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Amphibians Exhibit Extremely High Hydric Costs of Respiration

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Synopsis Terrestrial environments pose many challenges to organisms, but perhaps one of the greatest is the need to breathe while maintaining water balance. Breathing air requires thin, moist respiratory surfaces, and thus the conditions necessary for gas exchange are also responsible for high rates of water loss that lead to desiccation. Across the diversity of terrestrial life, water loss acts as a universal cost of gas exchange and thus imposes limits on respiration. Amphibians are known for being vulnerable to rapid desiccation, in part because they rely on thin, permeable skin for cutaneous respiration. Yet, we have a limited understanding of the relationship between water loss and gas exchange within and among amphibian species. In this study, we evaluated the hydric costs of respiration in amphibians using the transpiration ratio, which is defined as the ratio of water loss ($\text{mol H}_2\text{O d}^{-1}$) to gas uptake ($\text{mol O}_2 \text{d}^{-1}$). A high ratio suggests greater hydric costs relative to the amount of gas uptake. We compared the transpiration ratio of amphibians with that of other terrestrial organisms to determine whether amphibians had greater hydric costs of gas uptake relative to plants, insects, birds, and mammals. We also evaluated the effects of temperature, humidity, and body mass on the transpiration ratio both within and among amphibian species. We found that hydric costs of respiration in amphibians were two to four orders of magnitude higher than the hydric costs of plants, insects, birds, and mammals. We also discovered that larger amphibians had lower hydric costs than smaller amphibians, at both the species- and individual-level. Amphibians also reduced the hydric costs of respiration at warm temperatures, potentially reflecting adaptive strategies to avoid dehydration while also meeting the demands of higher metabolic rates. Our results suggest that cutaneous respiration is an inefficient mode of respiration that produces the highest hydric costs of respiration yet to be measured in terrestrial plants and animals. Yet, amphibians largely avoid these costs by selecting aquatic or moist environments, which may facilitate more independent evolution of water loss and gas exchange.

Introduction

The transition from water to land was a pivotal moment in the history of vertebrates that shaped the evolutionary trajectory of terrestrial life (Bray et al. 1997; Hsia et al. 2013; Cupello et al. 2022). Life on land presented major new challenges, which fundamentally reshaped the physiology, behavior, morphology, and life history of terrestrial taxa (Gans 1970; Graham and Lee 2004; Hsia et al. 2013). From a physiological perspective, organisms faced challenges related to ion balance,

nitrogen excretion, osmoregulation, breathing air, and maintaining water balance in a more desiccating environment (Little 1990; Cupello et al. 2022). Breathing air while staying hydrated posed particularly problematic obstacles given the inextricable link between water loss and gas exchange. Organisms breathe air across respiratory surfaces, which include the lungs, gills, and integument (e.g., skin or cuticle). To facilitate gas exchange, respiratory surfaces are often thin, highly vascularized, and saturated with body water, which also results in

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high rates of water loss (Maina 2002; Lillywhite 2006). Thus, the conditions necessary for gas exchange (both oxygen uptake and carbon dioxide elimination) are also responsible for high rates of water loss and produce a fundamental trade-off between the capacity to breathe and the capacity to remain hydrated (Woods and Smith 2010). The relationship between water loss and gas exchange is evident across a range of terrestrial organisms and persists even when accounting for body mass (Woods and Smith 2010). However, the relationship between water loss and gas exchange remains relatively unknown for amphibians—a clade that has successfully colonized most terrestrial environments on Earth and has a distinct respiratory strategy compared to all other tetrapod clades.

The simultaneous success and strict habitat requirements of amphibians presents a paradox. Modern-day amphibians (Lissamphibia) evolved approximately 300 to 360 million years ago (San Mauro et al. 2005; Zhang and Wake 2009) and consist of roughly 8,000 species with a global distribution that extends across most terrestrial ecosystems, spanning the tropics, deserts, and tundra (Duellman 1999; Wake and Koo 2018). Yet, amphibians are also known for their high susceptibility to desiccation and strict reliance on moisture or freshwater aquatic environments (Tracy 1976; Wake and Vredenburg 2008). How have such sensitive organisms persisted in so many environments for so long? In part, amphibians have used behavioral means to avoid dry environmental conditions (Inger 1957; Bertolucci 1998). By selecting moist environments, amphibians largely avoid the risk of desiccation that arises from their permeable, wet skin (Spotila and Berman 1976; Lillywhite 2006). These same properties of the skin also facilitate its use as an important respiratory surface (Tattersall 2007), often acting as the primary respiratory surface at rest for oxygen uptake and carbon dioxide elimination (Bentley and Yorio 1979; Burggren and Moallf 1984; Wygoda 1984; Burggren and Vitalis 2005; Lillywhite 2006). Thus, the physiological requirements and consequences of cutaneous respiration have shaped amphibian ecology for millions of years. Despite the unique physiology of amphibians, we have yet to understand how the relationship between water loss and gas uptake in amphibians compares with that of other terrestrial organisms. We also do not know whether the relationship between these physiological traits varies with abiotic factors, such as temperature and humidity.

Temperature has inescapable effects on rates of both water loss and gas uptake. As with any ectotherm, temperature directly affects respiration due to thermodynamic effects on molecular motion and activation energy (Schulte 2015), leading to higher rates of gas uptake at warmer temperatures to meet the rising de-

mands of respiration. The effects of temperature on water loss are indirect and determined by the vapor pressure deficit (VPD), defined as the difference between the ambient vapor pressure and the saturation vapor pressure (Stull 2000). The VPD determines the rates of evaporation and thus sets the evaporative demand of the air (Anderson 1936; Monteith and Campbell 1980). Because the saturation vapor pressure increases exponentially with temperature, warm air has greater drying potential than cool air, which explains why warmer climates tend to have higher VPDs across the planet (Riddell et al. 2019). From a purely biophysical perspective, organisms in warmer environments are expected to experience greater evaporative demand. To counteract these effects, some terrestrial organisms (including amphibians) increase their physiological resistance to water loss in response to warm temperatures, thereby limiting evaporative water loss (Maenpaa et al. 2011; Riddell and Sears 2015; Senzano and Andrade 2018). These responses potentially act as an adaptive strategy to minimize water loss in these warmer, drier environments. Evaluating hydric costs with respect to temperature and humidity might reveal adaptive responses or constraints in the evolution of gas uptake.

Here, we studied the hydric costs of gas uptake in amphibians by examining the relationship between water loss and gas uptake, which can be expressed as the transpiration ratio (the ratio of water loss rate [$\text{mol H}_2\text{O d}^{-1}$] to gas uptake rate [$\text{mol O}_2 \text{d}^{-1}$]). Species or individuals with a high transpiration ratio lose more water per unit of gas consumed and thus have high hydric costs of respiration. Across terrestrial animals, this ratio tends to be near unity (Woods and Smith 2010). The 1:1 relationship suggests that gas uptake (and thus respiration) might be constrained by water loss because higher respiration rates would require thinner, more “leaky” respiratory surfaces. First, we compared transpiration ratios between amphibians and the other terrestrial organisms from Woods and Smith (2010), which included plants, insects, mammals, and birds (and their eggs). With this comparison, we evaluated whether amphibians exhibited a relationship between water loss and gas uptake similar to that observed in other terrestrial organisms. Second, we conducted a phylogenetic analysis to evaluate variability in the transpiration ratio among amphibian species. We also evaluated whether the experimental body mass, temperature, VPD, and flow rate affected phylogenetic variation in the transpiration ratio. Finally, we evaluated how body mass, temperature, VPD, and flow rate influenced transpiration ratios within species. We predicted that transpiration ratios could either be positively or negatively associated with temperature, depending on whether water loss rates generally increased (Tracy et al. 2008; Mokhatla et al.

2019) or declined (Riddell and Sears 2015; Senzano and Andrade 2018; Riddell et al. 2019) with temperature after accounting for VPD. We also predicted that transpiration ratios would increase with VPD due to the potential for higher water loss rates at higher VPDs. In addition, we predicted that transpiration ratios would not be related to mass because both water loss rates and gas uptake rates increase with body mass. These results reveal the hydric costs of gas exchange in amphibians and provide insight into the evolutionary constraints on water loss and gas uptake in terrestrial organisms.

Methods

Data acquisition

We searched for studies on Web of Science and Google Scholar using the search terms “amphibians,” “water loss rate,” “metabolic rate,” “temperature,” “VO₂,” “EWL,” and “VCO₂.” We collected studies on resting amphibians with species-level measurements of oxygen consumption, carbon dioxide production, and water loss rate. In some cases, studies provided a single species-specific average for each trait, and in others (generally more recent studies), studies provided the datasets with measurements on each individual. We conducted a phylogenetic and intraspecific analysis on the relationship between gas uptake and water loss rates. For the intraspecific analysis, we required that studies measure gas uptake and water loss on the same individuals. Studies that measured water loss rates on one population and gas uptake on another population were not included in the intraspecific analysis because relationships between traits could not be evaluated at the level of the individual. We also searched for studies that measured each trait across a range of temperatures for the intraspecific analysis. For instance, some studies recorded their measurements at a single temperature (Messerman and Leal 2020). These measurements were included in the phylogenetic analysis but not the intraspecific analysis. We also required that the studies report the relative humidity (or VPD) for the experiment. In general, most studies did not control for the VPD by adjusting ambient vapor pressure across temperature treatments. Thus, temperature and VPD were confounded in several studies (but see variance inflation factors below). For the studies that only measured carbon dioxide production, we converted values to oxygen uptake using a respiratory quotient of 0.7 because individuals were always measured in a post-absorptive, resting state, similar to Woods and Smith (2010).

For the phylogenetic analysis, we found data on water loss and gas uptake measurements for 15 species of amphibian (4 frogs and 11 salamanders). Among the salamanders, 2 were lungless and 9 had lungs. For

the intraspecific analysis, we found 844 measurements of water loss and gas uptake (1 frog and 8 salamander species). The measurements were conducted on the same individuals across a range of temperatures (6–35°C), VPDs (0.5–5.8 kPa), and body masses (0.6–32.2 g). Of the eight salamander species, one was a hybrid between two parental lineages (Burger et al. 2024), and another was a unisexual lineage of salamander (Denton et al. 2017). Hybrids and unisexuals were not included in the phylogenetic analysis, and excluding these groups from the intraspecific analysis did not change the interpretation of our results. For the intraspecific analysis, measurements on water loss and gas uptake were collected at the same time, with the exception of one species (*Aneides aeneus*, Newman et al. 2022). All measurements in the intraspecific analysis were conducted using flow through respirometry. We used a recent consensus tree developed for all amphibians in our phylogenetic analysis (see below) (Jetz and Pyron 2018).

In the seminal study on the hydric costs of gas uptake (Woods and Smith 2010), the analysis included measurements of water loss and gas exchange across the respiratory surfaces, either lungs (mammals and birds), cuticle (plants), tracheal system (insects), or egg shell (bird eggs). Amphibians, however, rely mostly on the skin for gas uptake at rest and only use the lungs (when present) to support more energetically demanding activities or in response to warmer temperatures (Senzano and Andrade 2018). By including the whole-organism water loss and gas uptake rates, our analysis lumps respiratory and cutaneous gas uptake and water loss into the same trait values. Because these values were measured at rest and most (if not all) water loss and gas exchange occurs across the skin at rest (Bentley and Yorio 1979; Wygoda 1984; Young et al. 2005; Senzano and Andrade 2018), our analysis evaluates the skin as a respiratory surface. We are, nonetheless, unable to disentangle the relative costs of each respiratory surface.

Statistical analysis

We conducted all analyses in R (v. 4.3.1, R Core Team 2021). For our response variable, we calculated the transpiration ratio using the same approach as Woods and Smith (2010), which was calculated as the ratio of water loss (mol H₂O d⁻¹) to gas uptake (mol O₂ d⁻¹). We compared transpiration ratios between amphibians and the terrestrial taxa from Woods and Smith (2010) by providing average water loss rates, gas uptake rates, and transpiration ratios for each group. We also estimated the slope and intercept between log₁₀-scaled water loss and log₁₀-scaled gas uptake in separate linear regressions for each taxonomic group. These statistics help to compare transpiration ratios and evaluate the

strength of the relationship between these two traits among groups, which may reflect the degree of constraint (i.e., weaker relationships may have less constraints).

We also conducted two analyses to understand how transpiration ratios are related to temperature, humidity, and body mass. In the first analysis (called the phylogenetic analysis), we sought to understand the phenotypic diversity and evolutionary lability of transpiration ratios among amphibian species. Thus, we conducted a phylogenetic generalized least squares analysis using the RRPP package in R (Collyer and Adams 2018). In the analysis, we used the \log_{10} -scaled average transpiration ratio as the response variable, and we used the average temperature ($^{\circ}\text{C}$), VPD (kPa), \log_{10} -scaled mass (g), and flow rate (L/s) as covariates. We included flow rate as a covariate due to its effect on convective conductance to water vapor (Campbell and Norman 1998). We also analyzed water loss and gas uptake in separate models using the same approach and covariates. To assess significance, we used a Type-II analysis of covariance in the RRPP package. For the phylogenetic analysis, we used our focal species to trim a consensus tree from a comprehensive phylogenetic tree of amphibians (Jetz and Pyron 2018). In addition, we also estimated phylogenetic signal using Pagel's lambda (λ) in the *caper* package in R (Orme 2023, <https://cran.r-project.org/web/packages/caper/index.html>, last accessed Jan. 17, 2024), which we calculated using the same response variable and covariates as the models above.

The intraspecific analysis was designed to evaluate the effects of temperature, humidity, and body mass on transpiration ratio within amphibian species. We \log_{10} -scaled the transpiration ratio and body mass to meet the assumptions of normality. We also used a mixed effect modeling approach with the *lme4* package (Bates et al. 2023, <https://cran.r-project.org/web/packages/lme4/index.html>, last accessed Feb. 12, 2024) with each predictor variable as a covariate and species as a random effect. We assessed our model for collinearity using variance inflation factors from the *car* package (Fox et al. 2023) and found that all variance inflation factors were less than 2, indicating a lack of collinearity among predictors (Craney and Surlles 2002). We did not incorporate relatedness into this analysis because we explored species-level diversity in the phylogenetic analysis above and were interested in the relationship between the transpiration ratio and our predictors within species. Also, phylogenetic relationships among *Ambystoma* were mostly ambiguous. Given the goals of our study and limitations of the data, we used the mixed effects modeling approach for the intraspecific analysis.

The ratio between two flexible traits can change for many reasons. For instance, the ratio might decrease due to a decrease in the numerator, an increase in the denominator, or both. Thus, we also analyzed the effect of each predictor on water loss rate and gas uptake rate in separate models to understand how changes in the underlying traits resulted in variation in the transpiration ratio. These analyses were conducted using the same predictors (temperature, VPD, and \log_{10} -scaled body mass) and random effects (species) with a mixed effects model. Because our analysis was a multiple regression, we plotted our results using partial regressions to illustrate the effects of each variable while accounting for the other predictors in the model.

For each model, we conducted a Type-II analysis of covariance with Satterthwaite's method of degrees of freedom from the *lmerTest* package (Kuznetsova et al. 2017). To assess model fit, we reported marginal and conditional R^2 from the *MuMIn* package (Barton 2023, <https://cran.r-project.org/web/packages/MuMIn/index.html>, last accessed Feb. 2, 2024) to report the goodness-of-fit for fixed effects and fixed plus random effects in the model, respectively. We also reported an effect size (ω^2) to estimate the magnitude of the effect for each predictor in the model. We estimated the effect size using the *effectsize* package, which calculates the statistic using:

$$\omega^2 = \frac{SS_{\text{treatment}} - df_{\text{treatment}} \times MS_{\text{error}}}{SS_{\text{total}} + MS_{\text{error}}},$$

where $SS_{\text{treatment}}$ is the sum of squares for a given parameter, $df_{\text{treatment}}$ is the degrees of freedom for that parameter, MS_{error} is the mean square error, and SS_{total} is the total sum of squares (Olejnik and Algina 2003).

Results

Comparison among terrestrial taxa

Based on the transpiration ratio, hydric costs of respiration were two to four orders of magnitude greater for amphibians than for birds, bird eggs, insects, and mammals (Table 1). Specifically, hydric costs are over 67,000-fold greater in amphibians relative to mammals, which had the lowest hydric costs, and 144-fold greater relative to plants, which had the second highest hydric costs behind amphibians. The extremely high transpiration ratio of amphibians is due to both high water loss rates and low gas uptake relative to other terrestrial taxa (Fig. 1, Table 1). In general, the relationship between water loss and gas uptake was near or below unity for each taxonomic group, with amphibians exhibiting the shallowest relationship among terrestrial taxa (Table 1). Within amphibians, the species with the lowest hydric costs was the barred tiger salamander (*Ambystoma ma-*

Table 1. Summary of physiological values, transpiration ratios, and the relationship between physiological values for terrestrial organisms

| Taxa | Water loss | Gas uptake | Transpiration ratio | Slope | Intercept |
|------------|------------|------------|---------------------|-------------|--------------|
| Mammals | 9.78 | 43.0 | 0.805 | 0.79 ± 0.06 | -0.18 ± 0.08 |
| Birds | 3.04 | 2.41 | 0.815 | 1.17 ± 0.13 | -0.20 ± 0.11 |
| Bird eggs | 0.0405 | 0.0302 | 1.23 | 1.09 ± 0.03 | -0.24 ± 0.06 |
| Insects | 0.000233 | 0.000102 | 4.98 | 0.79 ± 0.08 | -0.42 ± 0.40 |
| Plants | 412.0 | 1.13 | 374.0 | 1.07 ± 0.11 | -2.50 ± 0.03 |
| Amphibians | 51.4 | 0.00211 | 54,112.0 | 0.59 ± 0.08 | -3.34 ± 0.27 |

Units: Water loss = mol H₂O d⁻¹; Gas uptake = mol O₂ d⁻¹; Transpiration ratio = mol H₂O mol⁻¹ O₂

Taxa are organized based on the value of the transpiration ratio, from lowest to highest. Amphibians demonstrate a remarkably high transpiration ratio due to a combination of low gas uptake and high water loss.

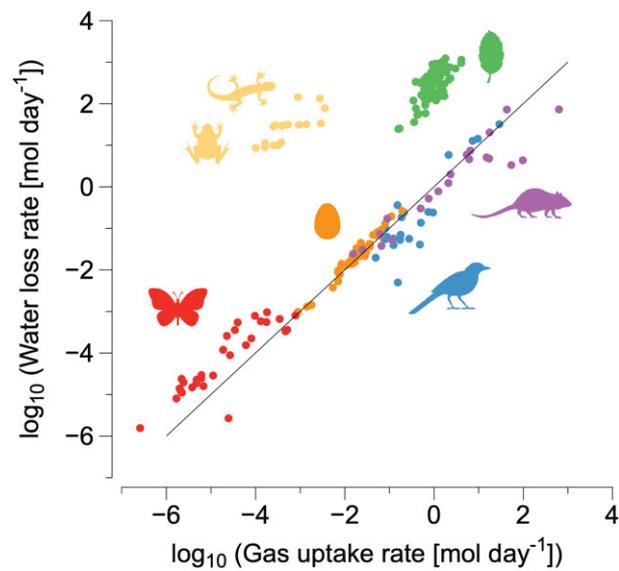


Fig. 1. Amphibians experience substantially higher hydric costs compared to other terrestrial species. The relationship between water loss rate and gas uptake rate for insects, eggs, birds, mammals, plants, and amphibians. The figure is modified from Woods and Smith (2010) to illustrate how amphibians compare to other terrestrial taxa.

vortium), which had costs 82.4% lower than the average hydric costs for amphibians. The amphibian species with the highest hydric costs was the canyon tree frog (*Hyla arenicolor*), which had costs 150% higher than the average hydric costs for amphibians. On average, hydric costs were similar between salamanders and frogs (1.9% lower for salamanders), and hydric costs for lungless salamanders were 27.3% higher compared to lunged salamanders.

Phylogenetic analysis

In the phylogenetic analysis, we found a significant negative relationship between the transpiration ratio and body mass (Table 2). In other words, larger species have lower hydric costs of gas uptake compared to smaller

Table 2. Results for the Type-II ANCOVA from the phylogenetic generalized least-squares for the effect of mass, temperature, VPD, and flow rate on amphibian transpiration ratio

| Variable | df | SS | Rsq | F | P |
|-------------|----|-----------|--------|-------|-------|
| log(Mass) | 1 | 0.0069517 | 0.2274 | 12.46 | 0.006 |
| Temperature | 1 | 0.0000069 | 0.0002 | 0.01 | 0.908 |
| VPD | 1 | 0.0000002 | 0.0000 | 0.00 | 0.983 |
| Flow rate | 1 | 0.0012234 | 0.0400 | 2.19 | 0.191 |
| Residuals | 10 | 0.0055775 | 0.1825 | | |
| Total | 14 | 0.0305593 | | | |

The analysis indicates that the transpiration ratio was significantly associated with body mass but not temperature or VPD.

species. Temperature, VPD, and flow rate were not associated with the transpiration ratio. In the separate analyses on water loss and gas uptake, we found that mass and temperature had significantly positive effects on gas uptake (mass: $P = 0.001$; temperature: $P = 0.019$), but VPD and flow rate did not affect gas uptake ($P > 0.717$). Mass, temperature, and flow rate had significant positive effects on water loss (mass: $P = 0.001$; temperature: $P = 0.004$; flow rate: $P = 0.049$), but VPD did not affect water loss ($P = 0.573$). Our analysis suggested that the transpiration ratio is phylogenetically labile (Fig. 2; $\lambda = 0.0$), with λ being significantly different from 1 ($P = 0.04$) but not 0 ($P = 1.0$). After accounting for mass and relatedness, our phylogenetic analysis identified substantial unexplained variation in the transpiration ratio (Fig. 2).

Intraspecific analysis on transpiration ratio

In the intraspecific analysis, temperature, VPD, and body mass had significant effects on the transpiration ratio. Our model also indicated a relatively high goodness of fit for those fixed effects (marginal $R^2 = 0.47$) and the random effect of species (conditional $R^2 = 0.73$). Temperature had a negative effect on the transpiration ratio (Fig. 3A, $P < 0.001$, $\omega^2 = 0.45$),

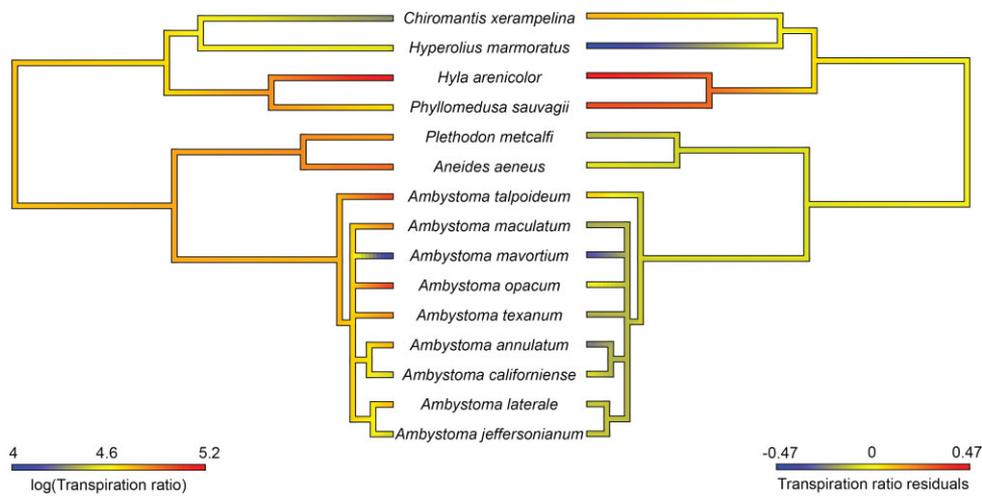


Fig. 2. The transpiration ratio (left) and the residuals of the transpiration ratio from the phylogenetic analysis (right). The figure demonstrates a high degree of variation in the transpiration ratio and the residuals of the ratio, which account for body mass and relatedness. The residuals figure demonstrates that our analysis leaves much variation to explain beyond body size, temperature, VPD, and flow rate. The phylogeny was generated by trimming the consensus tree from [Jetz and Pyron \(2018\)](#).

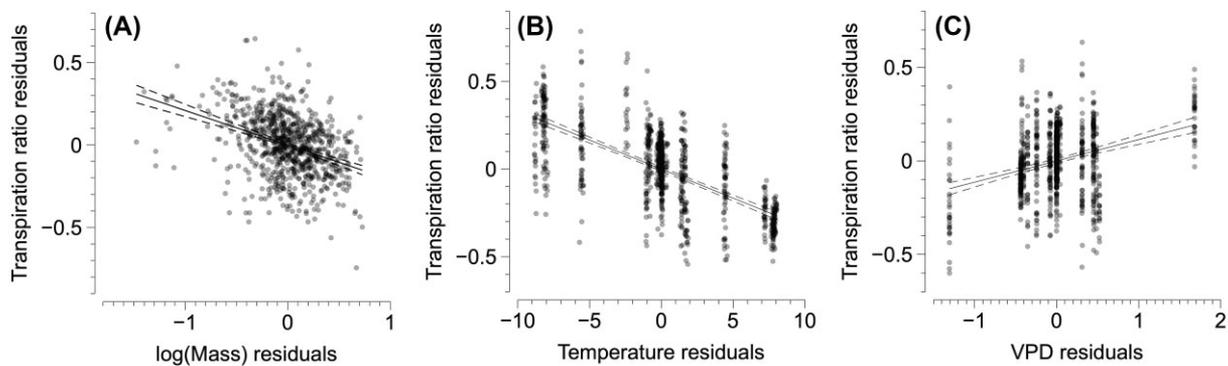


Fig. 3. Amphibians experience lower hydric costs with larger body size, at high temperatures, and low vapor pressure deficits (VPDs). The effects of (A) log-scaled mass on transpiration ratio, (B) temperature, and (C) VPD. These results were generated from a mixed effects model that include each variable as a covariate and species as a random effect. The results highlight the importance of temperature, humidity, and body size in driving the relationship between water loss and gas uptake. Partial regressions are shown for each plot.

indicating that hydric costs of respiration declined as temperatures warmed. VPD had a positive effect on the transpiration ratio ([Fig. 3B](#), $P < 0.001$, $\omega^2 = 0.10$), indicating that hydric costs increased as air became drier. In addition, body mass had a negative effect on the transpiration ratio ([Fig. 3C](#), $P < 0.001$, $\omega^2 = 0.17$), with larger individuals exhibiting lower hydric costs of respiration. Flow rate was not associated with the transpiration ratio ($P = 0.122$, $\omega^2 = 0.18$).

Thus, the negative effect of body mass on the tran-

Intraspecific analysis on water loss and gas uptake

The separate analyses on water loss and gas uptake revealed the underlying patterns that shaped variation in the transpiration ratio. Body mass had a positive effect on both response variables ([Fig. 4A, B](#), water loss: $P < 0.001$, $\omega^2 = 0.07$; gas uptake: $P = 0.001$, $\omega^2 = 0.26$).

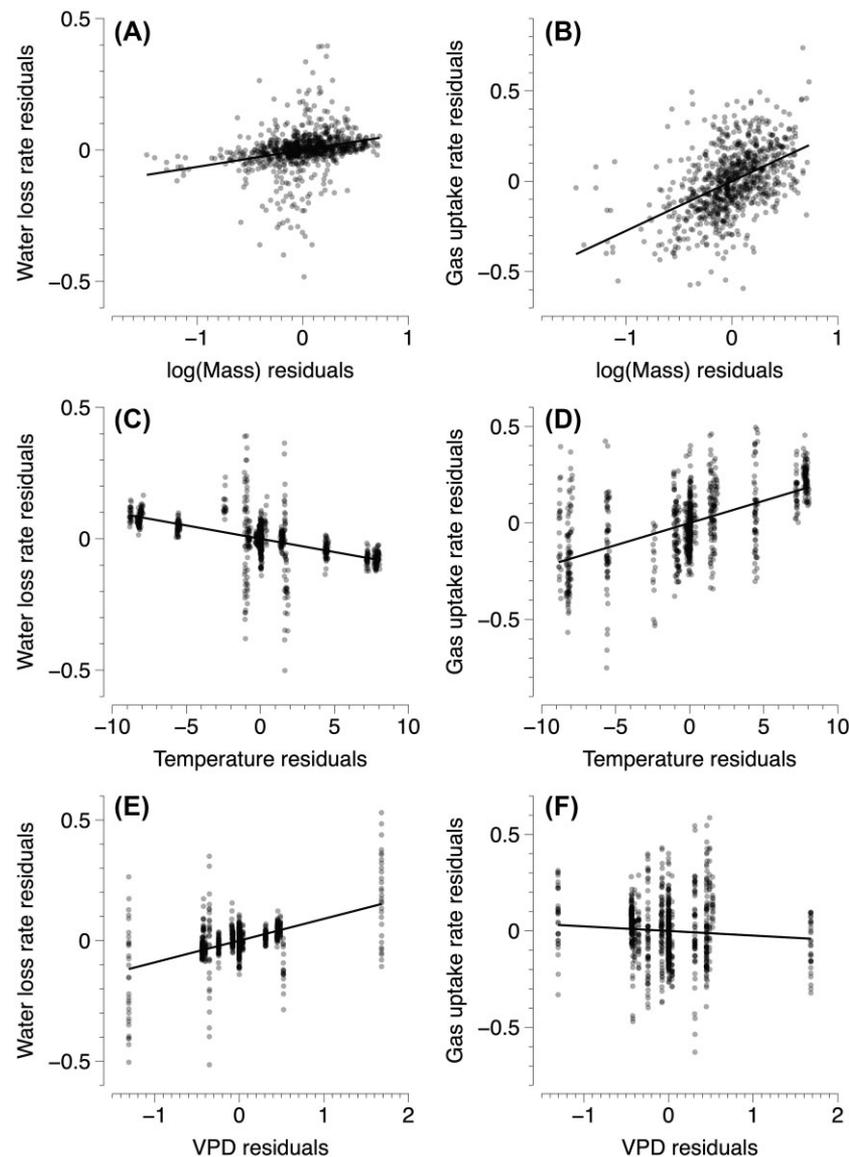


Fig. 4. Intraspecific analysis on water loss rate and gas uptake rate. (A) A positive relationship between water loss rate and log-scaled body mass. (B) A positive relationship between gas uptake rate and log-scaled body mass. (C) A negative relationship between water loss rate and temperature. (D) A positive relationship between gas uptake and temperature. (E) A positive relationship between water loss and VPD. (F) A weak, negative relationship between gas uptake rate and VPD. Partial regressions are shown for each plot.

spiration ratio was due to the slope between gas uptake and body mass being much steeper (and relatively less variable) than the slope between water loss and body mass (water loss = 0.066 ± 0.008 ; gas uptake = 0.278 ± 0.017). Therefore, larger individuals have lower hydric costs than smaller individuals because gas uptake is more sensitive to body mass than water loss. Temperature had a negative effect on water loss (Fig. 4C, $P < 0.001$, $\omega^2 = 0.27$), whereas temperature had a positive effect on gas uptake (Fig. 4D, $P < 0.001$, $\omega^2 = 0.31$). VPD had a positive effect on water loss (Fig. 4E, $P < 0.001$, $\omega^2 = 0.25$) and very small (though significant) negative effect on gas uptake (Fig. 4F, $P = 0.035$, $\omega^2 < 0.01$). Flow rate had a positive effect on water loss

($P < 0.001$, $\omega^2 = 0.86$) but did not affect gas uptake ($P = 0.137$, $\omega^2 = 0.16$).

Discussion

Our study revealed that the hydric costs of respiration in amphibians are two to four orders of magnitude greater than the hydric costs in other terrestrial organisms. The extremely high transpiration ratios are the result of both relatively high water loss and low gas uptake (Table 1), revealing a striking mismatch between gas uptake and water loss in amphibians not found in birds, mammals, or insects. Gas uptake and water loss are likely decoupled due to the consequences

of cutaneous respiration (or skin breathing). Though oxygen uptake is less effective across the skin compared to lungs, skin breathing provides an energetically cheap strategy for oxygen uptake that avoids the cost of ventilation. Accordingly, amphibians exhibit some of the lowest metabolic rates among terrestrial vertebrates (White et al. 2005; Uyeda et al. 2017), which likely reflects an adaptive strategy for cheap (albeit ineffective) gas uptake (Pough 1980). Having permeable skin for gas uptake has substantial costs from a hydration perspective, but by selecting aquatic or saturated environmental conditions, amphibians appear to avoid these costs and often experience little to no water loss despite having leaky skin (Lertzman-Lepofsky et al. 2020; Hoffmann et al. 2021). Amphibians are thus largely buffered from selective pressure on water loss, which explains the highly conserved and low physiological resistance to water loss among amphibians (Lillywhite 2006). Selecting moist environments may also allow metabolic rates to evolve more independently to other environmental variables, such as temperature or food availability (Feder 1978; Navas 1996; Podhajský and Gvoždík 2016; Enriquez-Urzelai et al. 2022). Therefore, the combination of skin breathing and habitat selection has produced an unexpected combination of water loss and gas uptake in amphibians relative to other animals.

Our phylogenetic analysis revealed a high degree of variation in the transpiration ratio among amphibians. Body mass is one of the most important factors driving variation in the transpiration ratio. Specifically, larger species (and individuals) exhibited lower hydric costs of respiration relative to smaller ones. Both water loss and gas uptake increased with body mass, which are commonly observed phenomena due to larger individuals having more surface area over which evaporation occurs and higher rates of respiration, respectively (White et al. 2019). The relationship between body mass and the transpiration ratio suggests that gas uptake increases with body mass at a higher rate compared to water loss (a pattern we also observed in the intraspecific analysis). The relationship could be explained by allometric scaling of surface area-to-volume ratios, which result in larger organisms having a lower surface area-to-volume ratio relative to smaller organisms (Lindstedt and Hoppeler 2023). This allometric scaling of surface area-to-volume ratios is potentially sufficient to explain the lower hydric costs of larger amphibians because the volume of cells consuming oxygen should increase at a faster rate with body mass compared to the surface area over which evaporation occurs. In a post-hoc analysis, we estimated the relationship between body mass and the ratio between surface area and metabolic rate from allometric relationships for amphibians in the literature

(surface area scaling coefficient = 0.6–0.77 [Klein et al. 2016]; metabolic rate scaling coefficient = 0.78–1.0 [White 2011]). The slope between mass and the estimated ratio varied between -0.01 and -0.4 , which encompassed our observed slope of -0.21 ± 0.02 (\pm standard error). However, alternative mechanisms (such as processes related to convection and oxygen diffusion) may also play a role (Weibel 1987). Nevertheless, even after accounting for body mass, our analysis revealed a high degree of unexplained variance in the transpiration ratio (Fig. 2B), which may be driven by responses to extrinsic factors, such as responses to environmental conditions, or factors not explored in our analysis, such as microhabitat selection.

Both temperature and VPD were associated with the transpiration ratio in the intraspecific analysis but not in the phylogenetic analysis. These variables likely failed to explain any variation in the phylogenetic analysis because of the low sample size ($n = 15$) and averaging over experimental temperatures and VPDs. In the intraspecific analysis, however, temperature and VPD had large, contrasting effects on the transpiration ratio. The positive effect of VPD on the transpiration ratio was driven by a positive effect on water loss and very small effect on gas uptake. Therefore, as air becomes drier, the costs of respiration increase as water loss increases and gas uptake stays the same. Temperature had a negative effect on the transpiration ratio due to the negative effect of temperature on water loss and positive effect on gas uptake. Thus, as temperatures warmed, water loss declined and gas uptake increased, thereby reducing the hydric costs of respiration. The increase in gas uptake is unsurprising given the effects of temperature on respiration in ectotherms; however, the reduction in water loss is more surprising. Typically, water loss is expected to increase with temperature (Verberk et al. 2016), though this effect is an indirect product of the exponential increase in saturation vapor pressure that lead to high VPDs. In most studies on the thermal sensitivity of water loss, temperature and VPD are confounded. In our analysis, variance inflation factors were low, providing the opportunity to tease apart the effects of VPD and temperature separately. Our results suggest that some amphibians physiologically reduce water loss in response to warming temperatures, which represents an adaptive physiological response to reduce desiccation risk in warm environments. Because warmer temperatures are correlated with drier air across the globe (Riddell et al. 2019), physiologically reducing water loss in response to warm temperatures may buffer amphibians from the drier conditions that frequently accompany warmer air. Some salamanders and frogs have been found to plastically increase their physiological resistance to water loss in response to warm temper-

atures (Riddell and Sears 2015; Senzano and Andrade 2018), though frogs also exhibit the opposite response (Tracy et al. 2008) or do not change at all (Davies et al. 2015). More often, however, temperature and VPD are confounded and preclude an independent assessment of these two variables. Therefore, more comparative experiments are needed to understand variation in the effects of temperature on water loss physiology. Similarly, the mechanisms driving these responses within organisms and between species will help to understand the relationship between water loss and gas uptake.

Whether an organism breathes across the lungs, integument, or both, there are several strategies and traits that have arisen within tetrapods to decouple gas uptake from water loss. Nasal turbinates in birds and mammals are known to conserve respiratory water loss and provide a means to decouple gas uptake from water loss (Hillenius 1992; Geist 2000). Across the integument, organisms (especially amphibians) often regulate gas exchange and water loss via perfusion of the underlying vasculature in the skin (Burggren and Moallf 1984; Burggren 1988), which provides a clear mechanistic basis for the trade-off between gas exchange and water loss. Properties of the capillary beds in the skin, such as density and degree of regulation, may provide insight into the ability of organisms to decouple water loss from gas uptake (Feder and Burggren 1985). However, there are alternative means of regulating water loss that likely have little to no effect on gas uptake. For instance, many organisms, including amphibians, regulate the composition of lipids in the skin to reduce the permeability of water (Mc Clanahan et al. 1978; Withers et al. 1984; Toledo and Jared 1993; Lillywhite et al. 1997; Lillywhite 2004, 2006). Similarly, melanin also lowers the permeability of the integument to water loss, potentially via pH-induced changes in lipids (Man et al. 2014). Exploring these mechanisms will help to predict the relative strength of the trade-off between species and the extent to which they can decouple gas uptake from water loss.

In this study, we compared the relationship between water loss and gas uptake between amphibians and several other terrestrial taxa that were previously presented in Woods and Smith (2010). Amphibians exhibited the highest hydric costs of gas uptake among any other terrestrial organisms examined and provide further evidence that more organisms (in addition to plants) deviate from the proposed 1:1 ratio of water loss to gas uptake (Woods and Smith 2010). The high hydric costs in plants might be related to the build-up of water vapor pressure within intercellular spaces that forces vapor out of the stomata (Leuning 1983; Woods and Smith 2010). The mechanisms driving high hydric costs in amphibians are different from plants, given the absence of evaporation within tissue and stomata. Rather, the

high costs in amphibians are likely due to the evolution of extremely thin skin to promote oxygen uptake and carbon dioxide elimination (Burggren and Vitalis 2005). Our analysis suggests that there are conditions by which gas exchange and water loss can evolve independently, at least toward high rates of water loss and low rates of gas exchange. Evolutionary responses in the opposite (and potentially adaptive) direction toward low water loss and high gas exchange appears to be more seriously constrained. Though beneficial from a gas uptake perspective, such a phenotypic combination would be likely have significant costs, such as production costs associated with water-proofing or greater demands for energy intake to support a high metabolism. Nevertheless, by maintaining exceptionally “leaky” skin, amphibians may have minimized the trade-off between water loss and gas exchange, possibly allowing gas exchange to evolve more freely. More observations and experiments that test this hypothesis could reveal the ecological and evolutionary importance of using a unique mode of respiration for decoupling a fundamental trade-off in terrestrial organisms.

Author contributions

E.A.R. conceived the project and analyzed the data, and E.A.R., I.J.B., M.M.M., S.J.W., and M.C.W. wrote and edited the manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

Data availability

Data and code are available on Zenodo (DOI: 10.5281/zenodo.10723872; link: <https://zenodo.org/records/10723872>).

References

- Anderson DB. 1936. Relative humidity or vapor pressure deficit. *Ecology* 17:277–82.
- Barton K. 2023. Package “MuMIn,” v. 1.18.

- Bates D, Bolker B, Walker S, Dai B, Fox J. 2023. Package “lme4,” v. 35.1.
- Bentley PJ, Yorio T. 1979. Evaporative water loss in anuran amphibians: a comparative study. *Comp Bio Phys Part A Phys* 62:1005–9.
- Bertoluci J. 1998. Annual patterns of breeding activity in Atlantic rainforest anurans. *J Herpetol* 32:607.
- Bray AA, Potts WTW, Milner AR, Chaloner WG, Lawson JD. 1997. The evolution of the terrestrial vertebrates: environmental and physiological considerations. *Phil Trans Royal Soc Lond Biol Sci* 309:289–322.
- Burger JJ, Carter ET, Magner LM, Muñoz MM, Sears MW, Fitzpatrick BM, Riddell EA. 2024. Assessing hybrid vigour using the thermal sensitivity of physiological trade-offs in tiger salamanders. *Funct Ecol* 38:143–52.
- Burggren W, Moallf R. 1984. ‘Active’ regulation of cutaneous exchange by capillary recruitment in amphibians: Experimental evidence and a revised model for skin respiration. *Respir Physiol* 55:379–92.
- Burggren WW, Vitalis TZ. 2005. The interplay of cutaneous water loss, gas exchange and blood flow in the toad, *Bufo woodhousei*: adaptations in a terrestrially adapted amphibian. *J Exp Biol* 208:105–12.
- Burggren WW. 1988. Role of the central circulation in regulation of cutaneous gas exchange. *Integr Comp Biol* 28:985–98.
- Campbell GS, Norman J. 1998. An introduction to environmental biophysics. 2nd ed. New York: Springer-Verlag.
- Collyer ML, Adams DC. 2018. RRPP: An R package for fitting linear models to high-dimensional data using residual randomization. *Methods Ecol Evol* 9:1772–9.
- Craney TA, Surlis JG. 2002. Model-dependent variance inflation factor cutoff values. *Qual Eng* 14:391–403.
- Cupello C, Hirasawa T, Tatsumi N, Yabumoto Y, Gueriau P, Isogai S, Matsumoto R, Saruwatari T, King A, Hoshino M et al. 2022. Lung evolution in vertebrates and the water-to-land transition. *eLife* 11:e77156.
- Davies SJ, McGeoch MA, Clusella-Trullas S. 2015. Plasticity of thermal tolerance and metabolism but not water loss in an invasive reed frog. *Comp Biochem Physiol A Mol Integr Physiol* 189:11–20.
- Denton RD, Greenwald KR, Gibbs HL. 2017. Locomotor endurance predicts differences in realized dispersal between sympatric sexual and unisexual salamanders. *Funct Ecol* 31:915–26.
- Duellman WE. 1999. Patterns of distribution of amphibians: a global perspective. Baltimore, MD: John Hopkins University Press.
- Enriquez-Urzelai U, Nicieza AG, Montori A, Llorente GA, Urrutia MB. 2022. Physiology and acclimation potential are tuned with phenology in larvae of a prolonged breeder amphibian. *Oikos* 2022:e08566.
- Feder ME, Burggren WW. 1985. The regulation of cutaneous gas exchange in vertebrates. In: Gilles R, editor. *Circulation, respiration, and metabolism. proceedings in life sciences Berlin, Heidelberg: Springer.* p. 101–13.
- Feder ME. 1978. Environmental variability and thermal acclimation in neotropical and temperate zone salamanders. *Physiol Zool* 51:7–16.
- Fox J, Weisberg S, Price B, Adler D, Bates D, Bolker B, Walker S. 2023. ‘Package “car,” v. 3.1.
- Gans C. 1970. Respiration in early tetrapods—the frog is a red herring. *Evolution* 24:723–34.
- Geist NR. 2000. Nasal respiratory turbinate function in birds. *Physiol Biochem Zool* 73:581–9.
- Graham JB, Lee HJ. 2004. Breathing air in air: In what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? *Physiol Biochem Zool* 77:720–31.
- Hillenius WJ. 1992. The evolution of nasal turbinates and mammalian endothermy. *Paleobiology* 18:17–29.
- Hoffmann EP, Cavanaugh KL, Mitchell NJ. 2021. Low desiccation and thermal tolerance constrains a terrestrial amphibian to a rare and disappearing microclimate niche. *Cons Physiol* 9:coab027.
- Hsia CCW, Schmitz A, Lambertz M, Perry SF, Maina JN. 2013. Evolution of air breathing: Oxygen homeostasis and the transitions from water to land and sky. *Compr Physiol* 3:849–915.
- Inger RF. 1957. Ecological aspects of the origins of the tetrapods. *Evolution* 11:373–6.
- Jetz W, Pyron RA. 2018. The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nat Ecol Evol* 2:850–8.
- Klein W, Dabés L, Bonfim VMG, Magrini L, Napoli MF. 2016. Allometric relationships between cutaneous surface area and body mass in anuran amphibians. *Zool Anz J Comp Zool* 263:45–54.
- Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest package: tests in linear mixed effects models. *J Stat Soft* 82:13.
- Lertzman-Lepofsky GF, Kissel AM, Sinervo B, Palen WJ. 2020. Water loss and temperature interact to compound amphibian vulnerability to climate change. *Global Change Biol* 26:4868–79.
- Leuning R. 1983. Transport of gases into leaves. *Plant Cell Env* 6:181–94.
- Lillywhite HB, Mittal AK, Garg TK, Agrawal N. 1997. Integumentary structure and its relationship to wiping behaviour in the common Indian tree frog, *Polypedates maculatus*. *J Zool* 243:675–87.
- Lillywhite HB. 2004. Plasticity of the water barrier in vertebrate integument. *International Congress Series, Animals and Environments . Proc Third Int Conf Comp Physiol Bioch* 1275:283–90.
- Lillywhite HB. 2006. Water relations of tetrapod integument. *J Exp Biol* 209:202–26.
- Lindstedt SL, Hoppeler H. 2023. Allometry: revealing evolution’s engineering principles. *J Exp Biol* 226:jeb245766.
- Little C. 1990. *The terrestrial invasion: An ecophysiological approach to the origins of land animals.* Cambridge, MA: Cambridge University Press.
- Maenpaa M, Riikonen J, Kontunen-Soppela S, Rousi M, Oksanen E. 2011. Vertical profiles reveal impact of ozone and temperature on carbon assimilation of *Betula pendula* and *Populus tremula*. *Tree Physiol* 31:808–18.
- Maina JN. 2002. Structure, function and evolution of the gas exchangers: comparative perspectives. *J Anat* 201:281–304.
- Man M-Q, Lin T-K, Santiago JL, Celli A, Zhong L, Huang Z-M, Roelandt T, Hupe M, Sundberg JP, Silva KA et al. 2014. Basis for enhanced barrier function of pigmented skin. *J Invest Dermatol* 134:2399–407.

- Mc Clanahan LL, Stinner JN, Shoemaker VH. 1978. Skin lipids, water loss, and energy metabolism in a South American tree frog (*Phyllomedusa sauvagei*). *Physiol Zool* 51: 179–87.
- Messerman AF, Leal M. 2020. Inter- and intraspecific variation in juvenile metabolism and water loss among five biphasic amphibian species. *Oecologia* 194:371–82.
- Mokhatla M, Measey J, Smit B. 2019. The role of ambient temperature and body mass on body temperature, standard metabolic rate and evaporative water loss in southern African anurans of different habitat specialisation. *PeerJ* 7:e7885.
- Monteith JL, Campbell GS. 1980. Diffusion of water vapour through integuments—Potential confusion. *J Therm Biol* 5: 7–9.
- Navas CA. 1996. Metabolic physiology, locomotor performance, and thermal niche breadth in neotropical anurans. *Physiol Zool* 69:1481–501.
- Newman JC, Riddell EA, Williams LA, Sears MW, Barrett K. 2022. Integrating physiology into correlative models can alter projections of habitat suitability under climate change for a threatened amphibian. *Ecography* 2022:e06082.
- Olejnik S, Algina J. 2003. Generalized eta and omega squared statistics: Measures of effect size for some common research designs. *Psychol* 8:434–47.
- Orme D. 2023. The caper package: comparative analysis of phylogenetics and evolution in R. v. 1.0.3.
- Podhajský L, Gvoždík L. 2016. Variation in winter metabolic reduction between sympatric amphibians. *Comp Bio Physiol Part A Mol Int Physiol* 201:110–4.
- Pough FH. 1980. The advantages of ectothermy for tetrapods. *Am Nat* 115:92–112.
- R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Riddell EA, Roback EY, Wells CE, Zamudio KR, Sears MW. 2019. Thermal cues drive plasticity of desiccation resistance in montane salamanders with implications for climate change. *Nat Commun* 10:1–12.
- Riddell EA, Sears MW. 2015. Geographic variation of resistance to water loss within two species of lungless salamanders: implications for activity. *Ecosphere* 6:1.
- San Mauro D, Vences M, Alcobendas M, Zardoya R, Meyer A. 2005. Initial diversification of living amphibians predated the breakup of Pangaea. *Am Nat* 165:590–9.
- Schulte PM. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J Exp Biol* 218:1856–66.
- Senzano LM, Andrade DV. 2018. Temperature and dehydration effects on metabolism, water uptake, and the partitioning between respiratory and cutaneous evaporative water loss in a terrestrial toad. *J Exp Biol* 221: jeb188482.
- Spotila JR, Berman EN. 1976. Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. *Comp Biochem Physiol A Physiol* 55: 407–11.
- Stull RB. 2000. *Meteorology for scientists and engineers*. Springer: New York, NY.
- Tattersall GJ. 2007. Skin breathing in amphibians. In: *Endothelial Biomedicine: A Comprehensive Reference*. 1st ed. Editor: William C. Aird. Cambridge, England: Cambridge University Press. p. 85–91.
- Toledo RC, Jared C. 1993. Cutaneous adaptations to water balance in amphibians. *Comp Biochem Physiol A Physiol* 105:593–608.
- Tracy CR, Christian KA, Betts G, Tracy CR. 2008. Body temperature and resistance to evaporative water loss in tropical Australian frogs. *Comp Bio Physiol Part A Mol Int Physiol* 150:102–8.
- Tracy CR. 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. *Ecol Monogr* 46:293–326.
- Uyeda JC, Pennell MW, Miller ET, Maia R, McClain CR. 2017. The evolution of energetic scaling across the vertebrate tree of life. *Am Nat* 190:185–99.
- Verberk W, Overgaard J, Ern R, Bayley M, Wang T, Boardman L, Terblanche JS. 2016. Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comp Bio Physiol Part A Mol Int Physiol* 192:64–78.
- Wake DB, Koo MS. 2018. Amphibians. *Curr Biol* 28:R1237–41.
- Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc Natl Acad Sci USA* 105:11466–73.
- Weibel ER. 1987. Scaling of structural and functional variables in the respiratory system. *Annu Rev Physiol* 49:147–59.
- White CR, Marshall DJ, Alton LA, Arnold PA, Beaman JE, Bywater CL, Condon C, Crispin TS, Janetzki A, Pirtle E et al. 2019. The origin and maintenance of metabolic allometry in animals. *Nat Ecol Evol* 3:598–603.
- White CR, Phillips NF, Seymour RS. 2006. The scaling and temperature dependence of vertebrate metabolism. *Biol Lett* 2:125–7.
- White CR. 2011. Allometric estimation of metabolic rates in animals. *Comp Biochem Physiol A Mol Integr Physiol* 158:346–57.
- Withers PC, Hillman SS, Drewes RC. 1984. Evaporative water loss and skin lipids of anuran amphibians. *J Exp Zool* 232:11–7.
- Woods HA, Smith JN. 2010. Universal model for water costs of gas exchange by animals and plants. *Proc Natl Acad Sci USA* 107:8469–74.
- Wygoda ML. 1984. Low cutaneous evaporative water loss in arboreal frogs. *Physiol Zool* 57:329–37.
- Young JE, Christian KA, Donnellan S, Tracy CR, Parry D. 2005. Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. *Physiol Biochem Zool* 78:847–56.
- Zhang P, Wake DB. 2009. Higher-level salamander relationships and divergence dates inferred from complete mitochondrial genomes. *Mol Phylogenet Evol* 53:492–508.