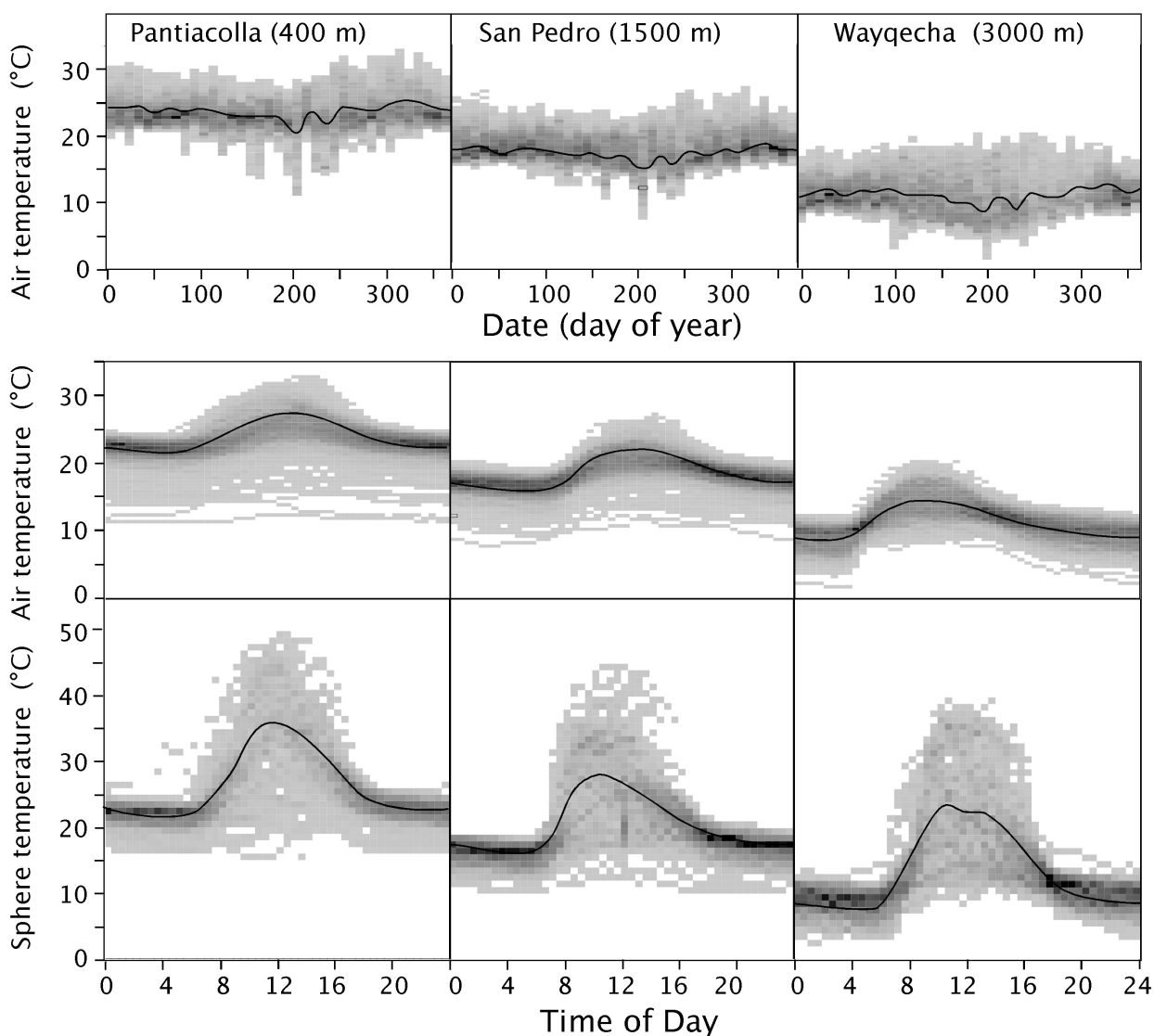
	Pantiacolla (400 m; $N = 90$)	San Pedro (1500 m; $N = 64$)	Wayqecha (3000 m; $N = 68$)	P (body mass)	P (native altitude)
Annual mean cost	2.07 \pm 0.051	1.73 \pm 0.062	1.72 \pm 0.059	0.418	< 0.0001
	2.11 \pm 0.054	1.86 \pm 0.064	1.88 \pm 0.063	0.192	0.0049
Highest 24 h cost	2.40 \pm 0.060	2.09 \pm 0.072	2.11 \pm 0.069	0.463	0.0015
	2.40 \pm 0.062	2.12 \pm 0.073	2.15 \pm 0.071	0.145	0.0068
Highest 12 h cost	2.63 \pm 0.067	2.28 \pm 0.081	2.30 \pm 0.078	0.204	0.0009
	2.66 \pm 0.068	2.35 \pm 0.082	2.38 \pm 0.080	0.161	0.0060
Highest 6 h cost	2.67 \pm 0.068	2.34 \pm 0.083	2.37 \pm 0.079	0.171	0.0037
	2.67 \pm 0.069	2.35 \pm 0.083	2.38 \pm 0.079	0.161	0.0060
Highest 30 min cost	2.91 \pm 0.075	2.57 \pm 0.090	2.61 \pm 0.087	0.138	0.0137
	2.98 \pm 0.078	2.69 \pm 0.095	2.75 \pm 0.090	0.414	0.0145
% of time thermoneutral	4.1 \pm 0.7	7.9 \pm 0.8	15.4 \pm 1.0		< 0.0001
	0.4 \pm 0.2	0.7 \pm 0.4	1.7 \pm 0.5		0.0187

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728 **Figure Legends**

729 **Figure 1:** Temperatures at our three field sites in southeastern Peru. Air temperature is shade
730 temperature; sphere temperature is the temperature of bird models (gray-painted aluminum
731 spheres) placed in open areas to receive sunlight and wind. The frequency of particular
732 temperatures is indicated by shading (pale = few, dark = many). Solid lines are smoothed
733 daily means (annual plots) or 30-min means (24-hour plots). Data were obtained in 2011-
734 2014. Mean air temperatures differ by about 12 °C between Pantiacolla and Wayqecha
735 (Table S1).

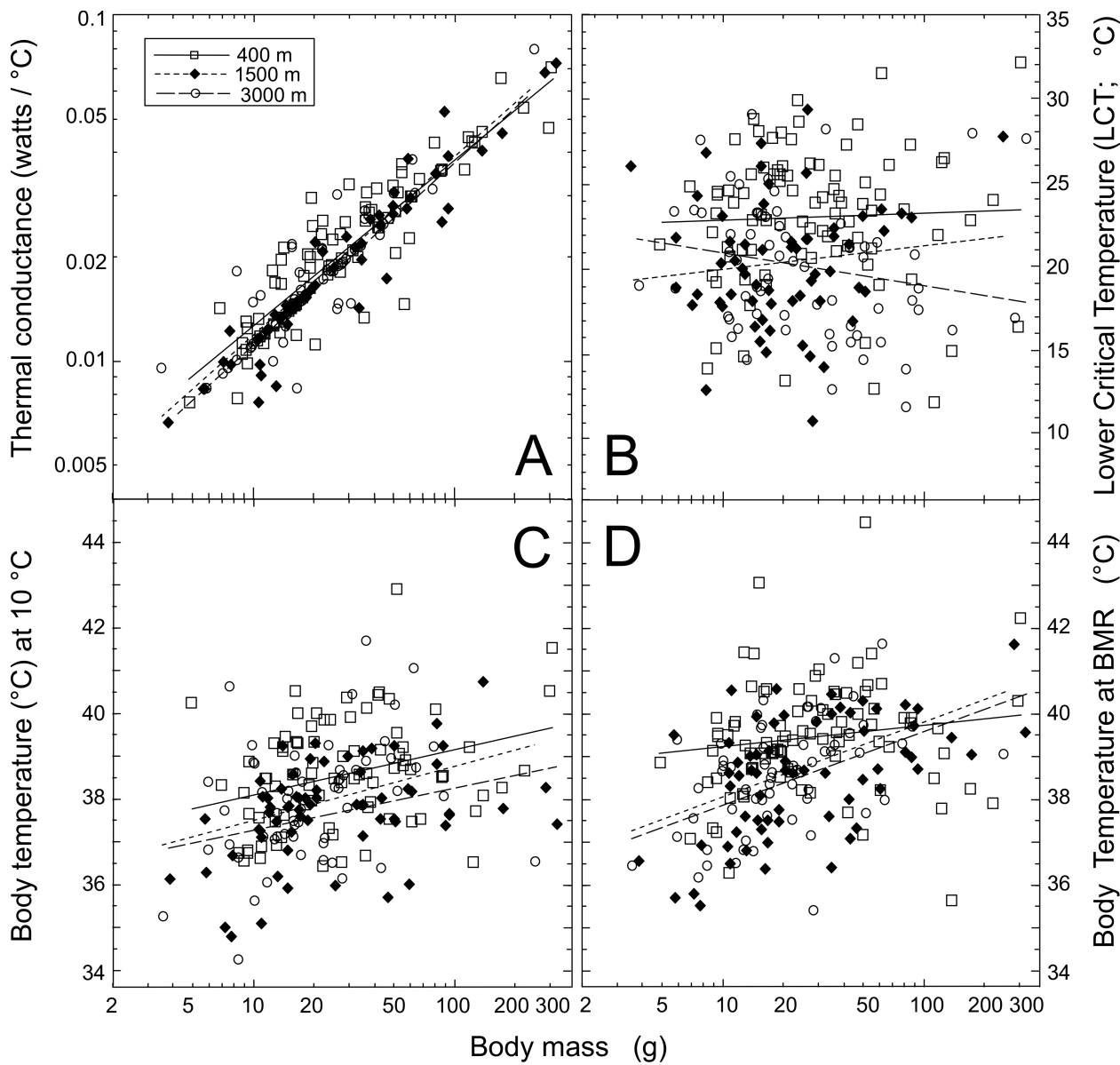
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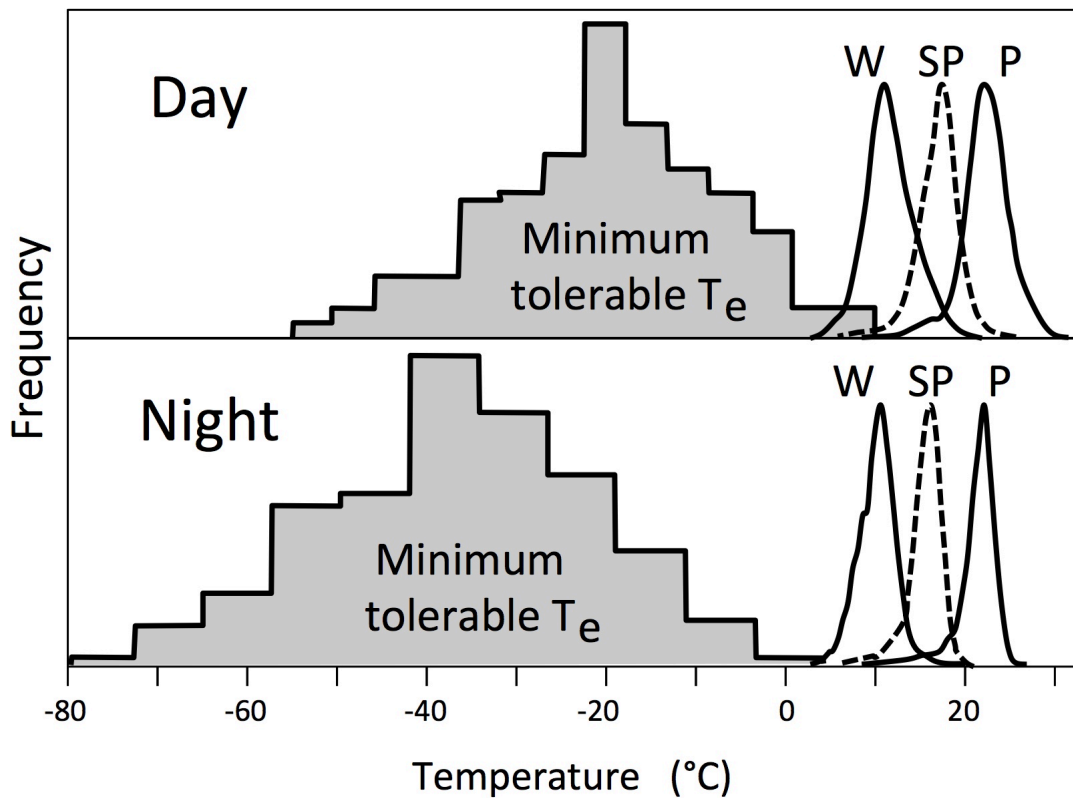
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739 **Figure 2:** Thermal physiology of Andean birds from three fields sites at different
 740 altitudes (Pantiacolla, 400 m; San Pedro, 1500 m; Wayqecha, 3000 m). A: thermal
 741 conductance; B: lower critical temperature; C: body temperature at an ambient
 742 temperature of 10 °C; D: body temperature at BMR (ambient temperature 30 - 34 °C).
 743 All traits differ among altitudes; all but lower critical temperature are affected by body
 744 mass (Table 1). Basal metabolic rates (BMR, not shown) are strongly positively
 745 correlated with body mass but are not affected by altitude.
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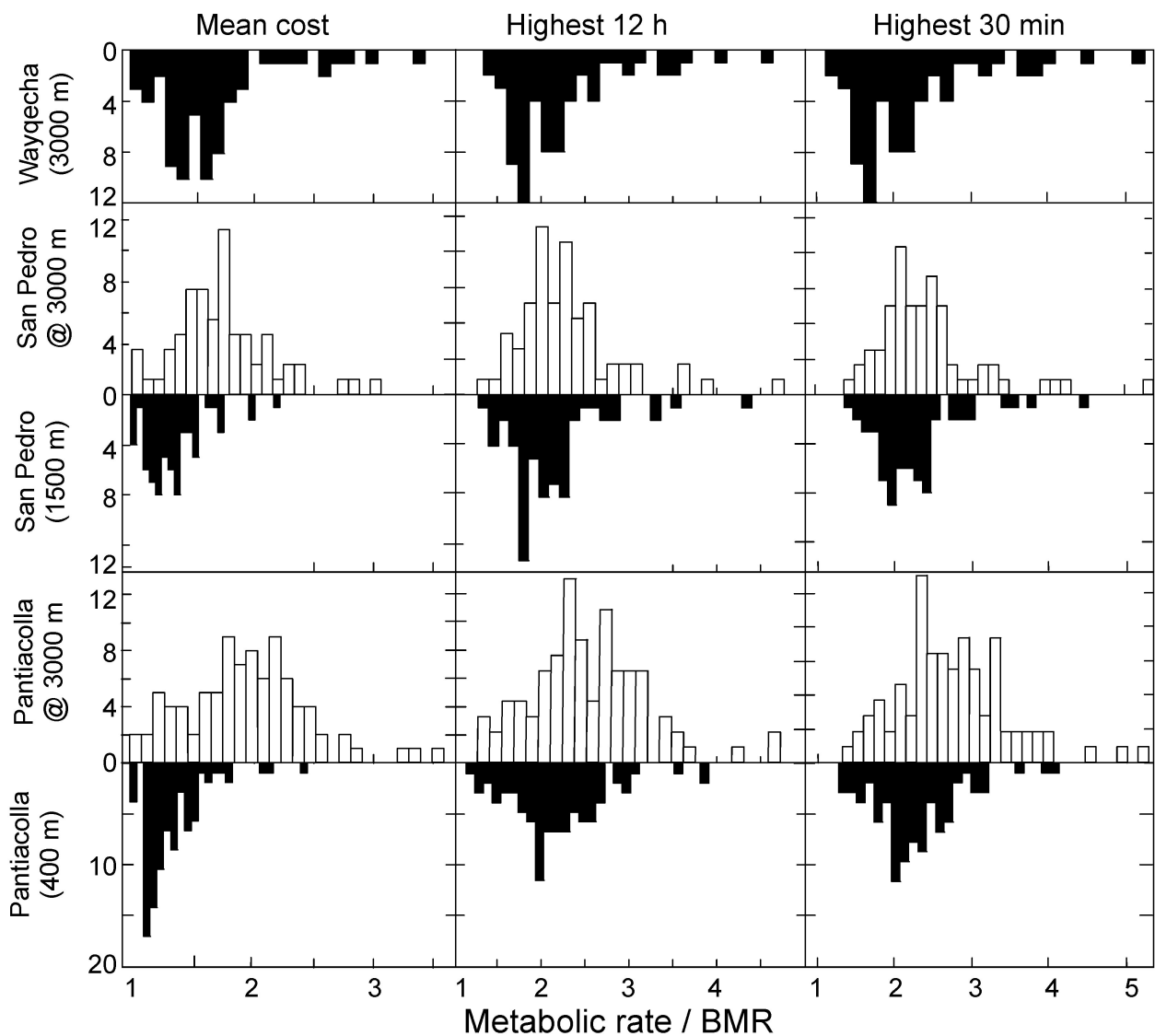
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750 **Figure 3:** Relationship between the estimated minimum tolerable environmental
751 temperature ($T_{e,min}$; gray shading) and shade temperature distributions (right) for 215
752 Andean bird species at three field sites, Wayqecha (W, 3000 m), San Pedro (SP, 1500
753 m), and Pantiacolla (P, 400 m). We computed $T_{e,min}$ from body temperature,
754 conductance, and a maximal thermogenic heat production of 4.5 X BMR (Wiersma,
755 Chappell & Williams 2007). $T_{e,min}$ was affected by native altitude (see text) but data
756 from different altitudes are pooled in this figure. $T_{e,min}$ differed between night and day
757 because both body temperature and conductance are higher during the active phase
758 (day) of the diurnal cycle (Prinzinger, Pressmar & Schleucher 1991; Schleucher & Withers
759 2001).
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763 **Figure 4:** Estimated thermoregulatory costs of bird species from low-, mid-, and high-
 764 elevation sites ($N = 88, 61,$ and 66 species, respectively). Costs (x-axis) are expressed as
 765 factorial increases above basal metabolic rate (BMR). Black bars show the number of
 766 species for each metabolic cost at native altitudes; open bars show the number of
 767 species for each cost at the high-altitude site. The three columns show the mean
 768 thermoregulatory cost (left), the highest cost averaged over 12 h (middle) and the
 769 highest cost averaged over 30 min (right). Costs were computed with a heat-budget
 770 model (Fig. S1) and the weather data in Fig. 1.
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