RESEARCH ARTICLE

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Trade-offs between water loss and gas exchange influence habitat suitability of a woodland salamander

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Abstract

1. Reversible acclimation increases resilience to environmental stress, but acclimation may have hidden costs due to underlying linkages between related physiological traits. These linkages might result in trade-offs that undermine whole-organism performance if the change in a related trait reduces the net benefits of acclimation or increases susceptibility to alternative environmental stressors.

2. Metabolic rate and water loss rate are two fundamental physiological traits that could interact due to their dependence on gas exchange across shared physical surfaces. Reductions in water loss rate in response to dehydration stress might reduce metabolic rate by constraining the flux of both water and oxygen.

3. We examined acclimation of metabolic rate and water loss rate using a species of woodland salamander (*Plethodon metcalfi*) in response to temperature and humidity using a full factorial experimental design. We controlled the evaporative demand of the air across temperatures to assess temperature and humidity as independent cues for acclimation. We predicted that reductions in water loss rate would coincide with reductions in metabolic rate in response to temperature due to shared physical and chemical pathways. We also assessed acclimation of heart rates as a potential compensatory mechanism used to promote oxygen delivery. We integrated these responses into a biophysical model developed from first principles to demonstrate the potential for these interactions to influence habitat suitability.

4. We found that reductions in water loss rates during thermal acclimation were associated with simultaneous reductions in metabolic rates, and we did not find a compensatory response in heart rates. We suggest that these linkages underlie whole-organism strategies (e.g. physiological dormancy or arousal) for reducing the energetic costs imposed by warm temperatures. The biophysical model suggested that the interaction between these two traits potentially structures phenotypic variation in our population because certain combinations of trait values were incapable of reaching positive energy balance.

5. Trade-offs between linked physiological traits potentially structure whole-organism strategies for responding to environmental stressors and constrain phenotypic variation. Therefore, predictions of the benefits of acclimation must be interpreted cautiously without knowledge of the underlying trade-offs among linked physiological traits.

KEYWORDS
acclimation, energy balance, metabolism, salamander, trade-offs, water loss

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1 | INTRODUCTION

Reversible acclimation represents a universal strategy among organisms that reduces stress caused by the environment (Seebacher, White, & Franklin, 2015). Organisms that acclimate increase resilience to environmental change by increasing thermal tolerances, homeostatic capabilities and physiological performance (Ghalambor, McKay, Carroll, & Reznick, 2007; Somero, 2010, 2015; Stillman, 2003). However, acclimation might result in trade-offs by reducing performance in a related trait due to shared chemical or physical pathways (Hochachka & Somero, 1973). In this context, we define trade-offs as a reduction in performance of a physiological trait as a direct consequence of competing demands in another physiological trait (Pörtner et al., 2006). Trade-offs during acclimation might expose organisms to alternative stressors or undermine the net benefits of the original acclimation response (Ricklefs & Wikelski, 2002). These "costs of phenotype" are difficult to detect (Murren et al., 2015), but they are critical to predicting whole-organism responses to environmental stressors and constraints on phenotypic evolution (Armbruster, Pélabon, Bolstad, & Hansen, 2014; Bozinovic & Pörtner, 2015; Verberk et al., 2016). By uncovering these trade-offs, we will improve our ability to predict an organism's susceptibility to environmental change.

Metabolic rate and water loss rate represent two fundamental and physiologically linked traits that maintain an organism's capacity to survive. Metabolic rate and water loss rate are fundamental to balancing energy flux with the environment and reducing thermal and hydric stress (Bartholomew, 1972; Porter & Gates, 1969). These traits also interact due to their shared physical pathway of gas exchange (e.g. skin, lungs, tracheal tubes or stomata) and dependency upon a moist respiratory surface to promote oxygen uptake (Maina, 1998). The physical and chemical linkages result in the simultaneous loss of water with the intake of oxygen (Addo-Bediako, Chown, & Gaston, 2001), particularly during bouts of activity (Chown, 2002). These dependencies are especially relevant for organisms in which most water loss and oxygen uptake occur over the same respiratory surface (i.e. some amphibians and plants), but the association also appears widespread among terrestrial taxa due to the universal pressures of gas exchange in terrestrial environments (Woods & Smith, 2010). Currently, we lack an understanding of the consequences of this link during acclimation to environmental stress.

Predicting the benefits of acclimation requires knowledge of the linkages between related physiological traits (Gunderson, Armstrong, & Stillman, 2016; Sinclair & Ferguson, 2013). These linkages might determine the optimal response to a stressor, such as the threat of dehydration (Bartholomew, 1972). Dehydration directly reduces performance (Hillman, 1984), but the threat of dehydration also limits the amount of time that animals can forage, find mates and defend territories (Levy, Dayan, Porter, & Kronfeld-Schor, 2016; Peterman, Locke, & Semlitsch, 2013), or plants can uptake carbon (Flexas, Bota, Loreto, Cornic, & Sharkey, 2004). Organisms might prolong activity by reducing water loss rates (Riddell & Sears, 2015), but reducing water loss rates might impede the uptake of oxygen required to sustain activities related to energy acquisition (Auer, Salin, Anderson, & Metcalfe,

2015). Respiratory systems help to regulate oxygen uptake and water loss independently, but the relationship between water and oxygen flux appears in organisms with lungs during acclimation (Williams & Tieleman, 2000) and across natural gradients (Tieleman, Williams, & Bloomer, 2003). Many studies cannot determine whether acclimation of water loss and metabolic rates are occurring independently or due to underlying linkages because thermal and hydric stress are often confounded. These experimental considerations are critical to identifying potential trade-offs and their downstream consequences for habitat suitability.

Habitat suitability can be predicted for any organism based on universal principles of energy flux (Porter & Gates, 1969). Approaches in ecological modelling incorporate biophysical and physiological processes to predict activity and energy budgets under local climatic conditions as proxies of habitat suitability (Kearney & Porter, 2004). Physiologically structured models have explored many ecological problems, including the functional traits that structure a species' geographic range (Riddell, Apanovitch, Odom, & Sears, 2017), the spread of invasive species (Phillips, Chipperfield, & Kearney, 2008), and the ecological consequences of evolutionary dynamics (Kearney, Porter, Williams, Ritchie, & Hoffmann, 2009), among many others. Models grounded in mechanism require intensive knowledge of the focal species (Kearney & Porter, 2009), but the underlying biophysical processes are universal among plants and animals (Gates, 1980; Monteith & Unsworth, 2013). Once basic knowledge on a species' physiology is known, these models provide the flexibility to predict the potential ecological and evolutionary consequences of physiological mechanisms from first principles (Helmuth, Kingsolver, & Carrington, 2005; Kish, Helmuth, & Wethey, 2016). Combined with empirical data, the biophysical framework is well suited to understand the potential ecological consequences of trade-offs between linked functional traits.

We conducted an acclimation study using a woodland salamander (*Plethodon metcalfi*) to evaluate trade-offs between metabolic rate and water loss rate during acclimation to warm temperature and dry humidity. Our study species provided the perfect opportunity to study the trade-off because, without lungs, we could leverage the simplicity of water loss and oxygen diffusion across a single respiratory surface. Although simplified, our system might identify the role of trade-offs in shaping variation of these traits across terrestrial taxa (Woods & Smith, 2010). In our study, we measured the volume of oxygen consumption ($\dot{V}O_2$) and skin resistance to water loss (r_i) during acclimation to temperature, humidity and their interaction in a full factorial design. We measured skin resistance to water loss because it represents the most physiologically relevant metric of water loss (Feder & Burggren, 1992). Our experimental design controlled temperature and the vapour pressure of the air to determine whether traits acclimated in response to different cues (i.e. independent acclimation to temperature or humidity) or the same cue. We also measured acclimation of heart rates because organisms can compensate for reduced oxygen diffusion by increasing heart rates to promote gas exchange at the respiratory surface (Franklin, Davison, & Seebacher, 2007; Lillywhite, Zippel, & Farrell, 1999). We predicted that reductions in $\dot{V}O_2$ would coincide with an increase in r_i (i.e. reduction in rates of water loss)

during acclimation to warm temperatures. We based our prediction upon previous experiments in which salamanders used warm temperatures as a cue to adjust water loss rates (Riddell & Sears 2015). However, if $\dot{V}O_2$ did not decline, we predicted that individuals might increase heart rates to promote oxygen delivery (Franklin et al., 2007). Then, we integrated the trade-off into a biophysical model built upon first principles to determine whether the trade-off influenced habitat suitability in our population. Our biophysical model suggested that the trade-off influences phenotypic variation in metabolic rates and water loss rates by constraining the combinations of traits that are capable of achieving positive energy balance in nature.

2 | MATERIALS AND METHODS

2.1 | Salamander collection and care

We investigated trade-offs between $\dot{V}O_2$ and r_i based on the change in phenotypes during acclimation to temperature and vapour pressure deficits. Salamanders for the study were captured from coordinates randomly generated using QGIS (v. 2.18) in the Nantahala National Forest (35°20′N, 83°4′W) during May 2016. We collected individuals between 2.5 and 4 g to minimize the influence of body size on variation of physiological traits. After collection, salamanders were acclimated to laboratory conditions in incubators for 1 month (Percival, Inc.; Model #I-36VL) in individual containers (17 × 17 × 12 cm) with damp paper towels under a cycling thermal regime. The cycling temperatures reflected the conditions that salamanders experience while underground (8.5°C) and on the surface of the forest floor (15°C) during the spring. Salamanders were provided crickets (*Acheta domesticus*) ad libitum during the month prior to the experiment, but we ceased feeding 1 week prior to the acclimation study to reduce the influence of digestion on physiological rates (Feder, Gibbs, Griffith, & Tsuji, 1984). Experiments were approved by the Institute for Animal Care and Use Committee at Clemson University (#2014-024), and collections were approved by the North Carolina Wildlife Commission (#16-SC00746) and United States Fish and Wildlife Service (#MA90761B-0).

2.2 | Acclimation study

Organisms acclimate by responding to specific environmental cues (Via et al., 1995). In our experiment, we exposed individuals to one of two temperature treatments, a cool (8.5–15°C) and warm temperature cycle (15–21.5°C) combined with a wet or dry humidity treatment, which resulted in four treatments: (1) cool, wet; (2) cool, dry; (3) warm, wet; (4) warm, dry. We programmed the incubators to adjust relative humidity with temperature to maintain the same vapour pressure deficits (0.25 kPa for the wet treatments and 0.5 kPa for the dry treatments) during the experiment. Cycling relative humidities with temperature ensured that temperature and evaporative demand of the air were not confounded. These methods allowed for testing whether acclimation occurred in response to temperature, humidity or both as cues. We simulated nocturnal activity by moving salamanders to activity containers (17 × 17 × 12 cm) with dried soil to mimic

substrate from the field and a mesh lid to circulate air from the incubator into the chamber. Activity was simulated for 3 hr each night to replicate bouts of activity on the forest floor. The mass of each salamander was recorded before and after each exposure in the activity chamber. We also recorded a baseline mass at the beginning of the experiment to ensure salamanders did not lose more than 10% of their baseline mass due to dehydration. During the study, salamanders were not exposed to their treatment a total of 1.1% of the total potential exposures (22 out of 1,921 exposures) to avoid lethal dehydration. We rotated the individual containers in the incubators daily with respect to the location of the shelf (e.g. top, middle, bottom) and position on the shelf (e.g. front, back, side) to ensure shelf and position were not confounded. We also rotated the treatments to different incubators once per week to avoid an incubator effect. Each individual was exposed to their treatment for 5 days, and the following day, we measured r_i and $\dot{V}O_2$ using flow-through respirometry.

2.3 | Flow-through system

Flow-through systems have the potential to simultaneously measure multiple physiological traits. In our flow-through system (Sable Systems Int. [SSI], Las Vegas, NV), air was constantly pumped through the flow-through system using a subsample pump (SS4; SSI) followed by a bubbler bottle to saturate the airstream. The airstream was then passed through a dew point generator (DG4; SSI) to control the vapour content. After the DG4, the airstream was separated using a flow manifold (SSI) that also controls flow rates (180 ml/min). The airstream then passed into the acrylic chambers (16 × 3.5 cm; volume *c.* 153 ml) with the salamander suspended over hardwire mesh to simulate posture during activity and minimize the potential for posture to influence water loss rates. Air was constantly flowing through all chambers, and we cycled through each chamber one at a time using a multiplexer (MUX8; SSI). After the chamber, the airstream passed through the water vapour analyzer (RH300; SSI) followed by a flow meter and a dual differential oxygen analyzer (Oxzilla; SSI). Prior to any measurements, we used a column of Drierite (™) on a daily basis to provide a baseline for the RH300, and during measurements, we scrubbed the air of water vapour and CO_2 using Drierite™ and soda lime prior to measuring flow rates and the partial pressure of oxygen. The series of equations for converting partial pressures of gases to meaningful physiological values can be found in the Supporting Information.

We determined acclimation by measuring changes in mean trait values over time in a common environment across different treatments (Leroi, Bennett, & Lenski, 1994). On a given night, we measured the physiology of an individual three times over a 2.5-hr period by cycling between baseline and chambers with salamanders. We analysed the average of the three measurements in the final analysis unless there were obvious signs of activity during a measurement. We ensured that salamanders were resting during the measurements based on the variation in water loss rates, which can be 18.5 times higher during activity (Riddell & Sears 2017), and any measurements with irregularities in vapour pressure were not included. Physiological measurements occurred between 19.00 and 6.00 to measure traits when individuals

would be active in the field. Prior to the measurement, salamanders were held at 18°C for 2 hr, and then each individual was weighed and placed into the chamber. We measured r_i and $\dot{V}O_2$ at 18°C and a vapour pressure deficit of 0.5 kPa for every measurement. Individuals were allowed one night without an activity exposure after measuring their physiology. We repeated these steps (five nights of exposure, one night of physiological measurements and one night of rest) for 1 month to measure changes in physiology over time. Each treatment consisted of 30 randomly assigned individuals (4 treatments × 30 individuals = 120), and each individual was measured five times over the course of the experiment, including the initial measurements. A detailed illustration of the physiological values and their relationship to mass can be found in the Supporting Information (Figure S1).

2.4 | Complementary study on cardiovascular function

We measured heart rates under the same environmental treatments as the acclimation study described above to assess compensatory response from the cardiovascular system. We measured cardiovascular performance by videotaping heart rates through the translucent dorsal skin of the salamanders with GoPro™ cameras (Hero4 Action Camera). Salamanders were placed on an acrylic glass surface, and we placed a small container with a moist paper towel over the top of the individual to limit water loss. The cameras were located below the acrylic glass surface and oriented to capture the dorsal surface of the salamander. Heart rates were determined by the time required for 10 complete ventricular contractions to occur at 10, 16, 24 and 30°C, which were visually counted in Adobe Premiere Pro (v. 10.4; Adobe Systems, San Jose, CA). In this experiment, we measured baseline and response values of r_i, $\dot{V}O_2$ and heart rate after exposure to the same treatments described above in 20 individuals spread across the four treatments ($N = 80$). Contrary to the previous experiment, acclimation to laboratory conditions occurred over 1 week, and the experiment lasted 24 days. We rotated the shelf, position and incubators to ensure that shelf, position and incubators were not confounded by treatment.

2.5 | Modelling the ecological consequences of physiology

Physiological traits determine habitat suitability by influencing the capacity of an organism to remain in positive energy balance (Kearney & Porter, 2004). We developed a biophysical model that incorporates physiology and biophysics to demonstrate the potential ecological consequences of trade-offs between water loss and metabolism. Our model is based on first principles and fundamental physiological processes, and therefore, it predicts suitability habitat without any statistical training. We estimated potential durations of activity and annual net energy balance for our study species across the spatial extent of the field site (10 × 10 km) in Cullowhee, NC. We used the *microclim* dataset to estimate temperatures experienced during nightly simulations of activity under contemporary climatic conditions (Kearney, Isaac, & Porter, 2014). We used estimates of surface temperature over soil in 90%

shade to reflect the typical conditions in a temperate forest. We corrected temperatures for the influence of elevation by using average adiabatic lapse rates (Supporting Information; Equation 5) for the Southern Appalachians to improve the spatial resolution of temperature data. Estimated values of temperature and humidity were validated with empirical measurements of surface temperature (Supporting Information).

Activity depends on a salamander's current state of hydration (Feder & Londos, 1984). The rate at which organisms lose water to their environment is a product of the evaporative demand of the air and physiological characteristics of the organism (Feder & Burggren, 1992). We estimated humidity by assuming minimum daily temperatures approach the dew point temperature, a common phenomenon in nature due to the effect of radiative cooling near the surface (Kimball, Running, & Nemani, 1997). The minimum dew point temperature provided an estimate of the total amount of vapour in the air, which can be constant across hundreds of kilometres at a given time (Bolstad, Swift, Collins, & Régnière, 1998). Then, we estimated the evaporative demand of the air by calculating the vapour pressure deficit (VPD), the physical factor that drives evaporation rates (Stull, 2000). The VPD is calculated from the difference between the amount of vapour present in the air (determined from the minimum dew point) and the total amount of vapour that the air can hold, termed the saturation vapour pressure (Stull, 2000). We validated our estimation of VPD and temperature data using iButton hygrochrons (Maxim Integrated) dispersed across our field site over two activity season in 2015 and 2016 (Figures S2 and S3).

We calculated rates of water loss based on average values of skin resistance to water loss. We also ran simulations of our model with higher vapour pressure deficits to identify the ecological significance of r_i under dry environmental conditions. In these simulations, we increased VPDs by 25% to reflect typical conditions that occur during drier periods during the year (Figure S3). With each iteration, we varied skin resistance to water loss to determine the sensitivity of potential activity time with respect to r_i. By varying r_i, we demonstrated the potential for adjustments of water loss physiology to increase durations of activity. Upon calculating the rate of water loss, we estimated the amount of time required to lose 10% of their body mass to water loss (Feder & Londos, 1984). Therefore, water loss rates influence energy budgets by determining the amount of time available to forage for prey.

We parameterized our model based on the physiological traits and tolerances of *P. metcalfi* from the literature and our empirical data. We restricted activity on the forest floor to night-time hours (21.00 to 06.00) between 5 and 24°C to reflect their nocturnal behaviour and thermal preferences for activity respectively (Petranka, 1998). While active, we estimated body temperatures based on humid operative body temperatures (T_{eh}) (Supporting Information; Equation 6), which accounts for the effect of evaporative cooling from water loss (Campbell & Norman, 1998). We estimated energy intake and assimilation based on an empirical relationship between temperature and prey intake from previous literature (Supporting Information; Equations 7 and 8) for the genus *Plethodon* (Merchant, 1970). We used maximum rates of energy intake to determine the potential limits of the trade-off imposed upon maintaining energy balance. We used T_{eh} to estimate

energetic costs from empirical relationships between temperature and metabolic rates for our study species (Supporting Information; Equation 9). The methods for estimating the thermal dependence of metabolic rates can also be found in the Supporting Information. We included water intake from prey items assuming an average content of water for an insect from the energy intake function. We assumed prey items consist typically of 70% water (2.33 ml H_2O/g dry mass) with an average energy content of 22 kJ/g dry mass (Bell, 1990). While inactive, we assumed that body temperatures were equivalent to soil temperatures 30 cm below the ground (Supporting Information; Equation 10). These simulations revealed how physiological traits influence energy balance in our species.

2.6 | Integration of the physiological trade-off

Trade-offs between physiological traits might offset the benefits of acclimation if a change in one trait simultaneously reduces the performance of a related trait. We used the relationship between the r_i and $\dot{V}O_2$ that was corrected for the effect of body size (see Section 2.7) to determine the influence of the trade-off on energy balance of *P. metcalfi* at our field site. The relationships between mass, $\dot{V}O_2$ and r_i for both experiments can be found in Figure S1a–d. We ran iterations of our biophysical model across a range of skin resistances (3.5–6.5 s/cm; Figure S1f) for an average size salamander (3.5 g in this study; Figure S1e). In each iteration, we then adjusted $\dot{V}O_2$ based on the correlation between the residuals of r_i and $\dot{V}O_2$ regressed against mass using the following equation:

$$\hat{p}\dot{V}O_2 = 0.14629 + (-0.3985 \times e_{ri})$$

where $\hat{p}\dot{V}O_2$ is the proportional change in $\dot{V}O_2$ relative to average $\dot{V}O_2$ and e_{ri} is the residual of r_i regressed against mass. Therefore, the value of $\dot{V}O_2$ was adjusted with different values of r_i assuming a proportional change in $\dot{V}O_2$ across the thermal performance curve. We ran simulations of the biophysical model without the trade-off between $\dot{V}O_2$ and r_i to demonstrate the consequences of ignoring the trade-off. These predictions can also demonstrate the potential role of interrelated traits in structuring phenotypic variation by identifying combinations of phenotypic traits that are capable (or incapable) of achieving positive energy balance.

2.7 | Statistical analyses

We analysed the results from the experiments separately using linear mixed-effects models with repeated measures in the NLME package in R (v. 3.2.4) to assess interactions and influence of treatments. For both experiments, we analysed the residuals of r_i and $\dot{V}O_2$ regressed against mass to account for the influence of body size on physiological traits (Addo-Bediako et al., 2001). We treated the residuals of $\dot{V}O_2$ as our dependent variable and the residuals of r_i as a continuous fixed effect. We treated total time in the experiment as a covariate, and we treated the temperature and humidity treatment as fixed factors. We also included an interaction between temperature and

humidity treatments due to previous studies demonstrating physiological responses to the interaction between temperature and humidity (Riddell & Sears, 2015). We used the individual as our random effect to account for individual variation between measurements. For the analysis on heart rates, we used a quadratic regression model to meet assumptions of normality. We then tested for the effect of temperature during the measurement of heart rates, the temperature during the measurement squared, temperature and humidity treatments, physiological traits and time point of the measurement. We then used type II Wald Chi-squared analysis to test for interactions among experimental treatments and physiological traits. We report marginal R^2 to describe the proportion of variation explained by the fixed effects (Nakagawa & Schielzeth, 2013) calculated from the MUMIN package in R.

3 | RESULTS

3.1 | Acclimation study

Our experiments revealed a trade-off between skin resistance to water loss and volume of oxygen consumption during thermal acclimation. During the experiment, r_i increased by 22% and $\dot{V}O_2$ decreased by 36% on average across all treatments (Figure 1a,b). Our analysis on the residuals demonstrated that acclimation of r_i was associated with acclimation of $\dot{V}O_2$ ($\chi^2 = 147.2$, $df = 1$, $p < .001$). Our experiment revealed that individuals that acclimated by increasing r_i (i.e. lowering water loss rates) also reduced $\dot{V}O_2$. Moreover, 36% of the variance was explained by the treatments and time in experiment as indicated by the marginal R^2. We also uncovered an effect of temperature in which the trade-off between r_i and $\dot{V}O_2$ persisted under the warm temperature during the experiment ($\chi^2 = 5.483$, $df = 1$, $p = .019$, Figure 1c,d). Prior to the experiment, the trade-off between the two physiological traits was ubiquitous (Figure 1c), but by the end of the experiment, only individuals in the warm treatment exhibited the trade-off between r_i and $\dot{V}O_2$ (Figure 1d). Therefore, our experiment revealed that temperature acted as a single cue for acclimation of the physiological traits.

We also investigated the potential for individuals to compensate for low oxygen flux by increasing heart rate. Heart rates were sensitive to the experimental temperature during measurements ($\chi^2 = 3,134$, $df = 1$, $p < .001$) and declined across the experiment ($\chi^2 = 35.23$, $df = 1$, $p < .001$). However, we did not find any relationships among heart rate, r_i, $\dot{V}O_2$ or the experimental treatments that would suggest a compensatory response from the cardiovascular system. We also found that the trade-off between r_i and $\dot{V}O_2$ was consistent with the complementary experiment on heart rates (Figure 2b; $\chi^2 = 32.89$, $df = 1$, $p < .001$).

## 3.2 | Ecological simulations
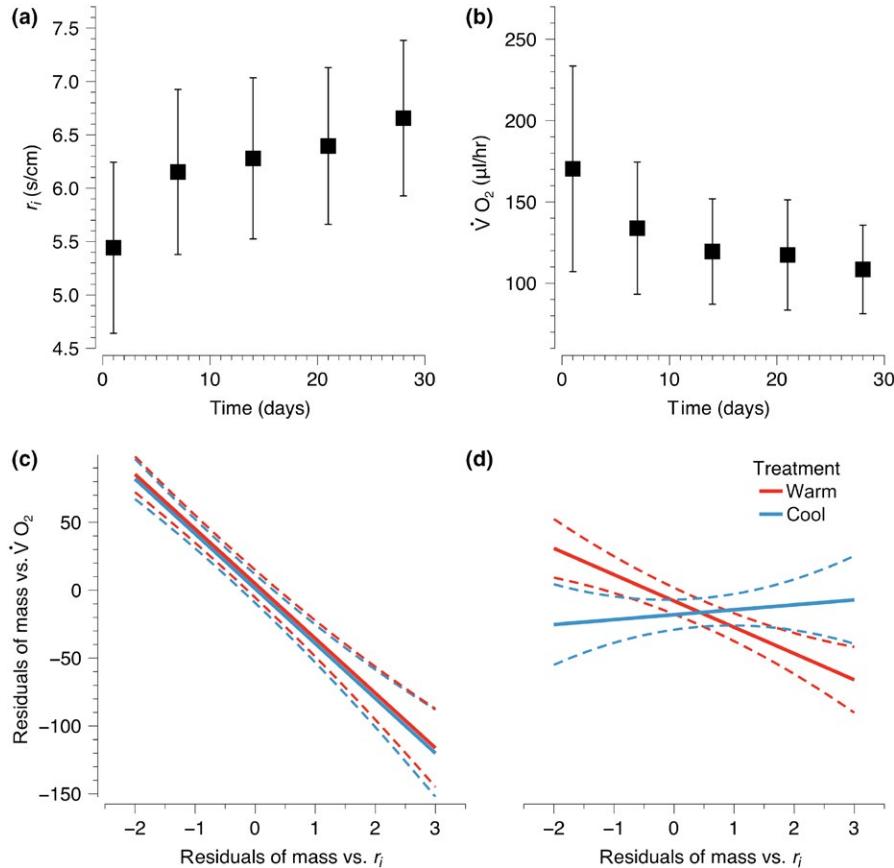
The biophysical model demonstrated that the trade-off between r_i and $\dot{V}O_2$ potentially shapes phenotypic variation by constraining energy budgets. Our model illustrated that without the trade-off, energy

balance did not vary with r_i because humidities are typically very high in this area (Figure 3). With the trade-off, we demonstrated a potential energetic cost due to the increase in metabolic rate associated with lower values of r_i. As a result, only certain combinations of physiological phenotypes were capable of achieving positive energy balance. The combinations that achieved positive energy balance also coincided with the observed variation of r_i that we measured in the acclimation experiment (Figure 3). In our simulations under dry conditions (determined from empirical estimates of vapour pressure deficits), raising r_i resulted in greater potential activity time but with diminishing returns of activity
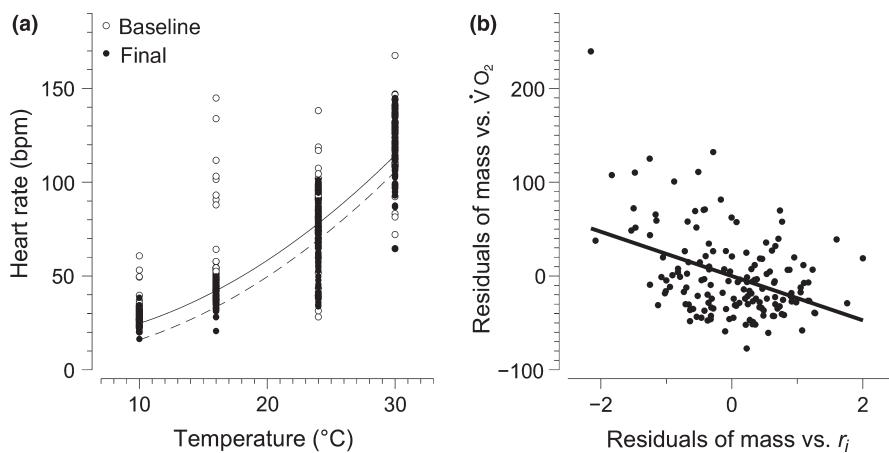
time (Figure 4). Potential activity time increased with r_i until c. 7 s/cm in which potential activity time reached an asymptote. Any increase of r_i beyond 7 s/cm does not appear to prolong durations of activity under the typical dry conditions that the population experiences.

4 | DISCUSSION
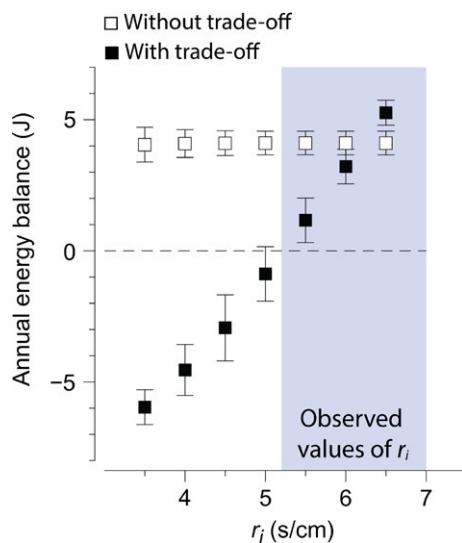
Our experiments uncovered a repeatable trade-off between $\dot{V}O_2$ and r_i that suggests predicting the benefits of acclimation requires knowledge
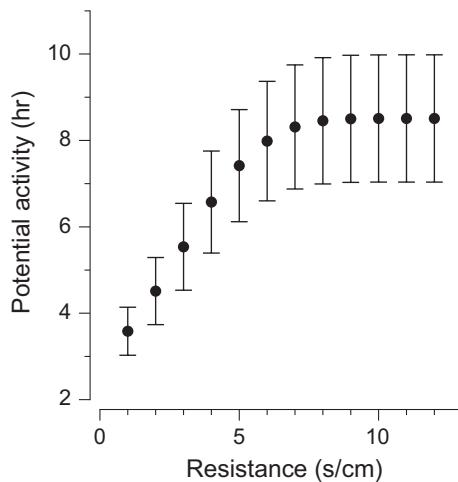

FIGURE 1 Physiological responses and trade-offs between r_i and $\dot{V}O_2$ reveal acclimation strategies. Change in means of physiological traits across the experiment for r_i (a) and $\dot{V}O_2$ (b), and the physiological trade-off between r_i and $\dot{V}O_2$ at the beginning (c) and end (d) of the experiment demonstrates acclimation strategy to temperature (blue = cool; red = warm). The interaction between r_i and $\dot{V}O_2$ persisted in the warm treatment at the end of the experiment, whereas the trade-off had disappeared in the cool treatment. We plotted the means and standard deviations of the physiological traits (a, b) and the regressions with 95% confidence intervals for the interaction analysis (c, d)

FIGURE 2 Thermal sensitivity of heart rates before and after experiment and trade-off in the complementary experiment. Heart rates increased with temperature before and after the experiments, and decreased during the experiment (a). Solid line and open circles are the initial measurements; the dotted lines and closed circles represent the final measurements. We also demonstrated a consistent interaction between r_i and $\dot{V}O_2$ in the complementary experiment (b)

FIGURE 3 Trade-off between $\dot{V}O_2$ and r_i influence energy balance. The figure displays mean values with standard deviations of energy balance incorporating or ignoring the trade-off under typical, moist climatic conditions. Energy balance does not change with r_i when ignoring the trade-off (open squares), whereas energy balance varies substantially by incorporating the interaction (solid squares). The values of r_i predicted to exhibit positive energy balance also coincided with the observed range of values ($M \pm SD$) measured in the acclimation study. Values above the dotted line are positive and below are negative

FIGURE 4 High resistance to water loss provides diminishing returns to potential activity in dry environments. Potential activity time increases with skin resistance to water loss until 7 s/cm, a typical maximum value of r_i from the acclimation experiments. The pattern suggests that salamanders can maximize potential activity time by maintaining a skin resistance of near 7 s/cm. The figure plots averages and standard errors of potential activity time (hr) from the biophysical model

of the linkages between related physiological traits. The trade-off supports our hypothesis that reductions in the flux of water vapour might impede the flux of oxygen as well. We cannot be certain that $\dot{V}O_2$ and r_i are changing independently of one another or changing due to underlying linkages, but there are several lines of evidence that support the

latter. As previously mentioned, these two traits share similar chemical and physical dependencies that appear across a diverse array of life, but more importantly, our experiment revealed that the linkage between the two traits occurred specifically in response to a single cue, temperature. The suite of physiological changes in response to a single cue suggests a relationship between these functional traits, either due to underlying dependencies or as part of whole-organism responses to environmental change. The lack of a response in heart rates suggests that individuals did not compensate for reduced metabolism by increasing blood flow to the skin to promote oxygen diffusion (Franklin et al., 2007). Rather, the pattern suggests that individuals preferred to minimize energetic costs of cardiac function by lowering heart rates during the experiment, potentially as part of a whole-organism strategy of acclimation. The degree of acclimation can be described as a continuum in which individuals either maintained high flux of water and oxygen across the skin or reduced the flux, and either strategy implies downstream consequences minimizing stress.

The trade-off between these functional traits might influence the expression of whole-organism acclimation strategies, such as physiological dormancy or arousal. Organisms commonly reduce the flux of water and oxygen with their environment as a survival strategy by seeking stable microclimates and dampening major physiological processes, such as during hibernation or dormancy (Storey, 2011). While most individuals eventually dampened r_i and $\dot{V}O_2$, our experiments also found that individuals maintained a high flux of water vapour and oxygen in the warm treatment. The "high-flux" strategy may seem non-adaptive due to the higher energetic costs and greater risk dehydration. However, foraging and reproductive activities require a high flux of oxygen (Barry, 2015; Seebacher, Ward, & Wilson, 2013), and if resources are available, increasing flux could support the energetic demand of activities that directly contribute to fitness. The trade-off between $\dot{V}O_2$ and r_i likely persisted under the warm treatment in our experiment due to the different strategies to either boost or depress oxygen flux as two ways to counteract rising energetic costs. These strategies demonstrate that the adaptive potential of acclimation depends upon whole-organism strategies in relation to available resources (e.g. prey) and environmental conditions (e.g. temperature; Bozinovic & Pörtner, 2015). However, the benefits of acclimation cannot be fully understood without knowledge of potential genetic correlations between traits across generations (Angilletta, Niewiarowski, & Navas, 2002).

Our study revealed potential constraints on the evolution of trait values due to the trade-off between r_i and $\dot{V}O_2$. We identified combinations of metabolic rates and water loss rates that were incapable of achieving positive energy balance, and therefore, selection against these combinations of phenotypes might explain the phenotypic variation that we observed in our population. However, without knowing the benefits of the high-flux strategy, any evolutionary conclusions are uncertain. High metabolic rates coincide with more efficient foraging behaviour (Finstad, Forseth, Ugedal, & Næsje, 2007) and a competitive advantage among conspecifics for resources (Nakano, 1995). Studies that relate metabolic expenditure to behaviour in terrestrial salamanders are non-existent, but if they are similar to other taxa, "high-flux"

strategies might increase rates of foraging, aggressive behaviour or dispersal movements. For "low-flux" strategies, our biophysical model suggests that increasing r_i beyond 7 s/cm provided diminishing returns for activity. The threshold for diminishing returns would increase under drier environments, but based on the estimates of VPD at our field site, these dry conditions appear rare. Therefore, our biophysical model combined with empirical data suggest that selection might have optimized r_i to simultaneously maximize activity and the delivery of oxygen required for activity. These interactions are specific to our focal species, but trade-offs among similar physiological processes likely occur across the diversity of life.

The link between metabolic rate and water loss rate appears broadly across a diversity of life (Woods & Smith, 2010), but correlations between these traits might become decoupled or linked to alternative traits due to specific physiological and morphological adaptations. For instance, endotherms have evolved complex turbinates in nasal passages to limit excessive water loss caused by their high ventilation requirements (Hillenius, 1992). The regulation of water loss also plays a critical role in heat balance for endotherms as the primary mechanism of evaporative cooling (Angilletta, Cooper, & Schuler, 2010). During heat stress, dehydrated endotherms will delay evaporative cooling to preserve water balance but at a cost of warming body temperatures (Cain, Krausman, Rosenstock, & Turner, 2009). For insects, respiration commonly occurs using discontinuous gas exchange cycles in which spiracles are opened for long durations to expel excess CO_2 (Hetz & Bradley, 2005). Even in our relatively simple system, salamanders exhibited a capacity to decouple metabolic rate and water loss rate during acclimation; however, the mechanism by which these traits are independently regulated is currently unknown. These adaptations and physiological requirements demonstrate the difficulty in generalization, but the concept of similar trade-offs persists across many taxa. Biophysical models can then extrapolate the potential ecological consequences of physiological dependencies.

Ecological models that include biological mechanism can accelerate the field by making explicit, quantitative predictions. Ecological studies often suffer from an approach that lacks quantitative predictions (Houlahan, McKinney, Anderson, & McGill, 2017). We addressed this growing problem by making specific predictions on the effect of physiological trade-offs on activity and energy budgets. Models based on first principles can be transformative for a field because they demonstrate an understanding of the proximate mechanisms underlying broad ecological and evolutionary questions, such as the physical processes shaping the thermal niche (Porter & Kearney, 2009) and potential for evolutionary responses to climate (Kearney et al., 2009). In our study, the biophysical model indicated that predictions on the benefits of acclimation must be interpreted carefully without knowledge of the underlying trade-offs between linked physiological traits. Therefore, biophysical models provide a clear utility in generating quantitative hypotheses that can be supported or refuted by subsequent field and laboratory-based experiments, a critical step to advance a scientific discipline (Angilletta & Sears, 2011).

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

AUTHORS' CONTRIBUTIONS

E.A.R., J.M. and J.D.D. conducted the experiments; E.A.R. and M.W.S. designed experiments; E.A.R. and M.W.S. wrote the manuscript and conducted analyses. All authors gave final approval for publication and agree to be held accountable for the work therein.

DATA ACCESSIBILITY

Data for the study are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.1r3s2, Riddell, McPhail, Damm, & Sears, 2017)

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