

The validity of ecogeographical rules is context-dependent: testing for Bergmann's and Allen's rules by latitude and elevation in a widespread Andean duck

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Consistent responses by various organisms to common environmental pressures represent strong evidence of natural selection driving geographical variation. According to Bergmann's and Allen's rules, animals from colder habitats are larger and have smaller limbs than those from warmer habitats to minimize heat loss. Although evidence supporting both rules in different organisms exists, most studies have considered only elevational or latitudinal temperature gradients. We tested for the effects of temperature associated with both elevation and latitude on body and appendage size of torrent ducks (*Merganetta armata*), a widespread species in Andean rivers. We found a negative relationship between body size and temperature across latitude consistent with Bergmann's rule, whereas there was a positive relationship between these variables along replicate elevational gradients at different latitudes. Limb-size variation did not support Allen's rule along latitude, nor along elevation. High-elevation ducks were smaller and had longer wings than those inhabiting lower elevations within a river. We hypothesize that temperature is likely a major selective pressure acting on morphology across latitudes, although hypoxia or air density may be more important along elevational gradients. We conclude that the effect of temperature on morphology, and hence the likelihood of documenting ecogeographical 'rules', depends on the environmental context in which temperature variation is examined. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, ●●, ●●–●●.

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INTRODUCTION

Adaptive strategies helping organisms to cope with challenges imposed by cold environments are diverse (Hopkins & Powell, 2001). Endothermic animals such as birds and mammals face a strong effect of cold environments because of their need to maintain a constant body temperature at the same time as minimizing energetic costs (Scholander, 1955; Bennett & Ruben, 1979; Porter & Kearney, 2009). Among the organismal responses assumed to represent adaptation by endotherms to cold environments, some of the most studied patterns are the so-called ecogeographical rules (Mayr, 1956). Bergmann's rule (Bergmann, 1847) and Allen's rule (Allen, 1877) indicate that organisms living in colder habitats, including those at high latitudes and elevations, tend to be larger overall and to have smaller appendages, respectively, to minimize heat loss (Blackburn, Gaston & Loder, 1999; Meiri & Dayan, 2003; Nudds & Oswald, 2007; Tilkins *et al.*, 2007).

Considerable debate has existed about the validity and applicability of ecogeographical rules such as Bergmann's or Allen's. First, the taxonomic scale to which such rules should be applied has been debated, where some studies claim that they should only apply to entire species assemblages at higher latitudes, whereas others argue that they can also be applied to closely-related groups of species or to different populations within a species (Blackburn *et al.*, 1999; Pincheira-Donoso, 2010). Second, there has been controversy regarding the environmental factors correlated with latitude (e.g. temperature, humidity, precipitation, primary productivity, seasonality, competition, resource availability, species richness or species range size) that ultimately promote changes in morphology (Graves, 1991; Jones *et al.*, 2005; Millien *et al.*, 2006; Yom-Tov & Geffen, 2006; Meiri, Yom-Tov & Geffen, 2007; Guillaumet *et al.*, 2008; Olson *et al.*, 2009), as well as about whether the cause involved in the response to those factors is the need for heat conservation or whether there are alternative physiological explanations (Scholander, 1955; Mayr, 1956; Irving, 1957; Olson *et al.*, 2009; Olalla-Tárraga, 2011; Watt & Salewski, 2011; Martinez *et al.*, 2013).

Multiple studies have attempted to determine whether Bergmann's rule applies to birds, finding evidence for it over a wide range of taxonomic levels (Zink & Remsen, 1986; Blackburn & Ruggiero, 2001; Ashton, 2002; Guillaumet *et al.*, 2008; Boyer, Cartron & Brown, 2010; but see also Perktas, 2011). Allen's rule has received considerably less attention (Cartar & Guy Morrison, 2005; Yom-Tov & Geffen, 2006; Symonds & Tattersall, 2010; Greenberg *et al.*, 2012a, b), frequently finding contradictory patterns

in the variation seen in different body appendages (beak, wings, and legs). However, considering that the original formulation of Bergmann's and Allen's rules states that the low temperatures characteristic of high-latitude environments pose the challenge of maintaining thermal balance and thus influence species' morphological traits, three important points have often been neglected by several of the studies focused on birds. First, few studies focused on single species (Snow, 1954; Power, 1969; Laiolo & Rolando, 2001; Meiri and Dayan, 2003) have examined the relationship between temperature and morphology directly; most studies typically correlate morphology with latitude and assume that any relationship observed is driven by temperature. Second, most studies on birds have focused on the effects of latitudinal variation in temperature on body proportions (Ashton, 2002), whereas few have considered elevational gradients in temperature as potential drivers of morphological variation (Blackburn & Ruggiero, 2001; Laiolo & Rolando, 2001; Bulgarella *et al.*, 2007; Milá *et al.*, 2009; Symonds & Tattersall, 2010; Wilson, Valqui & McCracken, 2010; Pitchers, Pool & Dworkin, 2012). Finally, almost all studies either use single morphological characters as a proxy for body size (e.g. wing length or tarsus length), or reduce morphological variation in several traits using multivariate statistical methods (Meiri & Dayan, 2003), without acknowledging that each character might be under particular selection pressures (e.g. flying, swimming, feeding or dissipating heat) (Raikow, 1973; Laiolo & Rolando, 2001; Altshuler & Dudley, 2006; Greenberg *et al.*, 2012a). Examining variation in different traits such as those utilized for locomotion or foraging separately may reveal patterns that are not obvious from the analysis of overall variation in body size.

The torrent duck (*Merganetta armata* Gould 1842, Aves: Anatidae) is a good species to address questions related to adaptation to contrasting thermal environments because it lives in Andean rivers over wide latitudinal (from approximately 54.8°S in Argentina to 8.9°N in Venezuela) and elevational (from sea level to over 4500 m) gradients (Carboneras, 1992). Three subspecies of *M. armata* are recognized based on differences in body size and in male plumage coloration, which occur in Venezuela, Colombia, and Ecuador (*Merganetta armata colombiana*), Peru and Bolivia (*Merganetta armata leucogenis*), and Argentina and Chile (*Merganetta armata armata*) (Carboneras, 1992). The distribution of the species provides a fine opportunity to test for (1) adaptive variation in morphology along a thermal latitudinal axis and (2) parallel evolution in morphological variation across populations of a single species by studying replicate elevational gradients located at different latitudes.

The examination of natural replicates of populations living under similar conditions allows for a better understanding of how environmental features affect the ecology of species and promote adaptation and plasticity (Rosenblum & Harmon, 2011).

We examined geographical variation in different morphological characters to evaluate whether torrent ducks follow Bergmann's and Allen's rules with respect to variation in temperature associated with latitude and elevation. We used different measurements reflecting variation in body (mass and skull size) and appendage size (beak, wing, and tarsus size) to separately examine the predictions of Bergmann's and Allen's rules. If these rules apply to *Merganetta*, then a trend of increasing body size and decreasing appendage size would be seen with lower environmental temperatures (i.e. at increasing latitudes and elevations). In this case, Bergmann's rule predicts a negative relationship between temperature and body size, and Allen's rule predicts a positive relationship between temperature and appendage size.

MATERIAL AND METHODS

Fieldwork was conducted between 2010 and 2012 in five different rivers inhabited by populations of all three subspecies of *M. armata*: Río Quindío, Quindío, Colombia (1500–2100 m; eight females and 15 males captured); Río Pachachaca, Apurímac, Peru (2200–3600 m; 20 females and 28 males); Río Chillón, Lima, Peru (1000–4000 m; 24 females and 85 males); Arroyo Grande, Mendoza, Argentina (1800–3200 m; 16 females and 30 males); and Río Malargüe, Mendoza, Argentina (1600–1900 m; seven females and 14 males) (Fig. 1). Because the elevational range covered by the samples of the Malargüe River was small compared to other rivers, and because it complements the elevational sampling of the nearby Arroyo Grande, both were treated as a single unit for analyses (hereafter Argentina, encompassing a gradient ranging from 1600 to 3200 m).

For each duck captured, we measured body mass (± 1 g), skull length (occipital condyle to beak tip; ± 0.1 mm), wing chord (carpal joint to longest primary feather unflattened; ± 0.1 mm), tarsal length (ankle joint to toe joint, ± 0.1 mm), and total culmen length (± 0.1 mm). Data corresponding to juveniles or molting adults were omitted from the analyses. To correct for overall size, appendage size was expressed as the residuals of a linear regression between each measurement (wing chord, tarsal length, and total culmen length) and skull length (results shown in the main text) or body mass (see Supporting Information, Fig. S1, Tables S1–S3). We georeferenced the site where each duck was captured and used geographical coordinates to obtain the mean annual temperature for

each locality from the WoldClim database (Hijmans *et al.*, 2005). We chose this variable to climatically characterize our sampling localities because it is widely proposed as the driver of body- and appendage-size differences observed across latitudinal and elevational clines (Mayr, 1956; Blackburn *et al.*, 1999; Meiri & Dayan, 2003).

All variables were tested for normality and homoscedasticity using Shapiro–Wilk and O'Brien tests ($\alpha = 0.05$), respectively. We used R, version 2.15.1 (R Core Team, 2012) to construct analyses of covariance (ANCOVA) to test for the overall effects of temperature on different morphological characters (body mass, skull length, corrected wing size, corrected tarsus size, and corrected culmen size), separately for males and females. The models incorporated the effect of locality (Colombia, Apurímac, Chillón, and Argentina) as a cofactor, and the interaction between temperature and locality. If temperature affects morphological traits in a similar way across replicated elevational gradients, then we expected ANCOVA models to be globally significant, showing a significant contribution of temperature and/or locality but no effect of the interaction term. We also fitted linear regressions only for the Chillón River, the locality in Peru with the largest sample size, to determine how variation in temperature associated with elevation relates to morphological traits. As a result of the large number of tests (20 in total), we applied a sequential Bonferroni correction for significance values (Holm, 1979) by pooling together ANCOVA and regression *P*-values, and ordering them from lowest to highest. We then assigned a rank of 20 to the lowest *P*-value and a rank of 1 to the highest *P*-value; to assess significance of tests, we divided the predefined 0.05 value of α by the rank corresponding to each *P*-value.

We analyzed variation in each morphological character separately because relationships with temperature may vary among characters. For example, because both body mass and skull length reflect body size, we expect both traits to show a negative relationship with temperature as predicted by Bergmann's rule. However, because body mass is a highly variable trait, which depends on several body and environmental conditions, we expect more variation in this trait than in skull length, which would be a more accurate proxy for body size (Wilson *et al.*, 2010; Wilson, Eaton & McCracken, 2012). By contrast, if Allen's rule applies to these birds, we expect a positive relationship between temperature and wing, tarsal or culmen size because these traits may be involved in heat dissipation (Hagan & Heath, 1980; Meiri & Dayan, 2003; Nudds & Oswald, 2007). Because the tarsus is involved in diving and swimming and the beak in foraging, there may be no observed pattern in relation to temperature if

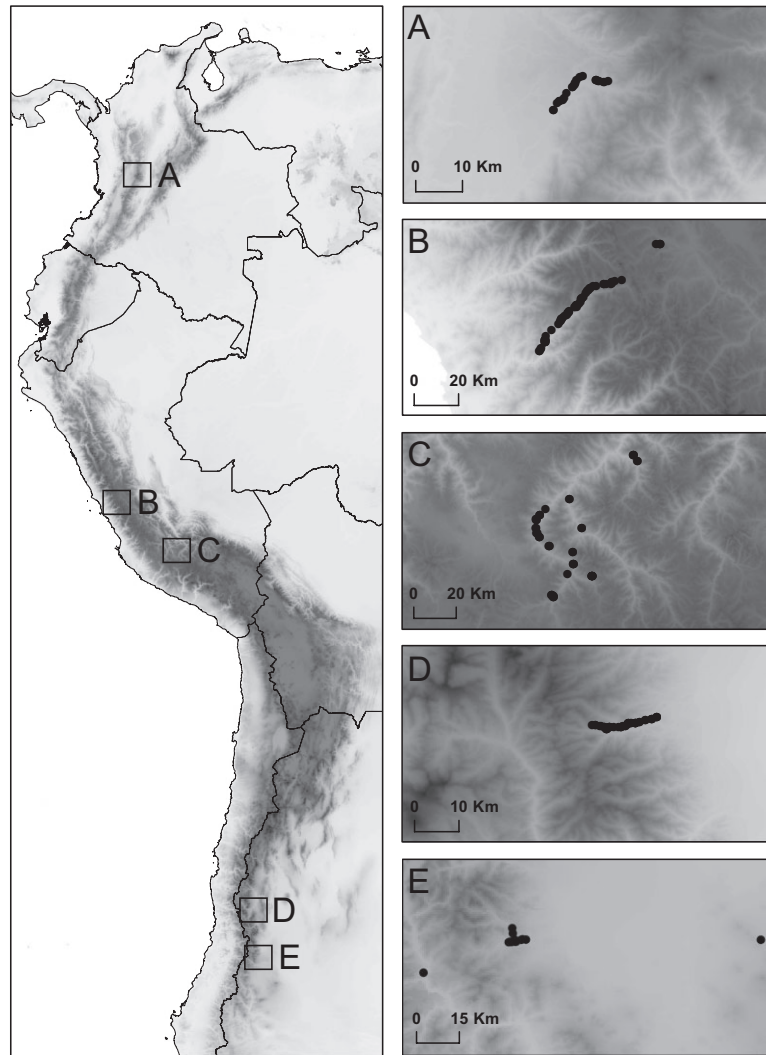


Figure 1. Map of South America showing the localities that were sampled for the present study. A, Río Quindío. B, Río Chilón. C, Río Apurímac. D, Arroyo Grande. E, Río Malargüe.

selection in relation to locomotion or feeding overrides any influence of selection in relation to thermal balance (Raikow, 1973). Similarly, wing size can be affected by the energetic demands of flight, which actually predict a negative (instead of a positive) relationship with temperature within each elevational gradient (i.e. proportionally larger wings are expected at higher elevations as a result of challenges of flying in colder environments with reduced air density; Savile, 1957; Altshuler & Dudley, 2002; Altshuler, Dudley & McGuire, 2004).

Additionally, to determine whether our assessment of Bergmann's rule was consistent even using a multivariate estimate of body size, we performed a principal component (PC) analysis with all uncorrected morphological variables evaluated. We then used the

resulting PC variables to conduct ANCOVA analyses aiming to test for the overall effects of temperature on body size, in accordance with the same procedure described above (see Supporting Information, Fig. S2, Tables S4, S5).

RESULTS

TEMPERATURE VARIATION

Mean temperature differed among our study sites: Argentina was the coldest locality (mean \pm SD 8.3 ± 2.0 °C; range 1.8–9.7 °C), followed by Apurímac (12.0 ± 2.5 °C; 8.7–16.3 °C), Chillón (12.2 ± 4.1 °C; 4.5–17.5 °C), and Colombia (17.7 ± 1.4 °C; 13.7–19.0 °C). The finding that Chillón had the largest

temperature range and Colombia the smallest is probably a result of these localities having the widest and the narrowest elevational ranges, respectively.

LATITUDINAL PATTERNS

ANCOVA models for mass, skull length, and wing size were globally significant for both males and females, and the culmen-size model was only significant for males (Fig. 2, Table 1). On average, significant models explained 40.2% (range 8.4–59.2%; Table 1) of the variation in body-size traits. Both temperature and locality had significant effects in the models describing variation in body mass, wing size, tarsus size, and culmen size; the only models not showing a significant effect of temperature were those examining variation in skull length in both males and females (Fig. 2, Table 1).

When analyzing all the data (i.e. including all localities from different latitudes), the relationship between temperature and body mass was negative (Fig. 2A, B, bold black lines), a pattern consistent with predictions of Bergmann's rule at the latitudinal level. In Argentina, temperatures were the lowest and ducks were the largest; in Colombia, temperatures were the highest and ducks the smallest, and Peruvian localities (Chillón and Apurímac) were intermediate for both temperature and body mass (Fig. 2A, B, Table 2). Although the ANCOVA model for skull length showed no significant effect of temperature (Table 1), there were significant differences between localities that might also be consistent with Bergmann's rule, with larger ducks at higher latitudes (Argentina; Fig. 2C, D, Table 2).

No apparent differences were observed in relative wing size between Peruvian and Argentinian popula-

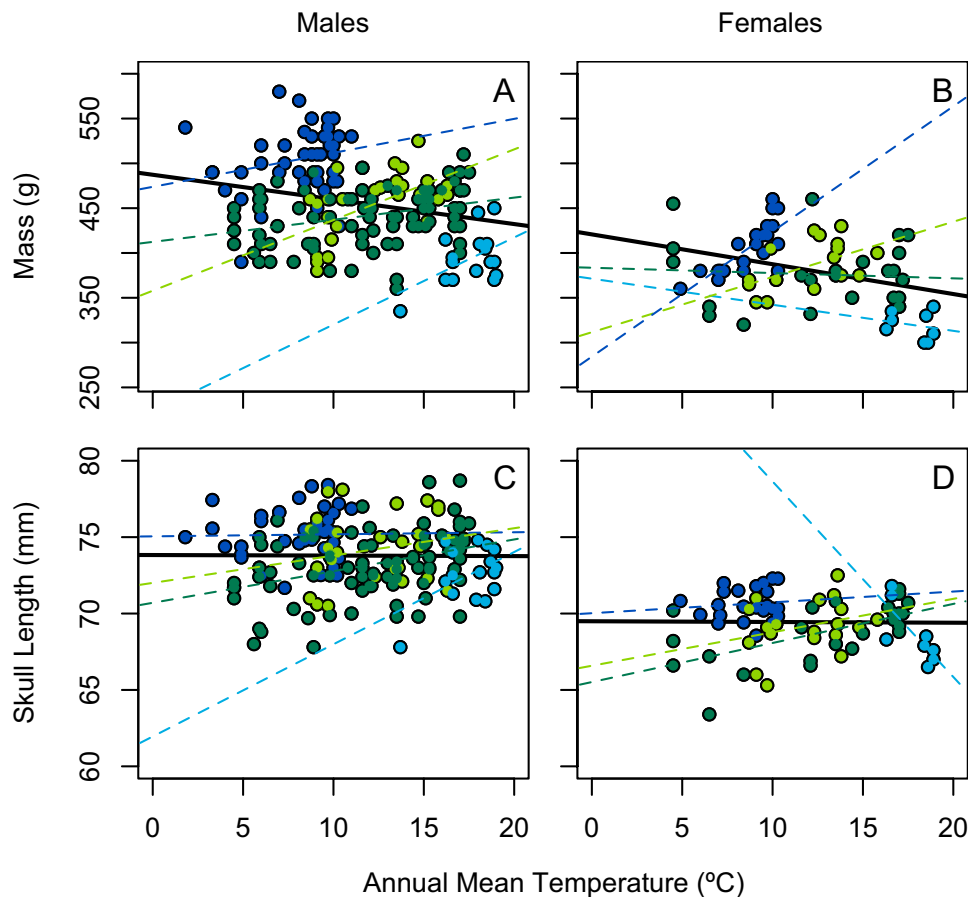


Figure 2. Variation in body mass (A, B) and skull length (C, D) in relation to annual mean temperature in torrent ducks. Light blue, dark green, light green, and dark blue dots and dashed lines represent Quindío (Colombia), Chillón (Peru), Apurímac (Peru), and Mendoza (Argentina), respectively. Bold black lines represent regressions fitted for all points, regardless of locality. Note that relationships existing within localities (i.e. along temperature gradients associated with elevation) are generally positive, whereas those existing across all localities (i.e. along the temperature gradient associated with latitude) are negative or nonsignificant.

Table 1. Significance level (*P* values) of the fitted models for each variable evaluated, and the partial effect of the covariable, the cofactor, and the interaction term

Sex	Variable	<i>N</i>	<i>p</i> rank	Overall	Adjusted <i>R</i> ²	Temperature	Locality	Temperature × Locality
Males	Mass	171	20	< 0.0001	0.5404	< 0.0001	< 0.0001	0.0941
	Skull length	172	16	< 0.0001	0.2068	0.9241	< 0.0001	0.3499
	Residuals wing size	163	19	< 0.0001	0.5457	< 0.0001	< 0.0001	0.2421
	Residuals tarsus size	169	6	0.2372	0.0136	0.0811	0.3232	0.4341
	Residuals culmen size	169	11	0.0029	0.0843	0.0058	0.0948	0.0407
Females	Mass	72	17	< 0.0001	0.4902	0.0001	< 0.0001	0.0030
	Skull length	75	15	< 0.0001	0.3557	0.9097	< 0.0001	0.0212
	Residuals wing size	69	18	< 0.0001	0.5924	< 0.0001	< 0.0001	0.3156
	Residuals tarsus size	75	3	0.5105	0.0861	0.7103	0.4104	0.3619
	Residuals culmen size	74	4	0.3920	0.0066	0.1239	0.4201	0.5345

Statistically significant values after applying a sequential Bonferroni correction ($\alpha = 0.05/p$ rank; see Material and methods and Table 3) are shown in bold.

Table 2. Mean ± SD values for each trait within each locality

Sex	Trait	Argentina	Apurímac	Chillón	Quindío
Males	Mass (g)	504.43 ± 36.97	453.86 ± 36.02	442.2 ± 31.79	397.67 ± 25.28
	Skull length (mm)	75.16 ± 1.63	74.19 ± 2.33	73.18 ± 2.17	72.88 ± 1.37
	Residuals wing size	6.20 ± 4.46	6.44 ± 7.26	0.86 ± 8.14	−17.33 ± 5.53
	Residuals tarsus size	0.26 ± 1.31	−0.22 ± 1.36	0.13 ± 2.24	0.42 ± 1.51
	Residuals culmen size	−0.12 ± 1.13	−0.06 ± 0.88	0.08 ± 0.97	0.91 ± 1.17
Females	Mass (g)	405.45 ± 27.90	385.56 ± 26.34	375.92 ± 37.42	319.38 ± 15.45
	Skull length (mm)	70.64 ± 1.04	69.15 ± 1.71	68.81 ± 1.97	68.6 ± 1.91
	Residuals wing size	−0.26 ± 5.73	3.54 ± 7.66	−4.72 ± 5.85	−22.59 ± 5.94
	Residuals tarsus size	−0.40 ± 1.63	−0.65 ± 1.87	0.13 ± 1.79	−0.46 ± 1.11
	Residuals culmen size	−0.35 ± 1.18	−0.58 ± 0.81	0.15 ± 1.74	0.24 ± 1.17

tions, although wings tended to be smaller in Colombian ducks (Fig. 3A, B, light blue lines and dots, Table 2). This is not consistent with what would be expected if Allen's rule applies to these birds at the latitudinal level. Conversely, a weak but positive relationship was found between temperature and culmen size in males (Fig. 3C, D, E, F, Table 1), as predicted by Allen's rule. Overall, the results obtained were very similar when correcting by body mass instead of skull length, or when using the PCs of body size instead of each variable separately (see Supporting Information, Figs. S1, S2, Tables S1–S5).

ELEVATIONAL PATTERNS

We found no interaction between temperature and locality in all but one of our ANCOVA models (Figs 2, 3, Table 1), which indicates that temperature is not related to morphology differently at each locality.

This result justifies our decision of choosing only our best-sampled locality in Peru (Chillón) to examine elevational variation in body-size traits. Because there were no statistical differences between regression slopes across sites, patterns observed at Chillón can be considered representative of patterns existing at other sites. The only exception was mass for females, although we consider that the small sample size and high variation within some localities (e.g. Colombia) could have affected the analysis of this character (Fig. 2B, Table 1).

Focusing then on the well-sampled Chillón River, we found a significant effect of temperature on body mass, skull length, and wing length in males (Figs 2, 3A, dark green dots and lines, Table 3) but only on skull length in females. Such difference between sexes is likely a result of the lower sample size for females and is not discussed further. The relationship between temperature and both mass and skull

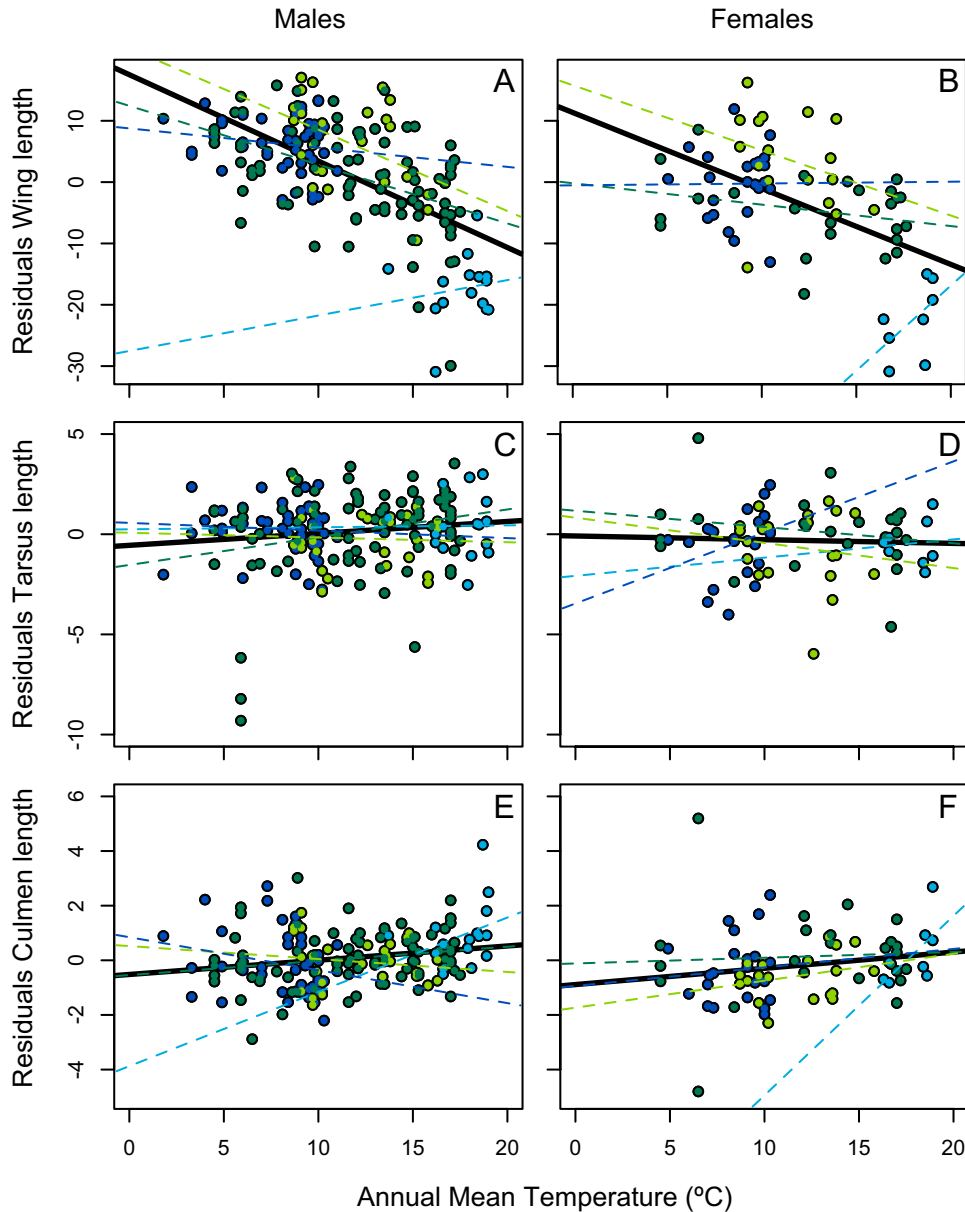


Figure 3. Variation in wing (A, B), tarsus (C, D), and culmen size (E, F) in relation to annual mean temperature in torrent ducks. Light blue, dark green, light green, and dark blue dots and lines represent Quindío (Colombia), Chillón (Peru), Apurímac (Peru), and Mendoza (Argentina) samples, respectively. Bold black lines represent regressions fitted for all points, regardless of locality.

length was contrary to that found at the latitudinal level and to that expected under Bergmann's rule: smaller-bodied ducks were found at lower temperatures (i.e. higher elevations; Fig. 2, Table 3).

The relationship between temperature and wing size was negative, with larger wings at lower temperatures (higher elevations; Fig. 3A), a pattern contrary to that predicted by Allen's rule. Tarsus length and culmen length did not show any relationship

with temperature (Fig. 3, Table 3), suggesting that Allen's rule does not apply at the elevational level. However, when correcting by body mass instead of skull length, two additional regression models (culmen and tarsus size for males) were significant after applying the Bonferroni correction, which would imply a weak support for Allen's rule over elevational gradients (see Supporting Information, Fig. S1, Table S3).

Table 3. Significance level (*P* values) of the linear regressions fitted for the Río Chillón data, with their corresponding adjusted *R*² and slope values

Sex	Trait	<i>p</i> rank	<i>P</i>	<i>R</i> ²	Slope
Male	Mass	10	0.0040	0.0855	2.4704
	Skull length	13	0.0003	0.1340	0.2073
	Residuals wing size	14	< 0.0001	0.2131	−0.9587
	Residuals tarsus size	9	0.0229	0.0501	0.1384
	Residuals culmen size	8	0.0518	0.0337	0.0519
Female	Mass	2	0.7350	0.0053	−0.5912
	Skull length	12	0.0020	0.3304	0.2558
	Residuals wing size	7	0.1928	0.0338	−0.3492
	Residuals tarsus size	5	0.3265	0.0003	−0.0812
	Residuals culmen size	1	0.7970	0.0031	0.0209

Statistically significant values after applying a sequential Bonferroni correction ($\alpha = 0.05/p$ rank; see Material and methods and Table 1) are shown in bold.

DISCUSSION

To our knowledge, only a few studies have assessed how various morphological characters vary in relation to temperature in a single bird species by evaluating both wide latitudinal and elevational gradients, thus providing a simultaneous test of Bergmann's and Allen's ecogeographical rules (Blackburn & Ruggiero, 2001; Wilson *et al.*, 2010). Our results are consistent with Bergmann's rule over the thermal gradient associated with latitude but not with respect to the thermal gradients associated with elevation in torrent ducks. In turn, Allen's rule does not appear to apply over thermal gradients associated with latitude (wing and culmen size), nor along gradients associated with elevation. Taken together, these results indicate that torrent ducks likely respond to pressures imposed by temperature differently over latitudinal and elevational gradients.

The significantly negative relationship between temperature and body mass, consistent with Bergmann's rule at the latitudinal level, was also supported by differences in mean body size (i.e. mass and skull length) between localities at different latitudes. Temperature is likely a main environmental factor determining latitudinal variation in morphology because mean temperatures are considerably lower at higher latitudes (Argentina) than at more tropical latitudes, and larger ducks are theoretically better suited to withstand lower temperatures by reducing surface heat loss (Bergmann, 1847; Mayr, 1956; Searcy, 1980). Also, because we found no overall significant relationship between temperature and skull length, the larger size at higher latitudes could be mostly the result of an increase in body fat or other structural changes, which would also be advantageous in cold environments. Increasing insulation is

one of many ways (e.g. modifying surface exposure or vascularization) to improve the thermal properties of a surface (Scholander, 1955).

Despite the strong negative relationship found between temperature and body mass at the latitudinal level, we found an opposite (i.e. positive) relationship between those variables along the individual elevational gradients. This is in contrast to what would be expected if Bergmann's rule applied consistently to these birds. Because we also found a positive relationship between temperature and skull size, we argue that the variation in body size seen along elevational gradients is probably driven by an increase in skeletal size rather than in body fat (Fig. 2; subcutaneous fat is quite low in torrent ducks in Colombia and Peru, N. Gutiérrez-Pinto and K.G. McCracken, pers. observ.). This also implies that thermal stress, as expected given assumptions of Bergmann's rule, is likely not a key selection pressure at increasing elevations. This reveals that there may be environmental factors other than temperature that have a more important role in shaping body size of torrent ducks at high elevations. Differences in body-size trends along latitudinal and elevational axes have been previously documented (including ectothermic animals; Gouveia *et al.*, 2013) and hypothesized to be a result of differences between such axes in the steepness of environmental gradients. In addition, we consider that differences in body-size trends associated with latitude and elevation can also arise from the interaction of multiple environmental factors affecting organisms differentially along latitudinal and elevational gradients, which would also explain the lack of consistency in patterns across various organisms (Brehm & Fiedler, 2004; Chown & Gaston, 2010; Pitchers *et al.*, 2012; Gouveia *et al.*, 2013).

For example, lower partial oxygen pressures at higher elevations present an obvious challenge by reducing overall oxygen uptake with respect to sea level; also, flight physiology is affected because air density is lower and thus more energy is required to sustain the bird in the air at higher elevations (Altshuler & Dudley, 2006). Thus, smaller (i.e. lighter) birds with larger wings (i.e. reduced wing load) are expected to have an aerodynamic and physiological advantage at high elevations (Savile, 1957; Altshuler & Dudley, 2002; Altshuler *et al.*, 2004) and this could explain our finding of a negative relationship between wing size and temperature over elevational gradients. Because our analyses focused only on wing length, this hypothesis needs to be examined further by characterizing wing shape and area. Furthermore, because torrent ducks spend much of their time diving (during foraging or when escaping from predators; Johnsgard, 1966), a small body size at high elevations could also represent benefits by reducing drag with surrounding water. We hypothesize that the increased diving performance that small body size may thus allow (Raikow, 1973; Lovvorn & Jones, 1991; Lovvorn, Jones & Blake, 1991) would be especially beneficial in the oxygen-poor and cold conditions existing at high elevations.

We found no support for Allen's rule (i.e. no variation in tarsus length and culmen size in relation to temperature) within individual elevational gradients. This is an indication that many different, possibly conflicting, selection pressures shape organismal phenotypes (Mayr, 1956), such that each character may be subject to different selective pressures shaping variation at the population level. For example, diving restrictions would favour a paddle and limb size sufficiently large to allow for efficient aquatic locomotion but small enough to allow for adequate movement on land (Raikow, 1973; Elliott *et al.*, 2013). In turn, feeding limitations may not allow major changes in the basic structure of torrent ducks. This species has a flexible bill used to probe among rocks and fissures when searching for food (Johnsgard, 1966; Cerón & Trejo, 2009), and such restrictions may over-ride the influence of any potential selective pressure acting on bills as heat radiators (Hagan & Heath, 1980; Greenberg *et al.*, 2012a). Of course, we cannot rule out the possibility that the morphological traits we studied are unaffected by differential selection along gradients in environmental conditions and resources associated with elevation.

The possible selective constraints resulting from trade-offs in the variation in individual traits with different functions noted above do not appear to be acting at the species-range (i.e. latitudinal) level: we found a significantly positive relationship between temperature and culmen size consistent with Allen's

rule. However, this ANCOVA model explained a small amount of variation (8%). There might be additional factors at each population that act in concert with temperature to influence variation in these characters. For example, larger body size might be related to variability in temperature (Boyce, 1979; Lindstedt & Boyce, 1985), such that larger animals occur in areas with greater seasonal (i.e. high-latitude) or daily (i.e. high elevation) variation in temperature (Janzen, 1967; Ghalambor *et al.*, 2006). It has also been argued that latitudinal variation in body size can be a product of humidity or of primary productivity (James, 1970; Blackburn *et al.*, 1999; Meiri & Dayan, 2003; Pincheira-Donoso, 2010). Alternatively, other physiological mechanisms helping organisms to cope with harsh environments may explain cases where we found no variation in morphology with respect to temperature. Such mechanisms include changes in insulation (feathers or body fat), a decrease in peripheral circulation, and countercurrent blood-flow systems, amongst others (Scholander, 1955).

Multiple sources of contrasting selective pressures shaping morphology may vary from one river to another, causing different outcomes in each population. Different Andean slopes show different climatic characteristics depending on wind currents, the climate of surrounding lowlands, and maximum elevation (Sarmiento, 1986). Also, terrain characteristics such as the slope of each river's course might not only have an influence on the steepness of the ecological gradients themselves, but also on the river's speed, the surrounding vegetation, and the abundance and diversity of invertebrate prey. These factors affect torrent duck abundance (Pernollet, 2010) and might also affect their morphology. Despite the multiple factors influencing morphology that vary between rivers and possibly causing different relationships between temperature and phenotypes, the fact that we found no significant interaction between temperature and locality in most of the ANCOVA analyses indicates that the effect of temperature is not statistically different among rivers. However, some variation in the relationship between temperature and different body-size characters can be observed by eye between rivers (Figs 2, 3), especially for females. This may simply reflect random variation, although we note that contrasting trends in female morphology may arise considering they could be under different selection pressures from those that apply to males (Mueller, 1986). For example, during the study of torrent ducks, we observed that, when flushed, males flew significantly more than females, which tended to escape more often by diving, particularly prior to the egg-laying period. Similarly, distinguishing between different possible selective agents is difficult without performing experiments that allow for an independent test of the

effects of each factor on morphological variation. Still, although we found statistically significant associations between variation in some morphological characters and variation in temperature, we cannot determine whether the observed patterns are adaptations in a strict sense, or rather a product of environmentally induced or developmental phenotypic plasticity (Gienapp *et al.*, 2008).

Finally, we note that, although some of our data are consistent with alternative adaptive scenarios described above, we cannot reject the possibility that some patterns of geographical variation in morphology have no adaptive basis and have resulted from historical factors unrelated to adaptation or from genetic drift (Martinez *et al.*, 2013). Specifically, the absence of clear patterns of variation in some traits in relation to elevation may simply suggest that the morphological traits we studied are unaffected by differential selection along gradients in environmental conditions and resources associated with elevation. Formal quantitative analyses are needed to test whether natural selection is indeed promoting morphological adaptations or whether alternative non-adaptive scenarios must be considered.

In conclusion, we found support for Bergmann's rule in torrent ducks in relation to variation in temperature existing across latitudes, although our data showed an opposite trend to that expected according to Allen's rule. Neither Bergmann's, nor Allen's rules are supported in relation to variation existing along elevational gradients. Our results thus highlight that a given environmental factor might have varying influences in terms of organismal morphology depending on its interaction with other potential agents of selection. Along latitudinal gradients, temperature is likely to be an important driver of differences in body dimensions. However, any similar effect as a result of elevational gradients in temperature may be masked (or even reversed) by covariation in other factors such as air density and oxygen availability. Contrasting results for elevational and latitudinal gradients have also been obtained in other studies on body-size variation (Gouveia *et al.*, 2013) and on the processes shaping species distributions (Halbritter *et al.*, 2013), suggesting that, although temperature varies across both types of gradients, they are not equivalent to each other as a result of variation in other environmental factors. Finally, we showed that taking into account the variation in different morphological characters separately might uncover the different responses to selection that act in conjunction to shape the phenotype of populations. Thus, comparative studies addressing variation in morphology across different types of environmental gradients are necessary to improve our understanding of the forces (i.e. natural

selection or phenotypic plasticity) driving processes at the population level.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Variation in wing (A, B), tarsus (C, D), and culmen size (E, F) in relation to annual mean temperature in torrent ducks. Measurements are corrected for body size using the residuals of a regression between each variable and body mass. Light blue, dark green, light green, and dark blue dots and lines represent Quindío (Colombia), Chillón (Peru), Apurímac (Peru), and Mendoza (Argentina) samples, respectively. Bold black lines represent regressions fitted for all points, regardless of locality.

Figure S2. Variation in principal component 1 in relation to annual mean temperature in torrent ducks. Light blue, dark green, light green, and dark blue dots and dashed lines represent Quindío (Colombia), Chillón (Peru), Apurímac (Peru), and Mendoza (Argentina), respectively. Bold black lines represent regressions fitted for all points, regardless of locality. Note that relationships existing within localities (i.e. along temperature gradients associated with elevation) are generally positive, whereas that existing across all localities (i.e. along the temperature gradient associated with latitude) is negative.

Table S1. Significance level (P values) of the fitted models for each variable evaluated, and the partial effect of the covariable, the cofactor, and the interaction term. Wing chord, tarsal length, and total culmen length are expressed as the residuals of a linear regression between each measurement and body mass. Statistically significant values after applying a sequential Bonferroni correction ($\alpha = 0.05/p$ rank; see Material and methods and Table 3) are shown in bold.

Table S2. Mean \pm SD values for each trait within each locality. Measurements are corrected for body size using the residuals of a regression between each variable and body mass.

Table S3. Significance level (P values) of the linear regressions fitted for the Río Chillón data, with their corresponding adjusted R^2 and slope values. Wing chord, tarsal length, and total culmen length are expressed as the residuals of a linear regression between each measurement and body mass. Statistically significant values after applying a sequential Bonferroni correction ($\alpha = 0.05/p$ rank; see Material and methods and Table 1) are shown in bold.

Table S4. Principal component analysis made with all the morphological characters measured in torrent ducks. The percentage of variation explained by the five components and their variable loadings is shown. Numbers in bold emphasize the two highest loadings for the first two principal components.

Table S5. Significance level (P values) of the fitted models for each variable evaluated, and the partial effect of the covariable, the cofactor, and the interaction term. Statistically significant values after applying a Bonferroni correction ($\alpha = 0.0125$) are shown in bold.