

RESEARCH ARTICLE

Anna's hummingbird (*Calypte anna*) physiological response to novel thermal and hypoxic conditions at high elevations

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ABSTRACT

Many species have not tracked their thermal niches upslope as predicted by climate change, potentially because higher elevations are associated with abiotic challenges beyond temperature. To better predict whether organisms can continue to move upslope with rising temperatures, we need to understand their physiological performance when subjected to novel high-elevation conditions. Here, we captured Anna's hummingbirds – a species expanding their elevational distribution in concordance with rising temperatures – from across their current elevational distribution and tested their physiological response to novel abiotic conditions. First, at a central aviary within their current elevational range, we measured hovering metabolic rate to assess their response to oxygen conditions and torpor use to assess their response to thermal conditions. Second, we transported the hummingbirds to a location 1200 m above their current elevational range limit to test for an acute response to novel oxygen and thermal conditions. Hummingbirds exhibited lower hovering metabolic rates above their current elevational range limit, suggesting lower oxygen availability may reduce performance after an acute exposure. Alternatively, hummingbirds showed a facultative response to thermal conditions by using torpor more frequently and for longer. Finally, post-experimental dissection found that hummingbirds originating from higher elevations within their range had larger hearts, a potential plastic response to hypoxic environments. Overall, our results suggest lower oxygen availability and low air pressure may be difficult challenges to overcome for hummingbirds shifting upslope as a consequence of rising temperatures, especially if there is little to no long-term acclimatization. Future studies should investigate how chronic exposure and acclimatization to novel conditions, as opposed to acute experiments, may result in alternative outcomes that help organisms better respond to abiotic challenges associated with climate-induced range shifts.

KEY WORDS: *Calypte anna*, Metabolic ecology, Range shifts, Trochilidae, Metabolic rate, Torpor

INTRODUCTION

Organisms are shifting their geographic distributions in response to anthropogenic climate change, with numerous plant and animal species shifting poleward (Parmesan and Yohe, 2003), upslope

(Chen et al., 2011) or deeper (Pinsky et al., 2013) to track their climatic niches. It is becoming increasingly clear, however, that range shifts are not always in the expected direction (Tingley et al., 2012) or at the expected rate (Currie and Venne, 2017). These deviations between modeling predictions and empirical realities highlight the problems of using temperature as the sole explanatory factor determining geographic range or predicting range shifts (Spence and Tingley, 2020). For example, the failure of species to shift in the direction predicted by rising temperatures may be due to many reasons, including species responding to other climatic factors such as changes in precipitation or water availability (Crimmins et al., 2011; Tingley et al., 2012), *in situ* behavioral or phenotypic plasticity (Riddell et al., 2021; Socolar et al., 2017), dispersal limitations (Bertrand et al., 2011; Comte and Grenouillet, 2013), biotic interactions (Alexander et al., 2018; HilleRisLambers et al., 2017), cooler microclimates (Benedict et al., 2020; Scheffers et al., 2013) or physiological limitations associated with non-climatic abiotic challenges (e.g. oxygen availability, air pressure or light; Spence and Tingley, 2020).

Montane habitats can be difficult to live in because, as organisms shift upslope, temperatures drop approximately 6°C per 1000 m while atmospheric pressure simultaneously decreases and limits the amount of available oxygen [e.g. approximately 23% less available oxygen at 2000 m above sea level (a.s.l.) and 39% less oxygen at 4000 m a.s.l.; Peacock, 1998]. Consequently, endothermic animals at high elevations face a physiological challenge: colder temperatures require increased metabolic rates to remain normothermic (McNab, 2002; Scholander et al., 1950), while simultaneous declines in oxygen can limit metabolic potential (Chappell et al., 2007). Furthermore, living in a hypoxic environment can cause physiological changes that affect individuals and cause downstream population effects, with organisms native to high elevations exhibiting lower maximum metabolic rates (Chappell et al., 2007; Shirkey and Hammond, 2014), higher locomotive costs (Harrison and Roberts, 2000; Welch and Suarez, 2008), reduced fecundity (Dahlhoff et al., 2019) and altered microbiomes (Herder et al., 2021) compared with their low-elevation counterparts. Consequently, lowland species attempting to move upslope must overcome hypoxic challenges, which organisms typically surmount only via millennia of evolutionary adaptation (Natarajan et al., 2016; Storz et al., 2010). As such, hypoxia-related physiological challenges may explain why elevational shifts tracking thermal isotherms under climate change are not reported as frequently as analogous latitudinal shifts (Chen et al., 2011).

To fully understand a species' capacity to shift in response to a warming climate, it is critical to both assess its physiological performance within its current range and compare this with performance beyond its current distribution (Hargreaves et al., 2014). By using this experimental framework, we can begin to answer questions about the ability of organisms to overcome novel environmental conditions they may face, such as low oxygen

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availability, if they continue to shift upslope to track thermal niches (Spence and Tingley, 2020). Alongside documenting changes in physiological performance outside of the current range, it is critical to assess whether the population of origin affects an individual's ability to colonize new environments (Duckworth, 2008). Organisms with large elevational ranges can show spatial heterogeneity in their physiological response to abiotic conditions along the elevational gradient; for example, individuals inhabiting the lower edge of an elevational range can display local adaptation or acclimatization to warmer temperatures (Keller et al., 2013). In contrast, individuals from upper edges of elevational ranges often show increased respiratory potential (Hammond et al., 1999; Shirkey and Hammond, 2014), alterations to internal and external morphology (Dillon et al., 2006; Sun et al., 2016), increased oxygen binding capacity (Storz et al., 2010) and, for flying organisms, changes in wing-beat amplitude (Altshuler et al., 2004). Studying how traits vary across an elevational gradient is important for understanding whether high-elevation populations are more likely to successfully colonize new areas (Jacobsen, 2020), or whether contraction of low-elevation populations can reduce genetic diversity (Rubidge et al., 2012) or local adaptation to non-climatic conditions through maladaptive introgression (Schiffers et al., 2013).

We tested these questions in a single species, Anna's hummingbird (*Calypte anna*), in the Sierra Nevada and White Mountains, CA, USA. Hummingbirds are a well-suited system to study the effects of temperature and oxygen simultaneously because they are at metabolic extremes, with the highest mass-specific metabolic rate of any vertebrate (Suarez, 1992). Changes in temperature and oxygen conditions are known to affect daily metabolic output and torpor use (Welch and Suarez, 2008; Spence and Tingley, 2021). Moreover, hovering is the most energetically expensive form of locomotion and becomes more difficult at higher elevations because of lower air viscosity (Chai and Dudley, 1996; Welch and Suarez, 2008). Despite these challenges, hummingbirds inhabit high-elevation environments across the Americas, and Anna's hummingbirds specifically have expanded their range northward and upslope, concurrent with a combination of warming temperatures and increased food availability (Battey, 2019; Tingley et al., 2009). However, it is unknown whether there is a limit to how far Anna's hummingbirds can continue to move upslope despite continued anthropogenic warming.

Here, we captured and housed Anna's hummingbirds from across their elevational range and tested physiological performance within and above their current elevational distribution. Specifically, we first measured hovering metabolic rate (HMR) to test for differences in metabolic performance across different oxygen concentrations. Next, we measured torpor use, duration and depth to test for differences in an energy-saving strategy under different thermal conditions. Individuals were all evaluated under natural conditions at a central location within their elevational range (1215 m a.s.l.) and were then transported, housed and tested again under novel conditions (3800 m a.s.l.) located approximately 1200 m above their upper elevational limit. We subsequently asked the following questions: (1) do hummingbirds captured at different elevations exhibit different HMR or torpor use within their range?; and (2) do hummingbirds from different elevations exhibit a change in HMR and torpor use above their natural elevational range? Finally, high-elevation environments can change internal morphology to help overcome low oxygen and thermal conditions (Shirkey and Hammond, 2014; Storz et al., 2010); therefore, we measured morphological characteristics of respiratory and digestive organs to

test for elevational associations in internal morphology for birds originating across a 2500 m gradient.

MATERIALS AND METHODS

Experimental design

Anna's hummingbirds, *Calypte anna* (Lesson 1829), are distributed across the western coast of North America from Baja California, Mexico to British Columbia, Canada. In California, USA, Anna's hummingbirds breed in the lowlands from November to May before dispersing upslope, with a summer elevational distribution ranging from sea level to approximately 2600 m a.s.l. During the summer of 2018, we captured 26 adult Anna's hummingbirds in California using Hall feeder traps (Tell et al., 2021) at sites spanning the entirety of their natural elevational range in the Sierra Nevada (69–2825 m a.s.l.). Immediately after capture, we transported hummingbirds to a central aviary located in the middle of their elevational distribution (1215 m a.s.l.; 37°10'24.3"N 118°17'26.8"W). We housed each hummingbird in individual 0.75 m³ cubicles made of wood with one side made of breathable mesh cloth to allow for ambient air and light conditions. We provided each hummingbird access to perches, water and *ad libitum* Nekton NektarPlus (NEKTON GmbH, Keltern, Baden-Württemberg, Germany). After an initial acclimation period (mean: 4.6 days; range: 12 h to 10 days), we measured the HMR and the frequency, duration and depth of torpor use (see below) to see how these physiological parameters respond to thermal and oxygen conditions within the natural range. Following these tests, we transferred each hummingbird to Barcroft Station in the White Mountains of California (elevation: 3800 m a.s.l.; 37°34'59.7"N 118°14'14.1"W) – approximately 1200 m above the current elevational range limit of Anna's hummingbirds. At Barcroft Station, we housed birds in cubicles identical in design to those described above and allowed the hummingbirds to rest (median: 12 h; range: 12 h to 4 days) before performing the same physiological tests. Because of the difference in elevation, mean minimum nightly temperatures were warmer at the central aviary within the birds' distribution (mean±s.d. 14.86±3.61°C) than at the high-elevation aviary above their distribution (5.04±2.16°C) and the partial pressure of oxygen was approximately 30% lower at the high-elevation site. After experimentation at the high-elevation site, we killed birds via thoracic compression (Tell et al., 2021). Treatment of birds (capture, housing and thoracic compression) was carried out under United States Fish and Wildlife Service Permits MB087454-0 and MB087454-2 and California Fish and Wildlife Service Permit SC 006598, and all methods were approved under University of Connecticut Institutional Animal Care and Use Committee Protocols A16-012 and A19-013.

Response to oxygen conditions: HMR and respiratory morphology

We measured HMR via O₂ consumption using a negative pressure open-flow respirometry 'feeder-mask' while the hummingbird ate from a modified feeder (Lighton, 2008; Tell et al., 2021; Welch, 2011). A plastic cylinder was placed over the opening of the feeder, and the hummingbird was forced to place its head into the cylinder to feed. A hole in the plastic cylinder was connected to plastic tubing (BEV-A-LINE, United States Plastic, Lima, OH, USA), and air was pulled at a rate of 1500 ml min⁻¹ with a mass flow system (MFS-5, Sable Systems, Las Vegas, NV, USA) to collect expired air from the hummingbird nares. Baseline ambient air was collected before the hummingbird began eating and expired air was collected from hummingbirds during feeding. We measured the percentage O₂ and

water content using a field metabolic system (2016, Sable Systems) and took quantitative measurements every second using Warthog LabHelper software (<http://warthog.ucr.edu>). Hummingbirds hovered for a mean \pm s.d. of 13.1 ± 5 s for each feeding event at the modified feeder. We calibrated the humidity sensor of the field metabolic system with nitrogen gas (Airgas, Radnor Township, PA, USA) as the zero-span and a bubbler set to produce humidified air at a constant dewpoint between 24 and 26°C. We used Warthog LabAnalyst to calculate \dot{V}_{O_2} and mathematically corrected our fractional gas composition values to account for water vapor (Lighton, 2008).

Response to thermal conditions: torpor frequency, duration and depth

We measured the frequency of torpor use, the duration of the torpor bout (measured as time in torpor to the nearest 15 min) and the torpor depth (measured as the mean torpor metabolic rate divided by the mean resting metabolic rate: TMR/RMR) by monitoring the metabolic rate of each hummingbird through the night. Hummingbird metabolic rate is highly correlated to body temperature and can indicate torpor use without the need for implanted probes (Shankar et al., 2020). To measure nightly metabolic rates, we used positive pressure open-flow respirometry (Lighton, 2008) with hummingbirds placed in a clear, sealed 1.2 l metabolic chamber with plastic tubing to measure CO_2 . Metabolic chambers were placed outside in a larger container to allow for ambient temperature and light conditions. We pushed incurrent air through the metabolic chamber at a rate of $350\text{--}400\text{ ml min}^{-1}$ with the mass flow system and subsampled excurrent air through a syringe barrel at a rate of 150 ml min^{-1} . We used ambient air as the baseline and measured the percentage CO_2 and water content in excurrent air using the field metabolic system. We used a TC-2K thermometer (Sable Systems) with type-T (Cu-Cn) thermocouples calibrated against a NIST-traceable thermometer to measure ambient air temperature. We placed the hummingbirds in the metabolic chamber around dusk (21:00–22:00 h), allowed the metabolic chamber to equilibrate for 30 min, and then measured nightly RMR until sunrise (05:00–06:00 h). We classified hummingbirds as using torpor if their nightly metabolic rate dropped below 50% of normothermic values (Lasiewski, 1963). Torpor duration was calculated as the length of time from torpor entrance to torpor emergence, rounded to the nearest 0.25 h. We calculated torpor depth as the relative TMR (i.e. mean TMR/mean normothermic RMR; (Spence and Tingley, 2021).

Dissection and organ mass

We dissected all experimental hummingbirds ($n=26$) and supplemented this sample with 26 additional Anna's hummingbirds collected in 2017 from a similar range in elevations as part of a previous experiment (Spence and Tingley, 2021). We collected the heart, lungs, liver and intestinal tract (from the end of the esophagus to the cloaca) and removed all connective tissues or fat. To calculate dry mass, we placed all organs in a drying oven at 60°C for at least 120 h before measurement on a scale (precision: 0.0001 g; Ohaus, Parsippany, NJ, USA).

Statistical analyses

We built generalized linear models with fixed and random effects within a Bayesian framework for all analyses, using JAGS via the R programming language version 3.6.2 (<http://www.R-project.org/>) and the package *R2jags* (<https://CRAN.R-project.org/package=R2jags>). We modeled HMR within and above the

natural elevational range as Gaussian-distributed random variables with a log-link with elevation of origin and sex as fixed effects. We modeled torpor use as a Bernoulli-distributed random variable using a logit-link with test location (categorical: within or above range), Julian date and days in captivity as fixed effects. We included random intercepts for individuals (to account for two measurements per individual) and elevational region of origin (categorical: low, medium or high elevation). We modeled torpor bout duration and relative metabolic rate as a Gaussian-distributed random variable with a log-link and a beta-distributed random variable with a logit-link on the moment-matched expected value, respectively, and included elevation of origin and days in captivity as fixed effects. We included total days in captivity as a fixed effect in torpor analyses because the amount of time in captivity can change RMR measurements (Auer et al., 2016). We did not include sex in any of the torpor analyses as previous experiments have shown sex is not related to torpor frequency, duration or depth in Anna's hummingbirds (Spence and Tingley, 2021). We modeled heart, lung, liver and intestine dry mass as a t -distributed random variable with a log-link as the t -distribution provides a more robust analysis in regard to the strength of the effect of outliers on the regression (Gelman and Hill, 2006). We included elevation of origin and sex as fixed effects and year of capture as a random effect. Organ statistical models exhibited normally distributed residuals (Shapiro–Wilk test: $P>0.05$) unless otherwise noted. For all models, we included vague priors on all slope parameters (normal: $\mu=0$, $\tau=0.001$). We ran three chains of 50,000 iterations thinned by 10 and a burn-in of 10,000, resulting in a posterior sample of 12,000 per model, and confirmed parameter convergence by visual inspection of traceplots as well as requiring a Gelman–Rubin statistic <1.1 (Gelman et al., 2013). We considered parameters strong predictors of responses if the 95% Bayesian credible interval (CrI) for a parameter did not overlap zero.

We used a Bayesian approximation of a Student's t -test in the *BEST* package (<https://CRAN.R-project.org/package=BEST>) to test whether the change in HMR, torpor duration and torpor depth between testing locations within and above the elevational range were different from 0. Model convergence was evaluated by a Gelman–Rubin statistic as <1.1 and the effective sample size of the posterior $>10,000$. Means were considered different from 0 if the 95% CrI did not overlap 0.

RESULTS

Response to oxygen conditions: HMR

Anna's hummingbirds exhibited lower HMR when tested above the elevational range limit versus within the elevational range (Fig. 1A; mean change in HMR $-15.78\text{ ml O}_2\text{ g}^{-1}\text{ h}^{-1}$; 95% CrI -23.11 , $-8.70\text{ ml O}_2\text{ g}^{-1}\text{ h}^{-1}$). HMR within the natural elevational range limit (Fig. 2) and above the natural range limit did not exhibit strong relationships with either elevation of origin or days in captivity (Table 1).

Response to thermal conditions: torpor frequency, duration and depth

Anna's hummingbirds were more likely to use torpor in novel thermal conditions above their range than within their natural elevational range (Fig. 3, Table 2). Hummingbirds were also more likely to use torpor later in the season near their migratory period and less likely to use torpor with increased time in captivity (Table 2). We did not find a relationship between elevation of origin and torpor duration or depth (Table 1). Among individuals that used torpor at both testing locations ($n=6$), individuals used torpor for longer at the above-range site (Fig. 1B; mean change in duration

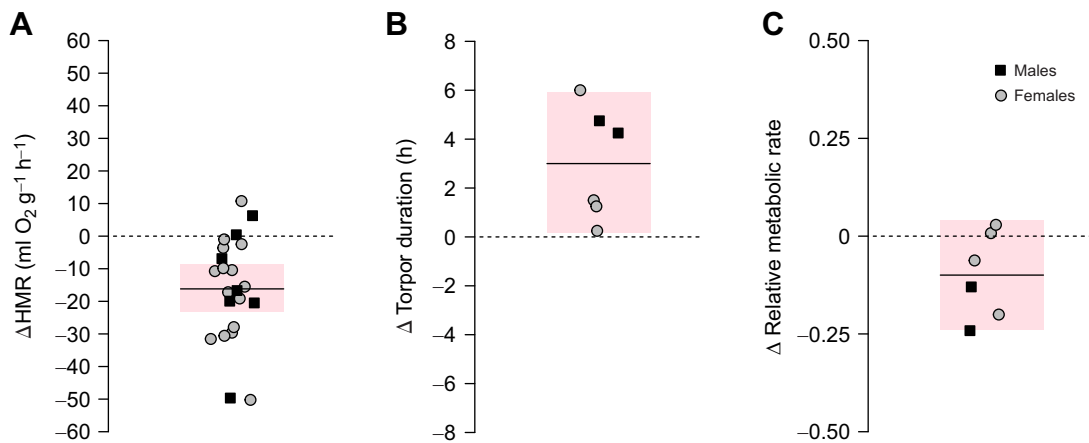


Fig. 1. Response of Anna's hummingbirds to oxygen conditions. Individual-level change in (A) hovering metabolic rate (ΔHMR ; measured as the change in \dot{V}_{O_2}), (B) torpor bout duration and (C) relative metabolic rate when tested 1200 m above the species' elevational limit. HMR was lower (A) and torpor duration was longer (B) when tested at higher elevations, but there was no difference in relative metabolic rate (C) between testing localities. The black line represents the mean, and the shading represents the 95% credible interval (CrI) of the change in mean from within elevational range to above range.

2.99 h; 95% CrI 0.01, 5.90 h). Individuals also generally used deeper torpor above their current range (Fig. 1C; mean change in relative metabolic rate -0.099), although the credible interval overlapped zero (95% CrI -0.23 , 0.04).

Hummingbirds from across the elevational gradient: organ mass

Anna's hummingbird heart dry mass had a positive relationship with elevation of origin (Fig. 4A) and was greater in males (Table 3). Liver dry mass had a negative relationship with elevation of origin (Fig. 4C) but did not show a relationship with sex. Lung and intestinal tract dry mass did not show relationships with elevation of origin (Fig. 4B,D) or sex (Table 3). The regression of lung dry mass did not result in normally distributed residuals ($P < 0.01$), suggesting the model may not adequately fit the data.

DISCUSSION

Our results provide insight into the physiological response of Anna's hummingbirds to an acute exposure to novel hypoxic and thermal conditions above their current elevational range limit. Hummingbirds from across their current elevational range exhibited

lower HMR under novel oxygen conditions. At the same time, hummingbirds showed the capacity to respond to novel thermal conditions associated with high elevations by using torpor more frequently and, generally, for longer periods of time. Although elevation of origin was not associated with HMR or torpor use, it was associated with organ mass, where hummingbirds originating from high elevations had larger hearts (but not lungs) and smaller livers than their low-elevation counterparts. Overall, oxygen availability and air pressure may be a greater challenge for hummingbirds shifting upslope than temperature. Hummingbirds showed the capacity to respond to colder thermal conditions through more frequent and longer torpor use, thereby reducing thermoregulatory costs, whereas flying in lower air pressure and oxygen conditions affected metabolic performance, thereby affecting daily energy expenditure and potentially impairing flight efficiency. While our results shed light on the potential for non-thermal abiotic challenges to slow or hinder climate-induced range shifts, it is also important to note that we only tested hummingbirds under conditions of acute exposure. Future work should investigate whether alternative experimental designs, such as chronic, long-term contact or a graded exposure to novel hypoxic conditions, would allow individuals to achieve the performance seen within their natural abiotic conditions.

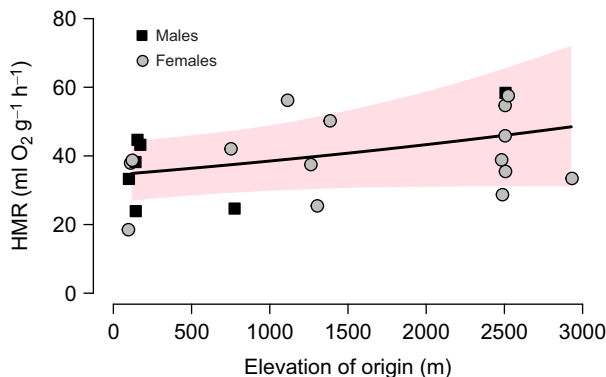


Fig. 2. HMR at elevation of origin. Anna's hummingbird HMR (\dot{V}_{O_2}) did not exhibit a strong relationship with elevation of origin within the natural elevational range (1215 m a.s.l.). The black line represents the predicted mean response, and shading represents the 95% CrI from a generalized linear model.

Response to oxygen conditions

HMR is an important indicator of daily energy expenditure in hummingbirds because hovering is the most metabolically expensive mode of animal transportation (Suarez, 1992) and daytime activities are the largest portion of a hummingbird's daily metabolic output (Shankar et al., 2019). We found Anna's hummingbird HMR was not related to an individual's elevation of origin, possibly because HMR is more dependent on local air pressure and thermal conditions than on intraspecific variation in physiology. In general, hummingbirds are expected to have higher HMR at higher elevations because it costs more to generate an equal amount of lift in less dense air (Altshuler and Dudley, 2003), and colder temperatures simultaneously necessitate increased thermoregulation (Welch and Suarez, 2008). For example, rufous hummingbirds (*Selasphorus rufus*), when tested within their natural elevational range, showed a positive relationship with elevation and HMR (Welch and Suarez, 2008). However, in our study, Anna's

Table 1. Parameter estimates for generalized linear mixed effects models examining the effects of elevation of origin (km), sex (female is baseline) and days in captivity on physiological measures within and above Anna's hummingbird elevational range

Response	Link function	Sample size (n)	Parameter	Coefficient (95% credible interval)
HMR within range	log	22	Intercept	3.60 (3.42, 3.78)
			Elevation of origin	0.11 (−0.04, 0.26) ml O ₂ g ^{−1} h ^{−1} km ^{−1}
			Sex (male)	0.06 (−0.27, 0.39) ml O ₂ g ^{−1} h ^{−1}
HMR above range	log	22	Intercept	3.12 (2.84, 3.39)
			Elevation of origin	−0.16 (−0.39, 0.07) ml O ₂ g ^{−1} h ^{−1} km ^{−1}
			Sex (male)	−0.18 (−0.70, 0.34) ml O ₂ g ^{−1} h ^{−1}
Torpor duration above range	log	24	Intercept	1.74 (1.59, 1.89)
			Elevation of origin	0.08 (−0.08, 0.08) h km ^{−1}
			Days in captivity	0.07 (−0.09, 0.22) h day ^{−1} in captivity
Torpor depth above range	logit	21	Intercept	−1.95 (−2.34, −1.57)
			Elevation of origin	−0.04 (−0.44, 0.35) relative metabolic rate km ^{−1}
			Days in captivity	−0.05 (−0.46, 0.31) relative metabolic rate day ^{−1} in captivity

Hovering metabolic rate (HMR; ml O₂ g^{−1} h^{−1}) was tested within and above the elevational range limit, while torpor duration (h) and depth (%) were only tested above the range limit. Bold indicates 95% credible intervals on parameter estimates that did not overlap 0, signifying strong model support.

hummingbirds exhibited a decline in HMR when tested above their elevational range limit, contrary to expectations and prior measurements within their natural range. An explanation for this counter-intuitive result is that we conducted an acute test to a novel hypoxic environment (i.e. Anna's hummingbirds have no evolutionary history with these environmental conditions), rather than testing solely within the natural range. In that regard, our results show similarity to mammalian studies where hypoxic environments reduce mammalian metabolic performance in individuals from low elevations (Chappell et al., 2007; Shirkey and Hammond, 2014). In birds, lower metabolic rates may also arise from the lack of force generated per wingbeat at lower air pressures. In our study, individuals were not able to fly as effectively or for as long when they were above their elevational range limit (A.R.S. and H.L., personal observation), corroborating laboratory studies showing that low-elevation hummingbirds display a loss in flying capacity in oxygen and air pressure conditions around 4000 m a.s.l. (Chai and Dudley, 1996), similar to our test conditions. Overall, these results suggest low air pressure and oxygen availability may reduce hovering performance in hummingbirds when exposed to the acute challenge of high-elevation conditions, but it is unknown whether this reduction is short or long term.

Despite no relationship between HMR and elevation of origin, we did observe differences in organ mass in birds across their elevational range. Anna's hummingbirds from higher elevations had larger hearts, which allows for increased circulatory capacity to deliver oxygen (Storz et al., 2010), a trait that can occur either

through local adaptation or as a plastic response through acclimatization (Van Bui and Banchero, 1980). Unlike mammals, which exhibit plasticity in lung mass and volume in response to hypoxic conditions (Shirkey and Hammond, 2014), Anna's hummingbirds did not exhibit differences in lung mass across elevations. This may be because avian lungs are much more efficient than mammalian lungs, and the rate of pulmonary blood flow may be more limiting than lung volume in hypoxic conditions (Yilmaz et al., 2007). Hummingbirds also showed a negative relationship between elevation and liver mass. More work is needed to understand whether this correlation is due to changes in diet with elevation (Rozenboim et al., 2016), the benefits of decreased mass toward reducing hovering costs, or other reasons. Regardless, as Anna's hummingbirds migrate seasonally to high elevations, further experimental work is needed to determine whether differences in heart and liver mass are due to local adaptation of individuals or populations that regularly return annually to high elevations or are a plastic response of individuals that disperse upslope.

Response to thermal conditions

Anna's hummingbirds showed the expected facultative response to colder temperatures and used torpor more frequently in conditions above their elevational range than within their elevational range. Torpor is known to be used more frequently during energetically stressful situations (Hainsworth et al., 1977), including cold temperatures, and Anna's hummingbirds appear to be able to change torpor frequency regardless of their elevation of origin.

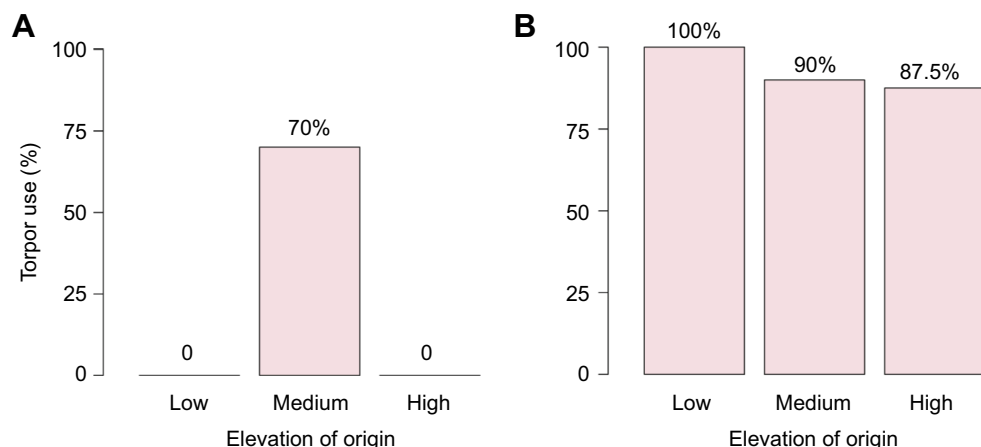


Fig. 3. Frequency of torpor use within and above the natural elevational range. (A) Torpor use within the natural elevational range, at 1215 m a.s.l., and (B) above the natural range limit, at 3800 m a.s.l., based on binned elevation of origin (low 69–99 m; medium 697–1324 m; high 2404–2825 m). There was a strong relationship with testing location, with hummingbirds more likely to use torpor above their natural range limit.

Table 2. Parameter estimates for a generalized linear mixed effects model with a logit-link, evaluating torpor use (yes/no) in 26 Anna's hummingbirds tested within and above their elevational range limit

Response	Parameter	Coefficient (95% credible interval)
Torpor use	Intercept	−9.56 (−48.36, 31.73)
	Testing location (above range)	50.13 (17.71, 95.28)
	Julian date	13.7 (0.12, 35.17)
	Days in captivity	−3.19 (−6.48, −1.06)

For testing location, within range is baseline. Day of year and length of time spent in captivity were controlled for. Bold indicates 95% credible intervals on parameter estimates that did not overlap 0, signifying strong model support.

These patterns match recent work showing Anna's hummingbirds are more likely to use torpor when they face experimentally manipulated colder temperatures (Spence and Tingley, 2021), as well as at later Julian dates, suggesting environmental conditions and time of year are key to predicting when Anna's hummingbirds will use torpor (Carpenter and Hixon, 1988; Hainsworth et al., 1977).

While Anna's hummingbirds responded to cold temperatures above the range limit as expected, they showed unexpected patterns of torpor use when tested within the elevational range. Hummingbirds from sea level and high elevations (>2000 m a.s.l.) did not use torpor at all when tested at the central-range aviary (Fig. 3). Previous work found Anna's hummingbirds use torpor approximately 60% of the time when tested in ambient temperature conditions at their native elevation (Spence and Tingley, 2021), and hummingbirds from the middle portion of the

range – those that were not transported far to the central aviary – used torpor at approximately this rate. The discrepancy between groups from different elevations of origin may be due to stress from transfer and captivity. The repeatability of metabolic rates is known to decline in captivity (Auer et al., 2016), and we found the number of days in captivity had a negative relationship with the frequency of torpor. Increased handling and transfer stress experienced by hummingbirds from the sea level and high-elevation capture sites may have affected the frequency of torpor use. As most experimental studies investigating torpor use only look at which conditions induce torpor (e.g. cold temperatures, food deprivation), future research should investigate how experimental design and animal handling, per se, may affect how individuals use torpor in different situations. Such research could help the interpretability and methodological standardization of torpor experiments.

When hummingbirds use torpor, duration and depth are the two dominant aspects that determine how much energy is saved throughout the night (Shankar et al., 2020). In our study, duration and depth showed different capacities to respond to novel thermal conditions beyond the current elevational range. Torpor duration is proportionally more important in contributing to energetic savings, with longer bouts of torpor providing more energetic savings than deeper torpor (Shankar et al., 2020). While previous work in Anna's hummingbirds found no relationship between temperature and torpor duration when manipulated within their natural elevational range (Spence and Tingley, 2021), our results suggest torpor bout duration may be plastic within the individual and change to accommodate novel thermal conditions. In contrast, torpor depth does not exhibit the same phenotypic plasticity. Although we found

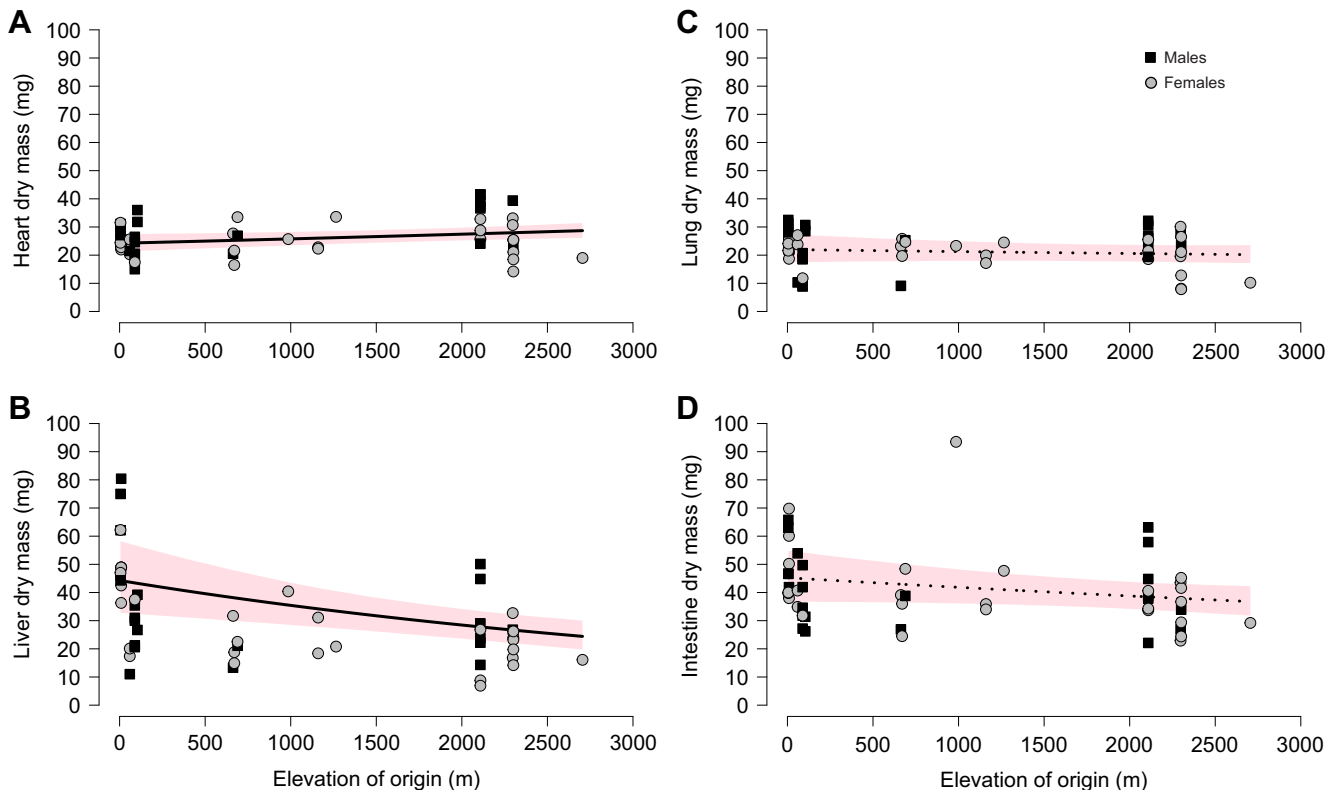


Fig. 4. Dry mass of organs as a function of elevation of origin. (A) Heart, (B) lung, (C) liver and (D) intestine dry mass were obtained from 52 hummingbirds. We found a strong positive relationship of heart mass and a strong negative relationship of liver mass with elevation of origin (solid lines), but only weak relationships for lung and intestine mass (dotted lines). Males had heavier hearts than females (Table 3); for clarity, the regression line for heart dry mass (A) is only plotted for the intercept for males. Shading represents the 95% CrI from a generalized linear model.

Table 3. Parameter estimates for generalized linear mixed effects models testing the organ dry mass (mg) of 52 Anna's hummingbirds as a function of elevation of origin (km) and sex (female is baseline)

Response	Parameter	Coefficient (95% credible interval)
Heart	Elevation of origin	0.06 (0.01, 0.12) mg km ⁻¹
	Sex (male)	0.12 (0.01, 0.23) mg
Lungs	Elevation of origin	-0.03 (-0.12, 0.06) mg km ⁻¹
	Sex (male)	0 (-0.19, 0.19) mg
Liver	Elevation of origin	-0.22 (-0.34, -0.10) mg km ⁻¹
	Sex (male)	0.05 (-0.2, 0.3) mg
Intestinal tract	Elevation of origin	-0.08 (-0.16, 0.01) mg km ⁻¹
	Sex (male)	-0.03 (-0.2, 0.14) mg

Dry mass was obtained for the heart, lungs, liver and intestinal tract. Bold indicates 95% credible intervals on parameter estimates that did not overlap 0, signifying strong model support.

a general trend of deeper torpor, this is likely because torpor depth is contingent on ambient temperature being close to a species-specific minimum torpor body temperature (Hainsworth and Wolf, 1970). Body temperature can only drop as low as local air temperature, so torpor depth is likely a passive response to the conditions experienced by an individual. Our current understanding of intraspecific variation in the frequency, duration and depth of torpor use in hummingbirds is limited, as more than half of all currently published torpor studies report on fewer than five individuals per species (Spence and Tingley, 2021). Therefore, while we acknowledge our results on intra-individual plasticity in torpor are based on only six individuals, such comparative studies are quite rare. Future studies should aim to understand how long-term conditions and acclimatization, as opposed to acute changes in thermal conditions, shape the frequency, duration and depth of torpor use.

Implications for future range shifts

Anna's hummingbirds have already responded to anthropogenic climate and land use change by expanding their distributions northwards and upslope (Battey, 2019; Tingley et al., 2009), with this expansion occurring relatively recently in the latter half of the 20th century. As such, there may not have been sufficient time for Anna's hummingbirds to show adaptive changes across the elevational gradient. We did not find strong relationships between elevation of origin and response of HMR and torpor use to novel hypoxic and thermal conditions. These results suggest that there may not be a strong pattern of spatial heterogeneity across the elevational gradient for these metabolic traits. Therefore, Anna's hummingbirds are likely to show little effect of loss of diversity or introgression from populations not adapted to high-elevation conditions regarding the traits we tested (Rubidge et al., 2012; Schiffers et al., 2013). One possibility for why elevation of origin was not a strong predictor may be this species' breeding ecology; Anna's hummingbirds breed during the winter at low elevations before dispersing upslope in the summer (Clark and Russell, 2012), thus limiting the chances of high-elevation traits being selected (Bay et al., 2021).

It is important to place our results in the context of our acute experimental design because acclimatization to novel conditions can occur, and this may change long-term inference. Phenotypic plasticity is often more pronounced after an acclimatory period (Seebacher, 2005; Storz et al., 2010), with some traits returning to levels of performance observed at lower elevations (Solari and Hadly, 2020). However, despite certain traits recovering efficiency, complex behaviors, such as flight or reproduction (Fraimout et al.,

2018; Westneat et al., 2009), often require multiple physiological systems working in tandem. As such, species often do not fully retain performance exhibited in normoxic environments after acclimatization, including with flight performance in birds (Sun et al., 2016). Moreover, these processes can be logistically difficult to study because acclimatization to new conditions can take weeks to months to occur (Storz et al., 2010). To the best of our knowledge, long-term acclimatization experiments have not been completed with hummingbirds. Despite the challenges, further physiological studies investigating how organisms will respond to abiotic conditions in future habitats will benefit from including an acclimatory period to better understand whether individuals have the ability to acclimatize or whether evolution is necessary to successfully colonize new habitats (Diamond, 2018).

Overall, our results highlight the importance of translocation and transplant experiments to understand how organisms will respond to the multitude of abiotic conditions in their predicted future ranges (Hargreaves et al., 2014). For many organisms, warming temperatures alone will not enable successful shifts upslope or poleward (Bjorkman et al., 2017). Our findings indicate that Anna's hummingbirds show an acute response to novel hypoxic conditions, which are certain to remain in the face of rising temperatures (Spence and Tingley, 2020). Understanding the acute and chronic responses to these novel conditions will be imperative to better predict how species will respond to continued anthropogenic climate change. The continued development of species distribution models that incorporate physiology (Kearney and Porter, 2004, 2009) and demography (Suárez-Seoane et al., 2017) will allow for a more realistic understanding of where species live (Austin, 2007; Gates, 1980; Kearney and Porter, 2009). By validating predictions through experiments in the field and creating more accurate expectations of where species will be in the future, we can better implement conservation policy to help preserve biodiversity in the face of unprecedented extinction risks (Urban, 2015).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.R.S., M.W.T.; Methodology: A.R.S., M.W.T.; Formal analysis: A.R.S., M.W.T.; Investigation: A.R.S., H.L.; Resources: M.W.T.; Writing - original draft: A.R.S.; Writing - review & editing: A.R.S., M.W.T.; Visualization: A.R.S.; Funding acquisition: A.R.S.

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Data availability

The data supporting this article are available from the Dryad digital repository (Spence et al., 2022): <https://doi.org/10.5061/dryad.59zw3r29t>.

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